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ABSTRACT BOOK

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ABSTRACT BOOK

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EHA was founded in June 1992 and today – with over 3,000 members from 100 countries – is a consolidated representative of European hematologists.

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- Promote the creation of a highly attractive market for practitioners and researchers in Europe thus fostering the mobility of hematologists in and to Europe.
- Reach out and offer a platform to countries that wish to further develop excellence in hematology.
- Promote education, training and scientific research in hematology in Europe.
- Exchange and disseminate knowledge and scientific information in the field of hematology.

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- Organizing of annual scientific and educational congresses in major European cities.
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- Collaborating with other leading organizations in the field of hematology and oncology.
- Providing postgraduate education through the annual congress, tutorials and workshops and scientific meetings.
- Supporting junior basic and clinical researchers in the development of their careers through the EHA Career Development Program.
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On behalf of the EHA Board and the Scientific Program Committee we are pleased to present the Abstract Book of the 18th Congress of EHA.

The Scientific Program Committee has compiled an exciting and topical program of Simultaneous Sessions and Poster Sessions from close to 3,000 submitted abstracts. Please join our expert moderators for a walk along the posters in your field of interest on Friday and Saturday. The six best abstracts have been selected for presentation during the Presidential Symposium on Saturday, June 15.

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

Jorge Sierra
Chair Scientific Program Committee

Abstract Book
18th Congress of the European Hematology Association, Stockholm, Sweden June 13-16, 2013

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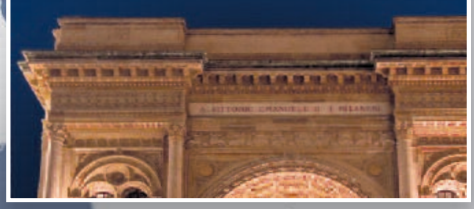

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POSTER SESSION I

Acute lymphoblastic leukemia - Biology

P001

INVESTIGATING THE BIOLOGY OF STIL-TAL1 FUSION POSITIVE T-ALL AND ITS GENETIC SUBCLONAL ARCHITECTURE

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Background: The *STIL-TAL1* fusion occurs in 25% of T-ALL as a result of a 1p33 deletion which removes the coding regions of *STIL* and places *TAL1* under the control of the *STIL* promoter. Our study aims to investigate the biology of this leukaemia subtype and its genetic sub-clonal architecture in order to aid rational design of future targeted treatment strategies. The hypothesis is that drugs targeted at events occurring at a sub-clonal level will likely fail due to Darwinian evolution and the emergence of drug resistance from a minor subclone selected out by environmental modifiers (drug therapy).
Aims: We aimed to determine the common genetic aberrations occurring in *STIL-TAL1* fusion positive T-ALL in children and young adults (age 1 – 25 years) and to investigate how the leukaemic clone evolves genetically in order to determine the early and founder genetic events amenable to therapeutic targeting. We describe our current approach to this issue using SNP arrays, mutation screening and single cell 4 colour FISH.
Methods: DNA from 17 *STIL-TAL1* fusion positive T-ALL cases and one cell line (RPMI 8402) was screened using the Affymetrix SNP 6.0 platform to detect copy number alterations (CNA) and was sequenced to detect mutations in *NOTCH1* (exons 26,27,34), *FBXW7* (exons 9, 10), *PTEN* (exon 7) and *IL7R* (exon 6). Genomic localisation of copy number alterations using CNAG version 3.3.0 software was used to design in house FISH probes for *STIL-TAL1* fusion and losses of *CDKN2A*, *PTEN*, *LEF1* and *CASP8AP2* in order to permit examination of single cells by multicolour FISH. Interphase FISH was performed on methanol-acetic acid fixed nuclei from diagnostic samples. The FISH probe to detect *STIL-TAL1* fusion consisted of two fosmids whose genomic localisation corresponds to the 1p33 deletion and two control fosmids located downstream of *TAL1*. This reliably detected the deletion in 8 samples screened to date.
Results: The most frequent CNA detected was *CDKN2A* loss (94% of samples). Other frequent CNA were losses of *PTEN* (22%), the region 6q13-6q16 including *CASP8AP2 (*22%), *LEF1 (*11%) and gain of *MYB (*11%). Mutation screening revealed mutation frequencies of 55% for *NOTCH1/FBXW7* and 38% for *PTEN*. Mutations in *IL7R* were not detected in this cohort. Single cell 4 colour FISH performed on 4 patients showed the presence of the *STIL-TAL1* fusion in all cells with an abnormal FISH signal pattern. Scoring of 100 – 200 cells per patient with 3 – 4 differentially labelled FISH probes allowed reconstruction of the evolution of the malignant clone through phylogenetic trees.
Summary / Conclusion: This study provides an overview of key genetic aberrations in this T-ALL subgroup. Results support the hypothesis that, in accordance with other types of leukaemia, the *STIL-TAL1* fusion is a founder event being present in all cells with an abnormal FISH signal pattern. Loss of *CDKN2A* occurred early in the evolution of the leukaemogenic clone in line with its key role in T-ALL. Copy number losses such as *PTEN, CASP8AP2* and *LEF1* occurred sub-clonally to *CDKN2A* loss. *PTEN* inactivation is frequent in *SIL-TAL1* T-ALL with either mutation or copy number losses detected in 50% of samples. Comparison of multicolour FISH results for two patients with *PTEN* loss suggests that the evolutionary timing of this event may vary between patients. Future work will incorporate gene mutations into the single cell analysis to determine the position of key mutations, such as *NOTCH1/FBXW7/PTEN,* in *STIL-TAL1* T-ALL evolution.

P002

DEVELOPMENT OF MINIMAL RESIDUE DISEASE (MRD) ANALYSIS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) BY NEXT GENERATION SEQUENCING (NGS)

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Background: Detection of sub-microscopic levels of disease (minimal residual disease; MRD) in childhood acute lymphoblastic leukaemia (ALL) after initial treatment is an important prognostic factor. Currently, stratification of therapy for the new frontline trial in childhood ALL (UKALL2011) is provided by MRD analysis using real time quantitative PCR (RQ-PCR) to identify and quantitate patient specific rearrangements of the immunoglobulin (Ig) and T-cell receptor (TCR) genes. Although MRD is a powerful and essential tool in stratification of ALL patients, the current methodology is expensive, time-consuming and complex to perform, and 8% of individuals in the current UKALL2011 trial do not have an informative MRD result.
Aims: Recently, Next Generation Sequencing (NGS) has led to the opportunity to improve Ig/TCR based MRD analysis by revealing the entire Ig/TCR repertoire. In addition, this technology may also have the potential to allow improved quantification of disease, by detecting lower disease levels, resulting in a potential increase in the specificity and sensitivity of MRD analysis in childhood ALL.
Results: To date, this project has concentrated on the IgH locus using the BIOMED 2 primers (van Dongen *et al.,* 2003) for target identification. These primers have been adapted in a novel manner to allow the deep sequencing of this locus using the Illumina MiSeq. Using a simple sequence analysis pipeline, this technology has been used in twelve patients, firstly, to identify the major clones revealed by current methodologies, and secondly, to detect many related and unrelated low-level clones. This NGS methodology has also been used to delineate patterns of IgH rearrangements in two patients previously shown to have oligoclonal rearrangements (greater than two). Such patients represent a time consuming and technical challenge for current technologies as it is important that all targets at the locus are followed by RQ-PCR to provide an informative MRD result. In addition to target identification, this approach can also be used for the quantification of MRD. Logarithmic dilution series of patient DNA in normal PBMC DNA have been analysed using this novel pipeline and reveal that stratification based on a clinical threshold of 1 in 10,000 is possible using this NGS methodology. An important consideration for both MRD target identification and quantification of disease is to undertake an assessment of the normal background repertoire, as well as the repertoire present at the end of induction therapy; both of which represent critical thresholds. Having established this technology it will be used to quantitate disease levels in end of induction samples, previously analysed by RQ-PCR, and the results compared.
Summary / Conclusion: This approach to target identification allows the study of multiple evolved clones, and therefore has the potential to reduce false negative results. Furthermore, this approach also represents a significant time saving (5-7 days) in comparison to established methods (3-4 weeks). Indeed, the interrogation of the entire Ig repertoire will further improve the predictive value of MRD testing. Additionally, further investigation into the clinical utility of NGS for MRD analysis will concentrate on analysing earlier timepoints in treatment and study the potential use of blood rather than bone marrow.

P003

NOVEL COPY NUMBER ALTERATIONS IN INFANTS WITH T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA (T-ALL)

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Background: Infant Acute Lymphoblastic Leukaemia (I-ALL) is a rare disease associated with *MLL* rearrangements, B cell precursor lineage with $CD10^-$ (pro-B ALL) and prenatal origin. In contrast to infant pro-B ALL, in which an *in utero* origin has been extensively demonstrated, the genetic origin and timing for infant T-cell ALL (T-ALL) has not been fully elucidated. Although very rare in infants, T-ALL is prevalent in older children, this raises the question of whether the aetiology of childhood T-ALL is likely to involve prenatal molecular alterations.
Aims: The overall aims of this study are to elucidate more definitively the early onset of these abnormalities in infants T-ALLs; to delineate the additional genomic hits required to accelerate the emergence of a frank leukaemia; and to compare infant cases with older children/adolescent T-ALL by tracing CNA accumulation.
Methods: The availability to us of a very rare series of 7 infant (≤12 months) T-ALL cases along with 6 remission samples and 4 Guthrie cards allowed us to genetically characterize infant T-ALL (Sanger sequencing, RT-PCR, Q-PCR,

FISH), to trace CNAs and LOH/UPD profiles (SNP-array) and to backtrack prenatal origin.
Results: The main clinical and demographic characteristics observed in our series were a median age of 7 months, no predominance of gender and high WBC count (≥50,000) in 6 out of 7 cases at diagnosis. The infants were treated according to Brazilian GBTLI protocol (n=3), BFM protocol (n=2) and INTERFANT (n=2). All samples have been analyzed at diagnosis for the main T-ALL abnormalities and our results showed 3 cases mutated for *NOTCH1*, all cases were WT for *FBXW7*, *PTEN* and *IL7R* mutations and we did not observe any *SIL-TAL1+* or *TLX3+* cases. *TCRG* & *D* were rearranged in 5 cases, 4 of them also being rearranged for *TCRB*; 1 case presented with only *TCRG* rearrangement and 1 case showed no receptor gene rearrangement at all. This latter case also had a classical ETP profile. *MLL* rearrangements were detected by FISH in 3 out of 6 cases. All diagnostic samples were analyzed by SNP-array to identify genomic losses and gains. As a summary of the SNP results, a recurrent chromosome 3 deletion was observed in 3 cases. This loss results in the complete deletion of *MLF1* and has not previously been described in T-ALL. *MLF1* plays a key role in AML and myelodysplasic syndromes leukaemogenesis. Q-PCR and FISH assays were used to confirm the copy number of *MLF1* in those cases analysed. Akin to paediatric T-ALL we observed a *PTEN* deletion in 1 patient and 2 further cases with a 11p13 deletion that could lead to activation of *LMO2*. Strikingly and unlike childhood T-ALL, none of the infants showed deletions in *CDKN2A*. We confirmed these results using a Q-PCR copy number assay for *CDKN2A* and FISH.
Summary / Conclusion: Since the long term aims of this study include exome sequencing, we cannot rule out the possibility of still having classic and relevant alterations yet to be discovered in these cases, however our preliminary data suggest that infant T-ALL in general presents different genetic abnormalities from childhood T-ALL.

P004

THE ROLE OF THE JANUS-FACED TRANSCRIPTION FACTOR PAX5-JAK2 IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: In B-cell precursor acute lymphoblastic leukemia (BCP-ALL) the transcription factor PAX5, a master regulator of B-cell commitment and maintenance, is fused to several different partner proteins including other transcription factors, structural proteins, and the tyrosine kinase JAK2. Mutations and translocations affecting JAK2 are found in a variety of hematopoietic malignancies and lead to its constitutive activation and cytokine-independent JAK-STAT signaling.
Aims: In this study, we aimed to determine the functional consequences of the chimeric PAX5-JAK2 protein, which contains the DNA binding paired domain of PAX5 and the JH1 kinase domain of JAK2.
Results: Indirect immunofluorescence displayed nuclear localization of PAX5-JAK2. Furthermore, the chimeric protein retains the ability to bind to wild type PAX5 target loci and to activate some PAX5 target genes, like *CD79A* but not *CD19*, as shown by chromatin immunoprecipitation and reporter gene assays, respectively. This suggests that the fusion protein has an influence on PAX5 target gene expression. In order to determine whether PAX5-JAK2 is tyrosine phosphorylated and whether it is capable of activating STAT proteins, intracellular phosphoprotein analyses using flow cytometry and / or Western blotting of different transfected cell lines were performed. Our data provide compelling evidence that PAX5-JAK2 itself is phosphorylated and acts as an active kinase, which in turn phosphorylates and activates downstream STAT proteins. Gene expression profiling of PAX5-JAK2 positive samples revealed high similarities with BCR-ABL1 and JAK2-mutated BCP-ALLs, further supporting an activation of the JAK-STAT pathway. Importantly, the kinase activity of the fusion can be efficiently blocked by JAK2 inhibitors rendering it a potential target for therapeutic intervention.
Summary / Conclusion: Together, our data show that PAX5-JAK2 may deregulate the PAX5 and the JAK-STAT transcriptional network at the same time and thus, by interfering with these two important pathways, may promote leukemogenesis.
This project is funded by the Austrian Science Fund FWF (P21554).

P005

THE PRE-B-CELL RECEPTOR PATHWAY IS A THERAPEUTIC TARGET IN SPECIFICALLY TCF3-REARRANGED B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: *TCF3*-rearranged B-cell precursor (BCP) ALL displays high levels of cytoplasmic Igm (CyIgm) which is a known component of the pre-B-cell receptor (pre-BCR).
Aims: We investigated whether an activated pre-BCR pathway in *TCF3*-rearranged BCP-ALL that may serve as therapeutic target.
Methods: The rearrangement pattern of immunoglobulin heavy chain (*IGH*), and light chains (*IGK* and *IGL)* was analyzed by multiplex PCR in leukemic cell samples of 190 non-*TCF3*-rearranged and 22 *TCF3*-rearranged childhood BCP-ALL patients. Expression levels of pre-BCR pathway components (EBF1, ZAP70, SLP65, BTK, PI3K-p110δ and IRF4) were measured by reverse-phase protein assays. Cytotoxicity of Ibrutinib (a BTK inhibitor) was determined in six *TCF3*-rearranged and six non-*TCF3*-rearranged cases by methyl-thiazol-tetrazolium (MTT) assays. The effect of Ibrutinib on Erk expression levels was tested by western blot.
Results: 84.6% (11/13) of *TCF3*-rearranged cases was highly positive for CyIgm-expression compared to 20.9% (68/325) of non-rearranged cases (P<0.001). The Ig-rearrangement pattern of the majority (86.4%) of *TCF3*-rearranged cases was arrested at IGH level without further processing of IGK/IGL genes. In contrast, only 17.9% of non-*TCF3* rearranged cases was blocked at *IGH* level (P<0.001). Expression levels of pre-BCR pathway proteins (ZAP70, SLP65, BTK, PI3Kd110, IRF4 and EBF1) were 1.2 to 5.2-fold higher in *TCF3*-rearranged compared to non-rearranged samples (P<0.05). The concentration of Ibrutinib lethal to 50% of primary leukemic cells was 16.7mM for *TCF3*-rearranged cases and >50 mM for non-rearranged cases (P<0.001). Ibrutinib effectively reduced the expression of Erk in the MHH-CALL3 (*TCF3*-rearranged) leukemic cell line whereas this level remained unaffected in the non-rearranged cell lines NALM6 and MHH-CALL4 .
Summary / Conclusion: This study indicates that *TCF3*-rearranged BCP-ALL is characterized by an immature pre-BCR signature with a concommitant activated pre-BCR signaling pathway. Inhibition of this pathway selectively reduced the cell viability of *TCF3*-rearranged BCP-ALL. These findings warrant clinical application of pre-BCR inhibitors (such as Ibrutinib) in the treatment of *TCF3*-rearranged BCP-ALL.

P006

ACUTE LYMPHOBLASTIC LEUKEMIA RECOGNITION BY NATURAL KILLER CELLS: CLINICAL AND THERAPEUTIC IMPLICATIONS
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Background: Natural killer (NK) cell recognition of malignant targets is finely tuned by activating and inhibitory receptors. The major receptors with activating functions are NCRs, NKG2D and DNAM-1. NCRs are orphan receptors, MICA/B and ULBPs are ligands for NKG2D, PVR and Nec-2 for DNAM-1. The pathway of NK cell-acute lymphoblastic leukemia (ALL) recognition is unclear. Differences in NK cell killing susceptibility among age-related subgroups of ALL patients have been described in the haploidentical stem cell transplant (SCT) context. Possible differences in the expression of NK cell activating ligands among molecularly defined subgroups of ALL patients have been also hypothesized.
Aims: In this study, we aimed at analyzing the pathways of NK-ALL recognition and at verifying whether differences in NK cell activating receptor ligands' expression among groups defined by age of patients or presence of cytogenetic/molecular aberrations correlate with susceptibility to NK cell recognition and killing.
Methods: We analyzed 103 newly diagnosed ALL patients: 46 adults, median age 34 years (18-74); 57 children, median age 4 years (0.1-17). Ninety patients had B cell precursor (BCP)-ALL: 39 were adults (15 BCR-ABL+, 7 MLL-AF4+, 2 E2A-PBX1+, 15 negative) and 51 children (6 BCR-ABL+, 3 MLL-AF4+, 1 MLL-ENL+, 10 TEL-AML1+, 31 negative); 13 patients had T-ALL (7 adults and 6 children). Phenotypic analyses to evaluate the expression of NKG2D and DNAM-1 ligands (MIC-A/B, ULBP1-2-3, Nec-2 and PVR) on primary ALL blasts and of NKG2D and DNAM-1 on a population of activated NK cells of donor origin were performed. Cytotoxic activity of activated NK cells against primary ALL blasts was also evaluated. For blocking experiments, NK cells were pre-treated with anti-NKG2D or anti-DNAM1 neutralizing mAbs. Student's paired *t* test was used for statistical analysis.
Results: ALL blast cells of children showed a higher expression of Nec-2 (P=.03), ULBP-1 (P=.01) and ULBP-3 (P=.04) compared to adult ALL samples (Figure 1A). The differential expression between adults and children was confined to BCP-ALL blasts with no known molecular alterations. The analysis performed within molecularly defined subgroups of ALL cases revealed a high surface expression of NKG2D and DNAM1 ligands on BCR-ABL+ blasts, regardless of the age of patients. In line with the phenotypic results, pediatric

BCP-ALL without molecular markers showed a higher susceptibility to NK cell killing than molecularly negative adult samples. In addition, primary blasts from BCR-ABL+ ALL appeared significantly more sensitive to NK-dependent lysis than BCP-ALL blasts without molecular aberrations (P=.01) (Figure 1B). Finally, when performing cytotoxic assays in the presence of neutralizing mAbs, the NK cell killing potential was significantly inhibited with the use of anti-DNAM-1 (P=.006), thus indicating a possible pathway of recognition of ALL blast cells in the setting of the Nec-2/DNAM-1.

Summary / Conclusion: For the first time a possible biological explanation responsible for the different role played by alloreactive NK cells in pediatric and adult ALL is suggested. The high expression of these ligands in BCR/ABL+ ALL cases, with the highest levels of cytotoxicity exerted by NK cells targeting this subgroup of ALL blasts, indicate a new possible immunotherapeutic strategy based on the *in vivo* infusion of activated NK cells for controlling/eradicating minimal residual disease in this subgroup of patients. The possible correlation between the level of activating receptor ligands' expression and susceptibility to lysis may help to identify those patients that may maximally benefit from NK-based immunotherapy and from NK alloreactive donors in the haploidentical SCT context.

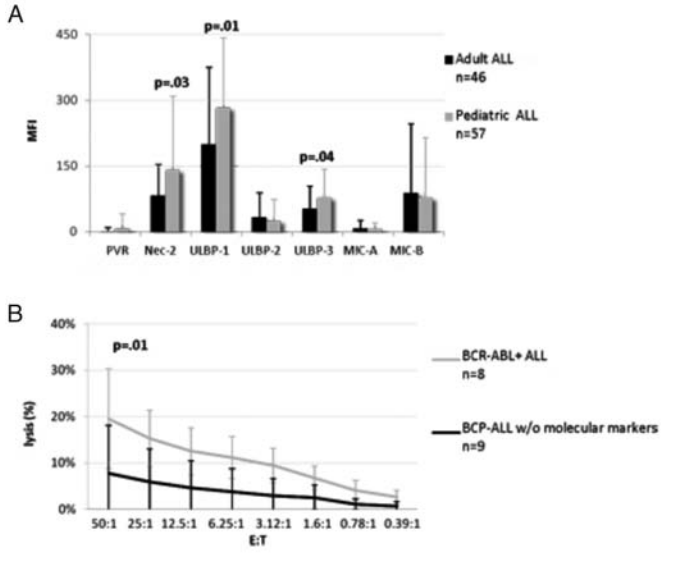

Figure 1.

P007

KRASG12D INITIATES ACUTE T-CELL LYMPHOBLASTIC LEUKEMIA IN COOPERATION WITH LOSS OF THE WILD-TYPE KRAS ALLELE

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Background: Activating mutations in RAS (NRAS, KRAS and HRAS) are found in patients of all major subtypes of hematopoietic malignancies including acute myeloid leukemia (AML), myeloproliferative disease (MPD) and acute lymphoblastic leukemia (ALL). Activation of KRAS in hematopoietic malignancy has been studied using the Mx1-*Cre*;*Kras*$^{LSL-G12D}$ mouse model, where expression of oncogenic *Kras*^{G12D} is induced by injection of polyinosinic-polycytidylic acid (pIpC). Injection with pIpC leads to rapid development of a 100% penetrant MPD. Surprisingly, when bone marrow from these MPD-mice is transplanted to wild-type recipients this leads to development of acute T-cell lymphoblastic leukemia (T-ALL). The cause for this lineage switch is not known. Injection with pIpC in the primary mice leads to expression of Kras^{G12D} also in non-hematopoietic tissues including the liver, colon and in the skin, which may affect the malignant phenotype. Alternatively, during transplantation, Kras^{G12D} tumor initiating cells of myeloid and T-lymphoid lineage might home differently.

Aims: In addition to understanding the cause of this lineage switch, this mouse model also gives an ideal situation for studying the effects of KRAS-activation both in myeloid and in T-lymphoid cells.

Methods: To investigate this and to address what causes the lineage switch we have performed cross bone marrow transplantations between Mx1-*Cre*;*Kras*$^{LSL-G12D}$ and wild-type mice.

Results: Expression of *Kras*^{G12D} has a greater proliferative driving force in myeloid compared to T-lymphoid cells. Expression of *Kras*^{G12D} also increases differentiation of myeloid cells which leads to induction of the primary MPD-phenotype. In parallel, expression of *Kras*^{G12D} expands immature T-cell populations in the thymus leading to a pre-leukemic state in T-cells. pIpC injected Mx1-*Cre*;*Kras*$^{LSL-G12D}$ cannot be rescued by transplanting wild-type bone marrow meaning that the cause of death in the primary mice is unrelated to their MPD. Transplanting the *Kras*^{G12D} bone-marrow extends the lifetime of the tumor, creating time for additional mutations to occur that transforms the pre-leukemic state into T-ALL. One of these additional mutations is loss of the wild-type *Kras* allele.

Summary / Conclusion: The effects of *Kras*^{G12D} expression differs between myeloid and T-lymphoid cells. In myeloid cells activated Kras increases proliferation and differentiation which induces MPD without the ability to transform into AML. In T-lymphoid cells expression of *Kras*^{G12D} leads to a pre-leukemic state with expanded immature populations that with time and additional mutations transforms into T-ALL. This indicates that activation of Kras in the development of acute leukemia, is a potent first genetic hit in the T-lymphoid lineage, but not in the myeloid lineage. Also, loss of the wild-type *Kras* allele in the T-ALL tumors indicates a tumor-suppressive role for wild-type *KRAS* in T-cell tumorigenesis.

P008

FREQUENCY AND PROGNOSTIC SIGNIFICANCE OF COPY NUMBER ALTERATIONS (CNA) IN HIGH-RISK B-LINEAGE ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS ENROLLED IN RISK-ADAPTED PROTOCOLS

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Background: In the last years, great advances in the understanding of ALL biology have been achieved. Several studies in pediatric patients have identified copy number alterations (CNA) in genes involved in cell differentiation, tumor suppression, cell cycle control and apoptosis as markers with prognostic significance. The frequency and prognostic value of CNA in adult ALL are less studied.

Aims: To analyze the frequency and prognostic significance of CNA in B-cell development genes in a series of high-risk B-lineage adult ALL patients enrolled in high-risk protocols from the Spanish PETHEMA Group.

Methods: Bone marrow or peripheral blood samples at diagnosis from 78 high-risk B-lineage adult ALL patients were studied by MLPA for the following genes: *IKZF1, IKZF2, IKZF3, EBF1, CDKN2A/B, PAX5, ETV6, BTG1, RB1,* X/Y PAR region genes (*CRLF2*, *CSF2RA* and *IL3RA*), 14q32.33 region genes (*IGH D*, *MTA1* and *KIAA0284*) and *hsa-miR-31*.

Table 1. Frequency of CNA in 78 higt-risk B-lineage adult ALL patients enrolled in PETHEMA protocols.

	Deletion, n (%)	Duplication, n (%)	Normal, n (%)
IKZF1	29 (37)	1 (1)	48 (62)
EBF1	8 (10)	3 (4)	67 (86)
CDKN2A/B	30 (38)	0	48 (62)
PAX5	27 (35)	1 (1)	50 (64)
hsa-miR-31	17 (22)	0	61 (78)
ETV6	3 (4)	0	75 (96)
BTG1	6 (8)	1 (1)	71 (91)
RB1	13 (17)	0	65 (83)
14q32.33 region	15 (19)	4 (5)	59 (76)
X/Y PAR region	6 (8)	9 (12)	63 (80)
IKZF2	0	3 (4)	75 (96)
IKZF3	1 (1)	4 (5)	73 (94)

Results: The median age was 46 [15-74] years, 40 (51%) males, median WBC count 15.4 x10^9/L [0.4-388]. Immunophenotype: 8 pro-B (10%), 44 common (57%), 15 pre-B (20%), 8 mature-B (10%), 3 undetermined (3%). Cytogenetics: 13 normal (17%), 6 hyperdiploid (8%), 2 hypodiploid (3%), 17 t(9:22) (22%), 5 t(1;19) (6%), 5 11q23/*MLL* (6%), 7 t(8;14)/*C-MYC* (9%), 1 complex (1%), 9 other translocations or deletions (12%), 13 no growth (17%). CNA results reported by MLPA are shown in table 1. Deletions of *IKZF1* were significantly associated with older age and Ph+ ALL. Patients with *EBF1* or *RB1* deletions showed higher WBC count at presentation and *RB1* deletions were associated with

high frequency of CNS infiltration. *IKZF1* deletions were also significantly associated with *EBF1* deletions. In the 9p region, we observed a high codeletion rate of *CDKN2A/B* with *PAX5*, *CDKN2A/B* with *hsa-miR-31* and *PAX5* with *hsa-miR-31*. We also observed concomitant deletions of *PAX5* with *BTG1*, *BTG1* with 14q32.33 region, *PAX5* with 14q32.33 region and *PAX5* with X/Y PAR region. Seventy six patients were evaluable for prognosis. In the whole and in the Ph-negative cohorts, patients with *BTG1* or those with ETV6 deletions had a significant lower complete remission (CR) rate than those not deleted. Concerning overall survival (OS), deletions of *IKZF1, CDKN2A/B*, *hsa-miR-31*and X/Y PAR region CNA were associated with significantly lower OS rates in the entire cohort. In Ph-negative patients, *IKZF1* deletions were also associated with lower OS, and X/Y PAR CNA showed a trend to reduced OS (P=0.052). In terms of CR duration, patients with deletions of *IKZF1* or *CDKN2A/B* showed significantly higher relapse rates in both the whole and the Ph-negative cohorts.
Summary / Conclusion: In high-risk B-lineage adult ALL patients the frequency of CNA is similar to that reported in childhood ALL. Resistance to therapy is associated with *BTG1* and *ETV6* deletions. *IKZF1* and *CDKN2A/B* deletions are associated with lower CR duration, and deletions in *IKZF1*, *CDKN2A/B*, *hsa-miR-31* as well as X/Y PAR CNA correlated with poorer OS.
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P009

P38ALPHA/BETA INHIBITION PREVENTS ALL PROLIFERATION *IN VIVO*
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Background: P38α/β stress-activated protein kinase (SAPK) has been described as an important regulator of dormancy and tumor cell dissemination in different epithelial malignancies. However, p38α/β also regulates other cellular processes such as inflammation, differentiation, apoptosis and, rarely, cell cycle progression.
Aims: Here, we study the role p38α/β as a targetable mediator of acute lymphoblastic leukemia (ALL) proliferation.
Methods: We performed *in vitro* and *ex vivo* flow cytometric analyses to study the role of p38α/β in ALL growth in cell lines. Biochemical analyses were conducted by Western Blotting. For *in vivo* studies, ALL xenografts were created by injecting fluorescently labeled ALL cells into large vessels in the chorioallantoic membrane of fertilized avian embryos (domestic turkey, *Meleagris gallopavo*). Engraftment in target organs was measured by flow cytometry and immunohistochemistry after 11 days. For *in vitro* and *in vivo* p38α/β inhibition, the kinase inhibitors SB203580 and BIRB-796 were used.
Results: P38α/β was highly phosphorylated during log-phase of ALL cell growth in culture. Importantly, intracellular flow cytometric analyses found that high phosphorylation of p38α/β correlated with the S-and G2/M-phases of the cell cycle and low phosphorylation of p38α/β with G0/G1. Remarkably, maximal p38α/β activation was found in ALL cells proliferating in permissive microenvironments like the bone marrow of avian xenografts. Additionally, growth suppressive measures such as L-Asparaginase therapy lead to an abrogation of p38 signaling while other MAPK pathways remained unaffected. Inhibition of p38α/β with SB203580 and BIRB-796 causing accumulation of cells in G0/G1 *in vitro*, markedly suppressed engraftment and proliferation *in vivo*.
Summary / Conclusion: We propose p38α/β as a mediator of important growth-stimulatory mechanisms in ALL cells and suggest p38α/β inhibition as a potential adjunct therapeutic approach in the treatment of this disease entity warranting further mechanistic exploration and validation in patient samples.

P010

A UNIQUE FAT1 CADHERIN ISOFORM REGULATES THE PROLIFERATION OF T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL) CELLS
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Background: The large 550 kDa FAT1 protein is a member of the cadherin superfamily and an ortholog of the Drosophila fat protein, which is implicated in cell proliferation during development. We recently reported that FAT1 cadherin is expressed by a range of leukemia cell lines, but not by normal peripheral blood and bone marrow cells from healthy donors. *In silico* analysis of expression data revealed significant expression levels of FAT1 in 11% of acute myeloid leukemia, 29% of T-ALL and 63% of B-ALL, and little or no transcript in normal blood cells. Using RNA-sequencing and Northern blot analysis of T-ALL cell lines, we now have identified a novel isoform of FAT1, which is unique to T-ALL and bears striking similarity to the drosophila allele Gull, an antimorph of the drosophila Fat.
Aims: To characterize the expression and function of FAT1 and the smaller unique isoform using *in vitro* and *in vivo* models.
Methods: RNA-seq data from a panel of leukemic T-ALL cell lines in combination with Northern blotting was mined focusing on members of the FAT cadherin family for mutations. Transcript and Protein expression of FAT1 and the isoforms was assessed through qPCR and Western blotting on both T-ALL lines and bone marrow aspirates from both primary B-ALL and T-ALL samples. The function of one of the isoforms, gulFAT1, was analysed *in vitro* using cell proliferation, colony forming and soft agar assays. To study the effects of the FAT1 isoforms *in vivo*, we expressed the gulFAT1 construct as well as the FAT1 cytoplasmic tail only in mouse hematopoietic cells through viral transduction. Transduced cells were injected into sublethally irradiated recipient mice.
Results: Using Northern blotting, we identified two novel FAT1 isoforms that occur as truncated transcripts at the RNA level (12 kb and 2.8 kb) compared to the wild type full length FAT1 mRNA (15 kb). The smaller truncated transcripts were found to be both unique and specific to T-ALL. RACE analysis showed the smaller 2.8 kb is the result of a cryptic transcription start site incorporating a retained intron and predicted protein to have a novel N-terminus. Interestingly the sequence is the striking ortholog of a gene mutation in drosophila (Gull) that results in developmental abnormalities of the wing [JC1] and we have therefore named this smaller human equivalent as gulFAT1. In murine NIH3T3 fibroblasts, gulFAT1 expression caused a significant increase in proliferation, which was abrogated upon removal of the carboxyl-terminal PDZ binding peptide. We have found that this motif can specifically bind to the DLG1, MAGI3 and SCRIB, proteins, which have previously been indicted in the etiology of T-ALL. Transient siRNA knockdown in human T-ALL lines decreased cellular proliferation, and on going experiments with expression of gulFAT1 in the bone marrow cells of mice caused increased white blood cell count.
Summary / Conclusion: The FAT cadherins have recently been implicated in a number of cancers, and our results have identified a short FAT1 transcript gulFAT1 that is potentially involved in the leukomogenesis of T-ALL. The exact role of gulFAT1 it yet to be determined, but may act by sequestering PDZ binding proteins from their normal function. With the expression of the shorter FAT1 isoforms unique to leukemic cells they could be considered as markers for diagnosis and minimal residual disease, and potentially future therapeutic targets.

P011

COMMON AND DISTINCT FEATURES OF PAX5 FUSION PROTEINS
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Background: In B-cell precursor acute lymphoblastic leukemia PAX5, a transcription factor pivotal for B-cell commitment and maintenance, is frequently affected by genetic alterations including deletions, mutations, and chromosomal rearrangements. The latter result in the expression of chimeric proteins that retain the PAX5 DNA-binding paired domain at the N-terminus, which is fused to the C-terminal domains of a heterogeneous group of partner proteins. PAX5 fusion proteins are hypothesized to act as aberrant transcription factors antagonizing wild-type PAX5 function.
Aims: To characterize PAX5 fusions we investigated the biochemical and functional properties of PAX5-DACH1, PAX5-DACH2, PAX5-ETV6, PAX5-HIPK1, and PAX5-POM121.
Results: Ectopic expression of these fusion proteins showed a predominant nuclear localization, which may be explained by the presence of the paired domain and at least one nuclear localization signal provided by PAX5 itself or the respective partner protein. Oligomerization was only observed for those fusion proteins that contain a known self-interaction motif like the sterile alpha motif of ETV6 or the coiled-coil domains of DACH1 and DACH2. In chromatin immunoprecipitation experiments all PAX5 fusion proteins exhibited binding to endogenous PAX5 target sequences. Notably, reporter gene assays showed that some fusions are capable of activating the *CD79A* promoter whereas others are not. However, none of the chimeras activated *Cd79a* and surface IgM expression in murine 558LμM plasmacytoma cells.
Summary / Conclusion: Our data show that PAX5 fusion proteins exhibit shared as well as distinct features, which may modulate their function and thus the development of the respective leukemia.
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P012

ANALYSIS OF CRLF2 EXPRESSION IN A COHORT OF 91 NEWLY DIAGNOSED ADULT PATIENTS WITH B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA: CORRELATION WITH CLINICO-BIOLOGICAL FEATURES AND OUTCOME
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Background: A deregulated expression of the cytokine receptor-like factor 2

(d-*CRLF2*) has been described in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) lacking recurrent chromosomal aberrations. An increased *CRLF2* expression is associated with activating *JAK1/JAK2* mutations and *IKZF1* lesions, seems to correlate with a "Ph-like" genomic profile and a poor outcome in high-risk patients. While the frequency and prognostic relevance of a *CRLF2* abnormal expression has been extensively investigated in pediatric cases, little is known about its role in adult ALL.

Aims: In order to evaluate the clinical relevance of *CRLF2* expression levels in adult ALL, we analyzed 91 newly diagnosed adult BCP-ALL cases (median age 44 years, range 19-81) negative for recurrent fusion genes, and correlated d-*CRLF2* with the clinico-biological characteristics and outcome. In addition, we assessed the incidence of the *P2RY8/CRLF2* transcript and its clinical impact on the same cohort of patients.

Methods: Total RNA was extracted from mononuclear cells of patients enrolled in different GIMEMA protocols. All samples were collected at diagnosis and contained at least 70% of leukemic blasts. Ninety-one BCP-ALL adult patients were studied: molecular screening, performed on all cases, proved negative for the recurrent fusion genes (*BCR/ABL1*, *ETV6/RUNX1*, *E2A/PXB1*, *MLL/AFF1*). To evaluate the expression of *CRLF2,* we performed quantitative PCR (Q-PCR) and the results were normalized on the housekeeping gene *GAPDH* ($\Delta Ct = Ct_{CRLF2} - Ct_{GAPDH}$). ΔCt values are inversely correlated to the expression levels of the gene. RT-PCR was carried-out to identify the presence of the *P2RY8/CRLF2* transcript.

Results: In our cohort, *CRLF2* showed an extremely heterogeneous expression (ΔCt range: 1.5-17.3). Correlation between *CRLF2* expression levels and the clinico-biological characteristics - i.e. age, white blood cell (WBC) and platelet (Plts) counts, and hemoglobin (Hgb) levels - showed a statistically significant association with hyperleukocytosis (P=0.0014) and thrombocytopenia (P=0.037), but not with age and Hgb levels. Next, by using a ΔCt ≤8 cut-off, based on martingale residuals, a statistically significant correlation was found with disease-free survival (DFS) and overall survival (OS) (P=0.004 and P=0.044, respectively); no association with response to induction chemotherapy was found. In addition, we evaluated the incidence of the *P2RY8/CRLF2* transcript: the fusion gene was identified in 7/91 patients (7.7%), with 3 cases showing a concomitant *CRLF2* overexpression. The correlation with outcome showed that of the 7 *P2RY8/CRLF2*+ cases, 2 experienced a relapse at 3 and 6 months, respectively, and 2 died during treatment.

Summary / Conclusion: These results indicate that *CRLF2* expression is highly heterogeneous in adult BCP-ALL cases and highlight a correlation with an increased WBC count and a decreased PLT count, as well as with a poor outcome. Furthermore, our study demonstrates that the incidence of the *P2RY8/CRLF2* transcript in adult BCP-ALL is similar to that reported in pediatric cohorts and confirms the lack of an absolute concordance between its presence and high *CRLF2* levels. Despite the small number of *P2RY8/CRLF2*+ cases, a negative role of this transcript on the outcome of adult patients is suggested.

P013

A CLINICALLY RELEVANT POPULATION OF LEUKEMIC CD34+CD19+ CD58- CELLS IN PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Xenotransplantation studies indicate that leukemia selectively develop from a faction of leukemia-initiating cells (LIC). Current therapies, despite actively targeting leukemic bulk blasts, often spare the quiescent and resistant LIC responsible for relapse. If clinically relevant, it is expected that LIC would be enriched in minimal residual disease (MRD) and predictive of relapse. Despite the considerable research on LIC over the past 2 decades, its clinical significance remains uncertain.

Aims: To investigate the leukemia-initiating capacity of CD34+CD19+CD58- cells and determine the prognostic significance of CD58 (lymphocyte function–associated antigen 3) expression by CD34+CD19+ cells in patients with *de novo* Philadelphia chromosome-positive acute lymphoblastic leukemia(Ph+ALL) treated in Peking University Institute of Hematology.

Methods: First, the leukemia-initiating potential of sorted $CD34^+CD19^+CD58^+$ and $CD34^+CD19^+CD58^-$ compartments was investigated *in vivo* using anti-mouse CD122 monoclonal antibody conditioned NOD/SCID mice by intra-bone marrow–injection. Second, we prospectively analyzed whether the $CD34^+CD19^+CD58^-$ compartment at diagnosis correlates with MRD frequency after therapy and clinical outcome in 49 adult patients (18-60 years) with *de novo* Ph^+ALL. According to ROC analysis, more than 20% of $CD34^+CD19^+$ cells with CD58 expression analyzed by flow cytometry at diagnosis were defined as $CD34^+CD19^+CD58^+$, all other cases were defined as $CD34^+CD19^+CD58^-$.

Results: Xenotransplantation of the sorted $CD34^+CD19^+CD58^-$ cells led to a repopulation of human B-ALL in recipient mice, which were phenotypically and clonally derived from the original Ph^+ALL patients analyzed by flow cytometry, as well as fluorescence in situ hybridization and quantitative reverse transcription-PCR for leukemia-specific cytogenetic abnormalities. In patients with Ph^+ALL, a $CD34^+CD19^+CD58^-$ phenotype at diagnosis significantly correlated with a lower complete remission rate and high MRD frequency. Additionally, it directly correlated with higher cumulative incidence of relapse(CIR, 51.79%±2.16% vs. 16.43%±0.49%, P=0.02) and unfavorable disease-free survival (DFS,40.18%±13.61% vs. 66.20%±12.17%, P=0.04) at 30-month. A good correlation between the MRD results detected by $CD34^+CD19^+$cells with aberrant CD58/CD123 expression and the BCR-ABL transcript levels was observed during the follow-up (n=360 pairs, Spearman r=0.88, P<0.0001). The $CD34^+CD19^+CD58^-$ group exhibited a higher rate of BCR-ABL mutations conferring higher level imatinib resistance than the $CD34^+CD19^+CD58^+$ group (42.86% *vs.* 5.71%, P=0.005). Multivariate analyses revealed that $CD34^+CD19^+CD58^-$ phenotype at diagnosis was an independent risk factor for relapse (HR=4.85, P=0.01) and DFS (HR=0.32, P=0.04) in Ph^+ALL.

Summary / Conclusion: Both the xenotransplantation data, as well as the clinical correlation studies, show that $CD34^+CD19^+CD58^-$ compartment enrich for leukemia-initiating cells in Ph^+ALL. $CD34^+CD19^+CD58^-$ phenotype at diagnosis independently correlates with an adverse prognosis, which promises to be an efficient tool for relapse prediction in Ph^+ALL patients.

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P014

TUMOR HETEROGENEITY IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: IMMUNOPHENOTYPE-DEFINED CELL SUBPOPULATIONS AND TCR GENE CLONALITY

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Background: The ability to distinguish leukemic cells based on their immunophenotype or on DNA sequences of rearranged clonal T-cell receptor (TCR) loci is a prerequisite for minimal residual disease (MRD) monitoring in T-cell acute lymphoblastic leukemia (T-ALL). A problem in T-ALL is discrepancies between flow cytometry (FC)- and PCR-based MRD results. Occurrence of blast subpopulations/subclones is a potential pitfall of MRD monitoring. Subpopulations might harbor different rearranged TCR DNA sequences leading to risk of underestimated PCR-MRD results which are based on sequence information from the main population at diagnosis.

Aims: The aim of this study is to characterize T-ALL leukemic blast immunophenotypic heterogeneity and the TCR gene rearrangement profiles for distinct cell subpopulations, and thereby evaluate whether PCR-based MRD monitoring detects these subpopulations.

Methods: We analyzed the leukemia-associated immunophenotype (LAIP) by multicolor FC in diagnostic bone marrow/peripheral blood samples from 41 consecutive Danish T-ALL patients treated according to the NOPHO ALL-2008 protocol. The analysis focused on characterization of heterogeneous FC expression patterns (bimodal defined as distinct populations in contour-plot, and broad). 24 patients, having leukemic cell subpopulations with heterogeneous expression of one/more markers, are currently being investigated by flow-sorting and screening of TCR clonality patterns (IdentiClone Clonality assay).

Results: 83% of the T-ALL patients had leukemic cell subpopulations (comprising <2% of total blast count) characterized by bimodal marker expression, most often of TdT, CD1a and CD4 (27-34% of patients), as well as cytCD3 and CD34 (15-17%). A common heterogeneity comprised of bimodal CD1a with ~2/3 of the blasts being negative and ~1/3 positive. Markers that often showed broad expression pattern were: TdT, CD34, CD4, cytCD3 and CD1a.

Results from TCR clonality testing in flow-sorted subpopulations in 24 patients will be presented. Preliminary data from 9 patients showed that the majority of patients (8 out of 9) have identical TCR gene rearrangements in flow-sorted individual leukemic cell subpopulations. But one patient had a CD1a-bright subpopulation negative for a TCRD and TCRB V-J gene rearrangement found in the bulk CD1a-neg. leukemic population. The TCRD and TCRB markers had been used for PCR-MRD, but FC revealed that the CD1a-pos. cell population had disappeared at treatment day 29. (The LAIP and gene rearrangement profile of the CD1a-pos. subpopulation showed to be identical to that of the leukemic cell population detected in the lymph node biopsy).

Summary / Conclusion: Our data show that the majority of T-ALLs have leukemic cell subpopulations by FC analysis, often characterized by heterogeneous expression of markers used for flow-MRD monitoring (eg. TdT, CD1a, CD34). Our preliminary data show that cell subpopulations may be negative for TCR clonal markers identified in the bulk cell population, thus leading to a risk of MRD underestimation. Using both FC and PCR-based monitoring could minimize this risk, which is important for correct treatment stratification.

P015

IMMUNOPHENOTYPE AND PROLIFERATION PROFILE OF NORMAL B CELL PRECURSORS IN REGENERATING BONE MARROW FROM PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Background: During follow up of B cell precursor (BCP) acute lymphoblastic leukemia (ALL) patients, the detection of minimal residual disease is the most important prognostic factor for relapse. The regeneration pattern of normal BCPs in bone marrow (BM) forms the background to which these remaining malignant blasts should be detected. During the treatment course of BCP-ALL, BCP numbers decrease dramatically. However, in between treatment blocks and after stop of treatment, massive regeneration of BCPs occurs in the BM.
Aims: BCP regeneration may hamper sensitive detection of MRD. In order to get more insight in the characteristics of regenerating BCPs in the BM of BCP-ALL patients, we compared the immunophenotype and proliferation profile of BCPs in regenerating BM and BM from healthy controls.
Methods: BM samples of healthy individuals (normal BM), patients with BCP-ALL after induction therapy (day 79) and patients with BCP-ALL one year after stop of therapy (month 36) were analyzed.For immunophenotypic analysis, 8 color flow cytometry was performed. Newly designed Infinicyt Software was used to define multiple maturation stages in the BCP population. For each individual marker, the expression pattern during these maturation stages was compared between regenerating BM and normal BM. To assess proliferation, four BCP subsets were sorted from each BM sample: pre-B-I, pre-B-II-large, pre-B-II-small and immature B cells. To determine the percentage of proliferating cells, propidium iodide (PI) was added and PI expression was measured using flow cytometry. The proliferation history of BCPs in the pre-B-II-small en immature subsets was assessed by performing a K-rearrangement excision circle (KREC)-assay. The ratio between the KREC and the rearranged *IGK*-gene represented the average amount of replication cycles the BCPs had undergone since *IGK*-rearrangement.
Results: The BCP expression patterns of CD19, CD10, CD34, CD58, CD66c, CD38, CD123, CD9, CD81, CD24, TdT, Igκ and Igλ of regenerating BM at day 79 and month 36 were similar to the expression patterns of BM from healthy individuals. As expected, most proliferation in normal and regenerating BM occurred in the pre-B-II-large subset. The average percentages of proliferation in respectively the pre-B-I and pre-B-II-large subsets were 13%±2% and 29%±3% at day 79 and 9%±2% and 30%±3% at month 36. This was comparable to the percentages of proliferating cells in the pre-B-I and pre-B-II-large subsets of normal BM. Regenerating BM at month 36 and normal BM showed no significant proliferation in the pre-B-II-small and immature BCP subsets. In regenerating BM at day 79, these two subsets were hardly present. KREC-analysis of the pre-B-II-small and immature subsets confirmed that no cell divisions had occurred after *IGK*-rearrangement in both normal BM and regenerating BM at month 36.
Summary / Conclusion: Regenerating BM in BCP-ALL patients seems immunophenotypically similar to normal BM. Proliferation in regenerating BM takes mainly place in the pre-B-II-large subset, like in normal BM. The percentage of proliferating cells and the number of cell divisions after *IGK*-rearrangement in the BCP subsets of regenerating BM are comparable to normal BM. Therefore, the regeneration of BCPs during and after therapy is unlikely the result of enhanced proliferation in the pre-B-I, pre-B-II-large, pre-B-II-small or immature subset, but might be due to a larger influx of non-committed stem cells into the B cell lineage. Knowledge on regenerating BCPs may support the design of sensitive flow cytometric MRD assays.

P016

INHIBITION OF S100A6 INDUCES GRAFT VERSUS LEUKEMIA EFFECTS IN MLL/AF4-POSITIVE ALL IN HUMAN-PBMC-SCID-MICE MODEL

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Background: *Mixed-lineage leukemia (MLL)/AF4*-positive acute lymphoblastic leukemia (ALL) is associated with poor prognosis even after allogeneic hematopoietic stem cell transplantation (allo-HSCT). The resistance to graft-*versus*-leukemia (GVL) effects may be responsible for the poor effect of allo-HSCT on *MLL-AF4*-positive ALL. Cytotoxic effector mechanisms mediated by tumor necrosis factor-alpha (TNF-α) was reported to contribute to the GVL effect. We have previously shown that *MLL-AF4*-positive ALL escapes from TNF-a-mediated apoptosis by up-regulation of S100A6 expression followed by interfering with p53-caspase 8-caspase 3 pathway *in vitro*.
Aims: We examined whether inhibition of S100A6 can induce effective GVL effect in a mice model.
Methods: To examine the long-term effects of inhibition of S100A6, we produced *MLL/AF4*- positive ALL cell lines (SEM) transduced with lentiviral vectors expresses both S100A6 siRNA and luciferase (SEM-Luc-S100A6siRNA). As a control SEM transduced with lentiviral vectors expresses both control siRNA and luciferase were produced (SEM-Luc-controlsiRNA). *In vitro* analysis, the growth of each cells were assessed with or without of TNF-α (1 and10 ng/mL). *In vivo* analysis,$1x10^7$/Body of SEM-Luc-S100A6siRNA cells were injected into five SCID mice and $1x10^7$/Body of SEM-Luc- control siRNA cells were injected into five SCID mice. After confirmation of engraftment of SEM cells by *in vivo* imaging system (IVIS), each groups of mice were injected $4.8x10^7$ of human- peripheral blood mononuclear cells (PBMCs). The serum concentrations of human-TNF-a three weeks after injection of human PBMCs were measured by ELISA (enzyme-linked immunosorbent assay) in each five mice.In addition to overall survival (OS) rate and tumor growth were assessed byIVIS.
Results: *In vitro* analysis, there were no significant differences between the growth of SEM-Luc-S100A6siRNA and those of SEM-Luc-control siRNA without TNF-a (P=0.890). However, the growth of SEM-Luc-S100A6siRNAwere significantly inhibited by TNF-α in comparison with the growth of SEM-Luc-control siRNA (P=0.012 for 1 ng/mL of TNF-α and P=0.005 for 10 ng/mL of TNF-α). *In vivo* analysis, although there were no significant differences between the serum concentrations of human-TNF-α after injection of human PBMCs of SEM-Luc-S100A6siRNA injected mice and those of SEM-Luc-control siRNA injected mice (145.0±5.0 pg/mL VS 150.0±40.0 pg/mL, P=0.95), significant differences were observed between OS of each mice (median >100 days VS median 54 days, P=0.002). As for tumor growth of 6 weeks after SEM cells engraftment, significant differences were observed between SEM-Luc-S100A6siRNA injected mice and SEM-Luc-control siRNA injected mice ($2.95±0.10x10^5$ p/s VS $1.42±0.05x10^7$p/s, P=0.001)
Summary / Conclusion: Our results showed thatinhibition of S100A6 can induce effective GVL effect in a mice model.These results suggest that inhibition of S100A6 may be a promising therapeutic target for *MLL/AF4*-positive ALL in combination with allo-HSCT because it induce effective GVL effectson *MLL/AF4*-positive ALL which is resistant to GVL effects.

P017

ETV6/RUNX1 TRANSCRIPT IS A POTENTIAL TARGET OF RNA-BINDING PROTEIN IGF2BP1

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Background: In our previously published study (Stoskus *et al*. Blood Cell Mol Dis 2011) we have determined that IGF2 mRNA binding protein 1 (IGF2BP1) is significantly overexpressed in t(12;21)(p13;q22)-positive leukemia compared to other acute lymphoblastic leukemia (ALL) subtypes and healthy controls. These findings prompted us to hypothesize about the interaction and potential functional relationship between RNA-binding protein IGF2BP1 and leukemia fusion gene *ETV6/RUNX1* (*TEL-AML1*) transcript.
Methods: To test the possible interaction between IGF2BP1 protein and *ETV6/RUNX1* mRNA, we employed RNA immunoprecipation (RIP) approach using cytoplasmic extracts form *ETV6/RUNX1*-positive leukemia cell line REH and clinical samples of t(12;21)-positive ALL. Cytoplasmic extracts from clinical samples were prepared using -80°C frozen WBC pellets. IGF2BP1/mRNA complexes were precipitated using RIP-grade rabbit anti-IGF2BP1 antibody (MBL International) and isolated with ProtA Dynabeads (Life Technologies) as described by Jain R. *et al* (Methods Mol Biol. 2011). Normal rabbit IgG was used as a negative control for RNA immunoprecipitation (isotype control). Enrichment of control transcripts – *ACTB*, *CTNNB1*, *MYC* – and a potential target - *ETV6/RUNX1* – was assayed by RT-qPCR method. The level of *IGF2BP1* expression in the RNA input aliquot was used as a reference for normalization in delta Cq calculation.
Results: By using RIP approach we have identified *ETV6/RUNX1* leukemia fusion gene transcript in the anti-IGF2BP1 immunoprecipitate of t(12;21)(p13;q22)-positive cell line REH cytoplasmic extracts (Figure 1). Quantitative analysis indicates that the enrichment of *ETV6/RUNX1* mRNA is comparable to the values obtained for the known targets of IGF2BP1 – *ACTB*, *CTNNB1*, and *MYC* (Figure 1). Furthermore, we have validated these results in t(12;21)(p13;q22)-positive ALL using frozen WBC pellets prepared from five different diagnostic samples of this leukemia type (Figure 1). Note, that a "title" target of IGF2BP1 protein – IGF2 mRNA – is not expressed in REH cell line and diagnostic samples of t(12;21)(p13;q22)-positive ALL (data not shown) indicating that other pathways might be modulated by this evolutionary highly-conserved protein in ETV6/RUNX1-positive leukemia.
Summary / Conclusion: Taken together, these data indicate that

ETV6/RUNX1 transcript is a potential target of RNA-binding protein IGF2BP1 and warrant further research of IGF2BP1's role in *ETV6/RUNX1*-mediated leukemogenesis.

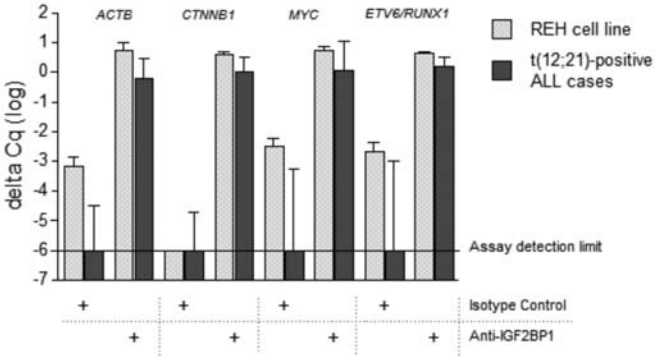

Figure 1.

P018

SNP ARRAY ANALYSIS OF ADULT ALL: A POPULATION BASED STUDY OF LITHUANIAN PATIENTS

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Background: The frequency of abnormal karyotypes in adult acute lymphoblastic leukemia (ALL) is 64-85%. The different outcomes of adult compared to children ALL may be related to different frequencies of specific abnormalities (Mrozek *et al*., 2004). There is a need for novel markers in adult and adolescent ALL to allow a better risk stratification of these patients and to identify new treatment targets (Paulsson *et al*., 2008). Considering high resolution and sensitivity of SNP array (SNP-A), it is possible to detect not only established but also new submicroscopic aberrations. The application of SNP-A method on a population level may give new information on distribution of known genomic aberrations and help to identify new candidate genes and loss of heterozygosity (LOH) related to the pathogenesis of ALL as well as their phenotypic and clinical relevance.
Aims: To characterize genomic aberrations of adult ALL on a population scale using SNP-A and identify new candidate genes relevant to the pathogenesis of ALL.
Methods: We assumed 70% (+/-8%) incidence of chromosomal abnormalities in a sample of ALL cases. A minimum sample size for the Lithuanian population was calculated to be 55. Adult ALL bone marrow samples were screened by Infinium HD whole-genome genotyping assay with the HumanCytoSNP-12 BeadChip (Illumina Inc., San Diego, CA, USA). Genotypes were called by GenomeStudio GT module version 1.7 (Illumina Inc.). QuantiSNP version 1.1 software was used for detailed analysis of structural and numerical chromosomal aberrations and loss of heterozygosity. All detected genomic lesions were checked and filtered using Database of Genomic Variants database.
Results: Bone marrow samples of 57 adult ALL cases diagnosed between 2007 and 2012 were analyzed. SNP-A analysis detected 694 genetic abnormalities not corresponding to known copy number polymorphisms (96% of all the patients, 55/57). These comprised 160 (23%) hemizygous and 34 (5%) homozygous deletions, 363 (52%) duplications and 137 (20%) amplifications (copy number >3). The most aberrations we detected in 1q21-q41 region. Most hemezygous and homozygous deletions were detected in chromosomes 5q and 9p respectively, whereas most duplications and amplifications were detected in 1q and 4p (Figure 1). The majority of aberrations was <5 Mb and hence expected to be cytogenetically cryptic. The median size of all aberrations was 75.2 Kb. The median size (Kb) of hemizygous deletions, homozygous deletions, duplications and amplifications was 113.9, 50.1, 97.3 and 41.9. Loss of heterozygosity (LOH) (>10 Mb) was detected in 27 genomic regions. The analysis revealed 170 recurrent copy-number changes. These copy number abnormalities encompassed several leukemia-related genes – *CDKN2A* (16 cases, 28%), *CDKN2B* (4 cases, 7%), *MLL* (7 cases, 12%), *IKZF1* (3 cases, 5%), *PAX5* (4 cases, 7%). We identified several new recurrent aberrations which included possible new target genes *TACC3* (22 cases, 38%), *FGFR3* (16 cases, 28%), *LRP1B* (10 cases, 17%), *FAM5C* (10 cases, 17%), *RAD51B* (7 cases, 12%).
Summary / Conclusion: SNP-A analysis provides the landscape of genomic lesions on population level of adult ALL. New candidate genes of adult ALL were identified.

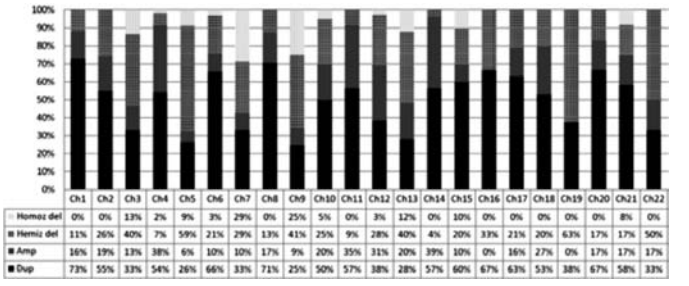

Figure 1.

P019

***IN VIVO* DUAL LUCIFERASE IMAGING ON PATIENTS' ACUTE LEUKEMIA CELLS GROWING IN MICE**

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Background: Acute lymphoblastic leukemia (ALL) is a frequent disease in children and adults which remains difficult to treat in a major part of patients. Novel therapeutic options are intensively desired.
Aims: The aim was to establish dual luciferase *in vivo* imaging in patient-derived xenograft ALL cells growing in mice for use in decisive preclinical treatment trials.
Methods: We transplanted primary tumor cells from patients with ALL into immuno-compromised mice as establisehd in the known individualized xenograft mouse model of ALL. We established lentiviral transduction of individual xenograft ALL cells for expression of transgenes. In patient-derived ALL cells, either Gaussia luciferase or an codon-optimized version of firefly luciferase was expressed.
Results: Using either Coelenterazine or Luciferine as a substrate in mice, patient-derived ALL cells could be easily monitored by *in vivo* imaging with either of both luciferases. Both luciferases enabled reliable and continuous follow up leukemic growth in mice. Sensitivity of both luciferases was high and allowed monitoring of minimal residual disease in mice as well as distinct disease phases and classical treatment outcome parameters. Most importantly, two distinct classes of leukemia cells could be monitored in parallel in a single mouse by use of the two different luciferases.
Summary / Conclusion: Taken together, dual luciferase imaging allows evaluating two different cell subpopulations in parallel in a single mouse in real time on patients' ALL cells *in vivo*. The method allows comparing, e.g., the therapeutic effect on genetically altered versus control cells in future preclinical treatment trials in mice.

Acute myeloid leukemia - Biology 1

P020

OVEREXPRESSION OF INTRONIC MIR-3151 AND ITS HOST GENE BAALC INCREASES LEUKEMOGENESIS IN ACUTE MYELOID LEUKEMIA (AML) BY DIRECT DEREGULATION OF TP53 AND MAY BE TARGETED BY BORTEZOMIB

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Background: High expression of both intronic *miR-3151* & its host gene *BAALC* associate with poor prognosis in cytogenetically normal (CN)-AML patients (Eisfeld *et al.* Blood 2012). The underlying mechanisms are unknown.are unknown.
Aims: To elucidate how the *miR-3151* & *BAALC* interplay contributes to aggressive AML.
Results: Pathway analysis of a previously derived gene expression profile that associates with *miR-3151*-expression in CN-AML patients (n=179; Eisfeld *et al.* Blood 2012) revealed TP53 signaling as the top canonical pathway. *TP53* is an *in silico* predicted direct *miR-3151* target. Luciferase assays proved a direct downregulating effect on the transcript from the *TP53*-3'-UTR by *miR-3151* (-40% mean [±1.6 standard deviation] *miR-3151* vs. scramble control, P<.001). Forced *miR-3151* expression reduced TP53 mRNA and protein compared to scramble in AML cells (KG1 cells: -70%, P=.007; MV4-11 cells: -86%, P=.002). Thus *miR-3151* directly deregulates TP53. We hypothesized that its host, *BAALC*, impacts the same pathway and tested the effects of *miR-3151* & *BAALC* overexpression on 30 TP53-pathway associated genes in primary AML blasts (n=4). *miR-3151* overexpression downregulated 15/30 genes compared to scramble. Overexpression of *BAALC* in comparison with scramble upregulated 8 genes, including the oncogene *JUN* (5.5 [±2.2]-fold). Thus, both *miR-3151* & *BAALC* deregulated the TP53-pathway. Caspase assays confirmed a decrease of apoptosis following *miR-3151* or *BAALC* overexpression, likely due to TP53 impairment, after 72h in KG1 (*miR-3151* vs. scramble: .44-fold [±.02], P=.002; *BAALC* vs. scramble: .36-fold [±.004], P<.001) and in MV4-11 (*miR-3151* vs. scramble: .70-fold [±.009], P<.001; BAALC vs. scramble: .80-fold [±.002], P<.001) cells. Next, we analyzed the impact of *miR-3151* & *BAALC* on AML leukemogenesis *in vivo* using a NOD scid gamma knock-out (NSG) mouse model, by injecting MV4-11 cells overexpressing *miR-3151*, *BAALC*, *miR-3151/BAALC*, or scramble (n=6 mice/group). Mice injected with *miR-3151* or *miR-3151*/*BAALC* had a significantly shorter survival compared to the scramble mice (*miR-3151*/*BAALC:* P=.006; *miR-3151*: P=.008; Figure 1). Survival of the mice injected with *BAALC* overexpressing cells did not differ from the scramble group (P=.4). Next, we sought to identify the causes of *miR-3151* & *BAALC* overexpression. We identified 2 possible transcription start sites (TSS-3151, -362 bp upstream of the stemloop; TSS-BAALC, -2038 bp upstream of the BAALC ATG). SP1 & NF-κB were *in silico* predicted to target both TSSs. Luciferase assays showed increased luciferase activity for both TSSs after addition of SP1 & NF-κB-constructs (TSS-3151: SP1: 4.9-fold [±.2], NF-κB: 2-fold [±.15]; TSS-BAALC: SP1: 5-fold [±.44], NF-κB: 1.8-fold [±.13]). Electromobility shift assays (EMSAs) confirmed SP1 & NF-κB binding. The proteasome inhibitor bortezomib abrogates SP1/NF-κB binding (Liu *et al.* Cancer Cell 2010). A decrease of *miR-3151* & *BAALC* expression was observed as early as 3 hours after bortezomib (100µg/ml) treatment compared to vehicle treated control cells (KG1a cells: *miR-3151* expression: -70%, P=.002; *BAALC* expression: -62%, P=.001).
Summary / Conclusion: We identify an interplay of an oncomiR, *miR-3151*, with its host gene, *BAALC,* that leads to deregulation of the TP53-pathway & thus promotes aggressive disease in AML. Overexpression of both *miR-3151* & *BAALC* may be targeted by bortezomib treatment in these high risk patients.

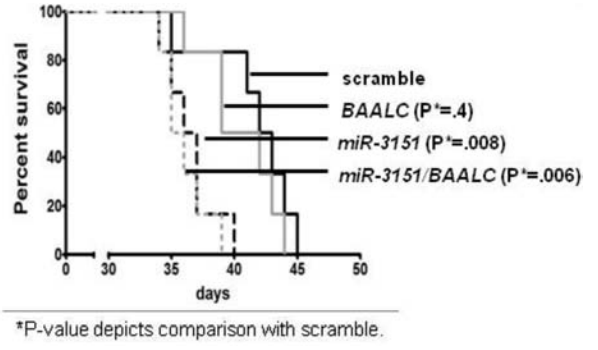

Figure 1. Survival of NSG mice after *miR-3151* and/or BAALC overespression.

P021

SP1 STABILIZATION IS REQUIRED FOR AML1-ETO LEUKEMOGENESIS

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Background: The AML1-ETO (AE) fusion protein generated by the t(8;21), present in 10% of Acute Myeloid Leukemias (AMLs), has been shown to alter the transcriptional pattern of leukemic cells trough chromatin modifications and interaction with transcriptional factors. In murine and human hematopoietic stem and progenitor cells (HSPCs) AE promotes self-renewal and blocks lineage differentiation, but does not by itself cause leukemic transformation. Recently, genome-wide analyses have demonstrated the importance of several transcription factors recruiting AE promoter binding to non-AML1 binding sites. We have identified the transcriptional factor Sp1, an essential transcription factor for haematopoiesis development, as a crucial element driving AE DNA binding pattern and thus supporting the role of Sp1-targeted therapy [1].
Aims: Determine the mechanism of Sp1 accumulation upon AE expression and the role of Sp1 protein in the leukemic transformation of HSPC-AE to study Sp1 targeting as a possible therapeutic strategy.
Methods: We made use of human hematopoietic stem cells retrovirally transduced with AE (HSPC-AE)[2] and the t(8;21) SKNO1 cell line. Several compounds were used: Mithramicyn A and a proteasome inhibitor (MG-132) from Sigma-Aldrich, JNK inhibitor II (SP600125) from Calbiochem. Lentiviral vector MISSION pLKO.1shRNA-puro constructs targeting human Sp1 were obtained from Sigma-Aldrich.
Results: Upon AE expression high Sp1 protein levels on the presence of low Sp1 mRNA levels were observed, pointing to Sp1 protein stabilization and accumulation. To elucidate the mechanism under Sp1 accumulation, JNK1 mRNA and protein levels were investigated, as JNK1 kinase has been shown to phoshporylate Sp1 inhibiting its degradation via proteasome. High levels of JNK1 mRNA and protein in HSPC-AE relative to HSPC controls and a positive correlation between JNK1 and Sp1 protein levels were observed. Indeed, pharmacological inhibition of JNK1 kinase activity on SKNO1 cell line abrogated Sp1 protein phosphorylation and accumulation. The exposure of SKNO1 to the proteasome inhibitor MG132, known to activate JNK1, leaded to Sp1 protein accumulation. These data further support JNK1 activation as a mechanism of Sp1 protein stabilization on AE expressing cells.The biological importance of Sp1 on t(8;21) leukemia was further studied by lentiviral shRNA knockdown system. A complete abrogation of self-renewal of two independent HSPC-AE clones and a total impairment of proliferation in SKNO- Sp1 knockdown cells were observed. Interestingly, on THP1 cell line, harbouring the MLL-AF9 fusion protein, little effect upon Sp1 knockdown was observed. Furthermore, we evaluate the sensitivity of inhibiting Sp1 DNA binding using Mytramicin A. Higher sensitivity to this drug in the presence of the AE fusion protein compared to cells harbouring other fusion proteins was also confirmed. Finally, genome-wide expression profiling has been performed upon pharmacological Sp1 binding inhibition to determine the role of Sp1. A complete analysis of the changes induced by this pharmacological inhibition is warranted.
Summary / Conclusion: We have shown that, through JNK1 phosphorylation, Sp1 is stabilized in AE expressing leukemia cells, leading to a Sp1 accumulation that is required for AE leukemogenesis. These results further support Sp1 as a potential drugable target

P022

PML/RAR-ALPHA INHIBITS PTEN EXPRESSION IN HEMATOPOIETIC CELLS BY COMPETING WITH PU.1 TRANSCRIPTIONAL ACTIVITY

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Background: The chimeric PML/RARA protein contributes to the pathogenesis of APL by impairing the formation of functional PML nuclear bodies (PML NBs), downregulation and displacing of tumor suppressors and by deregulating genes that are critical to myeloid differentiation. All-trans retinoic acid (ATRA) treatment antagonizes PML/RAR effects, reverts the leukemic phenotype and enables leukemic cells to undergo terminal differentiation. Inactivating mutations of p53 and PTEN (phosphatase and tensin homologue deleted in chromosome 10) are uncommon in patients with APL and AML. Instability and downregulation caused by PML/RARA have been demonstrated for p53, while displacement to cytoplasm with loss of its nuclear function has been shown for PTEN in APL. The anti-oncogenic activity of PTEN is dose-dependent and down-regulation of the protein may be an additional relevant mechanism contributing to its impaired function. We found that primary blasts from patients with APL have significantly lower PTEN expression than blasts from other AML subtypes. To determine whether PML/RARA could directly down-regulate PTEN expression, we performed computational analysis of its promoter region and

found two RARE plus PU.1 and one RARE half plus PU.1 motifs. Ets transcription factor PU.1 is essential for myeloid development, and its silencing produces dysfunction and maturation arrest of hematopoietic stem cells.
Aims: The aim of this work is to asses PTEN expression in patients with APL and its modululation by PML-RARA protein.
Methods: Twenty eight APL and 28 unselected AML patients were analyzed by quantitative PCR using Taqman Probes to measure mRNA PTEN level expression. PTEN protein levels were also analysed in 15 AML and 12 APL cases using Western Blot. In addition, PTEN mRNA and protein levels were studied in: i) PR9 cells, a Zn inducible PML/RARA cell line; ii) APL patient primary blasts and NB4 cells after treatment with ATRA and ATO. ChIP-qPCR was used to analyse the regulatory PTEN sequence for the presence of PU.1 protein.
Results: PTEN mRNA and protein levels were significantly lower in APL as compared to AML samples (P=0.036 and P<0.0001, respectively). PTEN protein expression increased in primary patient blasts and in NB4 cells after *in vitro* treatment with ATRA. By analyzing the PR9 cell line transfected with a zinc inducible PML-RARA construct, we detected a decrease of PTEN mRNA at 2 and 4 hours, simultaneously with PML-RARA maximal expression. Treatment with arsenic trioxide (ATO) also enhanced PTEN expression for the first 1-2 hours, corresponding with degradation of PML/RARA protein. The upstream promoter sequence of PTEN gene locus contained several RARE and one RARE half plus PU.1 motifs. Treatment of NB4 cells with ATRA resulted in increased binding of PU.1 to PU.1-RARE half site as demonstrated by ChIP-qPCR analysis.
Summary / Conclusion: This is the first report showing that PTEN expression is suppressed in APL, and restored upon treatment with ATRA. In leukemic cells PU.1 enhances PTEN expression via binding to two specific motifs on the PTEN promoter sequence. We show that PML/RARA inhibits PTEN expression by directly binding to the protein promoter and displacing a pro-expression PU.1-RARA complex. The binding causes a major decrease of PTEN production thus contributing to compromise its antioncogenic activity.

P023

ACUTE MYELOID LEUKEMIA WITH T(8;21)/RUNX1-RUNX1T1 DEMONSTRATE A HIGH NUMBER OF SECONDARY GENETIC LESIONS: FREQUENCY AND IMPACT ON CLINICAL OUTCOME
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Background: The translocation t(8;21) with the resulting *RUNX1-RUNX1T1* rearrangement is one of the most common chromosomal abnormalities in acute myeloid leukemia (AML). Although it is generally associated with a favourable prognosis, impact of additional mutations is poorly understood.
Aims: In the present study, we aimed to assess the frequency and clinical impact of secondary mutations in AML patients (pts) harbouring t(8;21)/*RUNX1-RUNX1T1*.
Methods: We analyzed 139 pts which were referred to our laboratory for first diagnosis of AML between 2005 and 2012 (65 females, 74 males; median age 53.3 years, range 18.6 - 83.8 years) and were proven to have t(8;21)/*RUNX1-RUNX1T1*. Diagnosis was established using cytomorphology and multiparameter flow cytometry according to FAB and WHO classifications. Chromosome banding analysis and fluorescence in situ hybridisation were performed for detection of t(8;21) and/or *RUNX1-RUNX1T1*. Mutation analysis of *ASXL1, FLT3*-ITD, *FLT3*-TKD, *KIT (*D816V, exon8 and 9-11*), NPM1, MLL*-PTD, *IDH1* and *IDH2, KRAS, NRAS, CBL,* and *JAK2* was performed in all pts.
Results: 107/139 were classified according to FAB criteria (77.0%). 34/107 had AML M1 (31.8%) and 73/107 AML M2 (68.2%). 117/139 had *de novo* AML (84.2%), 22/139 therapy-related AML (t-AML) (15.8%). 69/139 (49.6%) had at least one molecular alteration in addition to *RUNX1-RUNX1T1*, 23/139 (16.5%) had two or more additional mutations. Most common were *KIT* mutations (23/139; 16.5%), followed by *NRAS* (18/139; 12.9%) and *ASXL1* (16/139; 11.5%). *FLT3*-ITD, *FLT3*-TKD*, CBL,* and *KRAS* were found in 4.3% - 5.0% of all pts, whereas *IDH2* and *JAK2* were detectable in 3.6% and 2.9%, respectively. *IDH1* mutations were found in only 0.7% (1/139), and, notably, *NPM1 and MLL*-PTD were never found in *RUNX1-RUNX1T1*. *FLT3*-ITD as well as *FLT3*-TKD were exclusive of *ASXL1*. Although not significant, *KIT* mutations were frequently associated with *NRAS* and *FLT3*-ITD, when compared to wildtype (*NRAS*: 21.7% vs. 11.2%; *FLT*-ITD 28.6% vs. 15.9%). With exception of *FLT3*-ITD, which was only present in *de novo* AML, there was no difference in mutation frequencies between *de novo* AML and t-AML. Cytogenetic data were available in all pts. 69.8% (97/139) had at least one chromosomal aberration in addition to t(8;21)(q22q22). Most frequent was the loss of either X- or Y- chromosome (together 46.8%), followed by del(9q) (15.1%), and trisomy 8 (5.8%). *FLT3*-ITD*, FLT3*-TKD and trisomy 8 were found to be mutually exclusive. The number of secondary chromosomal aberrations did not differ between pts with *de novo* AML or t-AML, showing a trend towards higher frequency of -Y, del(9q), and trisomy 8 in pts with t-AML. Survival was calculated in pts with intensive treatment (n=111/139, 79.9%; median follow-up 26.9 months; 2-year survival rate 73.4%). With exception of *KIT*D816V, which had a negative impact on survival in *de novo* AML (2-year survival rate 64.2% vs. 82.3%, P=0.05; pts censored on the day of transplantation), we found, that none of the above mentioned mutations influenced outcome. This also holds true for pts with 2 or more coexistent secondary molecular lesions. Moreover, no influence of secondary chromosomal aberrations on survival was found.
Summary / Conclusion: We analysed a sizeable cohort of pts with AML t(8;21)/*RUNX1-RUNX1T1* and screened for a broad panel of mutations as well as chromosomal aberrations. With exception of *KIT*D816V, which negatively influenced survival, our results emphasize the impact of the prognostic favourable t(8;21) status, which was not altered by secondary molecular or chromosomal lesions.

P024

CELLULAR AND MOLECULAR TARGETS OF MLL-AF9 IN A NOVEL CONDITIONAL MOUSE MODEL
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Background: Gene fusions involving the mixed lineage leukemia 1 (MLL1) gene on the long arm of chromosome 11 are associated with infant, pediatric and adult *de novo* and therapy related leukemia that are often linked to poor prognosis. Expression of several leukemia-associated mixed lineage leukemia (MLL) fusion genes transform human and mouse bone marrow cells *in vitro* and *in vivo*.
Aims: To dissect the molecular and cellular targets of the MLL-AF9 fusion using a novel inducible doxycycline (DOX)-regulated transgenic mouse model.
Results: Conditional *ex vivo* activation of MLL-AF9 induced aberrant self-renewal and impaired differentiation capacity of long-term hematopoietic stem (LT-HSC) and various progenitors including granulocyte-macrophage progenitor (GMP) cells. MLL-AF9 expression in LT-HSC or GMP cells caused accumulation of immature blast-like cells that displayed distinct origin-associated properties in colony formation, differentiation capacity and resistance to chemotherapeutic drugs. Turning-off of fusion expression resulted in multi-lineage differentiation of LT-HSC-derived cells, whereas differentiation of GMP-derived cells was restricted to mature macrophages and granulocytes suggesting maintenance of origin identity during MLL-AF9-mediated transformation. *In vivo*, conditional MLL-AF9 expression induced an aggressive and transplantable disease characterized as acute myelo-monocytic leukemia closely mimicking the human MLL-AF9 expressing disease. Fusion gene expression and leukemia induction was DOX dosage dependent and reversible upon DOX removal. LT-HSCs induced a more aggressive leukemia than GMPs. Notably, 10% of LT-HSC derived leukemia had a 50% shorter median latency (LT-"Early") as compared to other LT-HSC-, or GMP-derived leukemias. Cytarabine (Ara-C) treatment significantly delayed leukemia induction by GMP- but not LT-HSC-derived blasts. Gene expression profiling in immortalized pre-leukemic cells revealed MLL-AF9-dependent expression of over 300 genes, including well-known MLL targets such as *Meis1*, *HoxA5*, *HoxA9* and *HoxA10*. LT-HSC-derived blasts displayed distinct gene signatures that clearly separated them from the GMP-derived blasts. *Evi-1* and *Erg*, two known prognostic markers in patient-derived leukemia gene signatures where expressed differentially in LT- and GMP-derived disease. The aggressive "early" LT-derived murine leukemia displayed high *Evi-1* and *Erg* expression levels (*Evi-1* high, *Erg* high) as compared to the "late" LT-derived (*Evi-1* low, *Erg* high) or the GMP-derived leukemias (*Evi-1* low, *Erg* low).
Summary / Conclusion: These observations suggest that the previously reported poor prognosis associated with elevated *EVI-1* and/or *ERG* expression might directly reflect the cellular origin of the disease. We are currently exploiting this highly informative MLL-AF9 disease model to evaluate the functional relevance of novel MLL-AF9 origin-dependent genes and to identify novel prognostic markers and potential therapeutic targets.

P025

STAT3 AND 5 ARE CONSTITUTIVELY ACTIVATED IN T(6;9)(DEK/CAN)-POSITIVE ACUTE MYELOID LEUKEMIA MODELS AND EFFICIENTLY INHIBITED BY EXPOSURE TO ARSENIC TRIOXIDE
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Background: Acute myeloid leukemia (AML) is characterized by an abnormal accumulation of hematopoietic progenitors in the bone marrow (BM). The AML phenotype is maintained by an accelerated proliferation of blast cells and is supported by the aberrant stem cell capacity of poorly defined leukemic stem cells (LSC) along with a block in differentiation. AMLs are frequently associated with specific chromosomal translocations, such as t(15;17), t(8;21) or t(6;9) which

encode aberrant fusion proteins (FPs). These Fps, i.e. PML/RARα (PR), AML-1/ETO (AE) or DEK/CAN (DC), recapitulate the leukemic phenotype in the mouse. Constitutive STAT activation is frequently found in AML and it is postulated that the aberrant stem cell capacity in leukemia is correlated to the activation of STATs. A relationship between STAT-activation and response to arsenic trioxide (ATO) has been documented in models of t(15;17)(PR)-positive AML. The t(6;9)(DC)-positive AML represents a separate entity with a young age of onset and poor prognosis. The leukemogenic mechanisms of DC-induced AML are poorly understood.

Aims: Here we analyzed the activation status of STAT3 and 5 in models of DEK/CAN-positive leukemia and investigated whether the STAT-activation plays a role for the pathogenesis of t(6;9)-positive AML.

Methods: The expression and activation status of STAT3 and 5 was investigated by western blotting and intracellular flow cytometry with antibodies against the phosphorylated and total form of STATs. Apoptosis was measured by 7-AAD staining of U937 cells retrovirally transduced to express DC or PR and treated for 5 days with 1 μM ATO. As models of DC-induced AML we used primary Sca1+Lin- murine hematopoietic stem and progenitor cells (HSPCs) which were retrovirally transduced to express DC or PR as control. For the *in vivo* studies, we used DC-positive primary as well as 2° leukemic cells from established DC-induced AML. The effect of constitutively active STAT3 (STAT3*) on the stem cell capacity was investigated in serial replating and CFU-S12 assays using transduced mHSPCs. ATO treatment of the mice inoculated with DC-positive primary leukemic cells started at day 5 after transplantation and was performed for a period of two weeks. The leukemia development and the response to ATO treatment was assessed 30 days post-transplantation by spleen size and the effect on STATs activation was analyzed in BM and spleen.

Results: Here we show that i) in the pre-leukemic state of DC-positive AML the activation of the STATs was hardly detectable; ii) both STAT3 and 5 were strongly activated in primary blasts of DC and PR from mouse leukemia as well as in retrovirally transduced primary HSCPs; iii) the expression of STAT3* in HSPCs led to an increased replating efficiency in the presence of cytokines and slightly increased the CFU-S12 colony number as a read out for short-term stem cell and early progenitor potential; iv) similar to PML/RARα, DEK/CAN also sensitized the U937 cells to arsenic-induced apoptosis *in vitro*; v) ATO treatment led to a significantly decreased spleen size as compared to solvent treated controls; vi) exposure to ATO abolished the phosphorylation of STAT5 *in vivo.*

Summary / Conclusion: Our results indicate a direct relationship between the expression of the leukemogenic fusion proteins DC or PR and the activation of STATs which seem to play an important role for the maintenance of the leukemic stem cell. The STAT5 activation was efficiently targeted by ATO. Consequently, ATO treatment represent a novel therapeutic concept for the t(6;9)-positive AML.

P026

THE GENE SIGNATURE IN C/EBPA DYSFUNCTIONAL AML PREDICTS RESPONSIVENESS TO HDAC INHIBITORS

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Background: C/EBPa plays a crucial role in granulocytic development, and defects in this transcription factor have been reported in acute myeloid leukemia (AML). K562 cells stably transfected with an inducible C/EBPa-estrogen receptor fusion protein (C/EBPa-ER) have been used as a model for human granulocytic differentiation. When stimulated with b-estradiol (E_2) to induce nuclear translocation of C/EBPa, these cells differentiate towards neutrophils.

Aims: The aims of this project are: 1. To identify genes upregulated upon C/EBPa activation, and therefore involved in granulocytic development. 2. To determine whether this set of genes, referred as the C/EBPa signature, is downregulated in AML patient samples. 3. To identify small compounds that could reactivate the C/EBPa signature, and promote granulocytic differentiation.

Results: K562 C/EBPa-ER expressing cells were stimulated with 1 uM E_2 or EtOH vehicle control, and gene expression profiles were determined by microarrays. By using prediction analysis of microarrays (PAM), we identified the C/EBPa signature, characterized by a set of 33 genes which are upregulated upon C/EBPa activation. We analyzed the expression of the C/EBPa signature in a cohort of 525 *de novo* AML patients, and identified a subset of 110 patient samples characterized by low expression of this signature. We referred to this group of patients as the C/EBPa dysfunctional subset. Remarkably, a large percentage of samples harboring C/EBPa biallelic mutations clustered inside this subset. We hypothesize that re-activation of the C/EBPa signature in the C/EBPa dysfunctional subset could have therapeutic potential. In search for small molecules able to reverse the low gene expression of the C/EBPa signature we applied the Connectivity Map. This analysis predicted a positive connectivity between the C/EBPa activation signature and histone deacetylase (HDAC) inhibitors. We showed that HDAC inhibitors (trichostatin A and vorinostat) reactivate expression of the C/EBPa signature in K562 cells. Next, we determined that patient samples with biallelic mutations in C/EBPa from inside the dysfunctional group cultured in the presence of HDAC inhibitors showed upregulation of cell surface granulocytic markers such as CD15 and CD11b. On the contrary, patient samples with biallelic mutations in C/EBPa, but clustering outside the dysfunctional group, had no significant changes in the same conditions. In addition, quantitative RT-PCR showed upregulation of granulocyte specific genes such as G-CSF-R (*CSF3R*), Gelatinase A (*MMP2*), C/EBPe (*CEBPE*), and lysozyme (*LYZ*) in HDAC inhibitor treated cells compared to vehicle control (EtOH) in samples from inside, but not from outside, the C/EBPa dysfunctional group.

Summary / Conclusion: Altogether, our data identify HDAC inhibitors as potential candidates in the treatment of certain AMLs characterized by the downregulation of the C/EBPa signature.

P027

AXL, A THERAPEUTIC TARGET IN AML MEDIATES STROMA-INDUCED CHEMORESISTANCE

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Background: Novel targets with potential to improve treatment of AML are urgently needed. Members of the Tyro3, Axl, Mer receptor (TAMR) tyrosine kinase family are abundantly expressed in physiological and malignant hematopoiesis and circumstantial evidence in literature links Axl expression to AML pathobiology. We discovered that Axl, the receptor for Growth Arrest-specific protein 6 (Gas6), represents a novel prognostic marker and potential therapeutic target in AML (Blood (ASH Annual Meeting Abstracts), Nov 2011; 118: 940).

Aims: Validate Axl as a new therapeutic target in AML and investigate its involvement in chemoresistance.

Methods: Gas6 levels were measured by ELISA and immunohistochemistry. Axl expression was detected by flow cytometry. Co-cultures of (murine) BM stroma cells (primary, OP9, S17) with Mv4-11 and OCI-AML5 cell lines were performed.

Results: By investigating primary AML and healthy BM (BM)(i) higher expression of Axl in AML BM compared to healthy BM donors (66.20±10.87 vs. 0.65±0.10%; n=8/6; P<0.05); (ii) Axl expression by 68±31% of AML blasts and (iv) higher expression of Axl by $CD34^+CD38^-$ AML stem cells compared to healthy $CD34^+CD38^-$ BM stem cells (58.43±4.63% vs. 6.00±2.01%; n=7/6; P<0.05). Thus, Axl blockade might be useful to inhibit AML stem cells. Subsequently we determined therapeutic efficacy of BGB324 on primary human AML cells. We incubated AML cells from patients at primary diagnosis with different concentrations of BGB324 and found a dose dependent inhibition of proliferation with a mean IC50 of 1.8 μM (range 0.2341-3.711 μM). Interestingly, sensitivity towards BGB324 (i.e. a lower IC_{50}) correlated with Axl expression on leukemia cells (Pearson's r = -0.9656, P<0.05). To investigate effect of BGB324 treatment on chemosensitivity of human AML cells we incubated primary AML cells with BGB324 in combination with cytarabine and found additive therapeutic effects of both treatments. Notably, BGB324 was also effective in cytarabine-refractory primary AML cells and could sensitize them for chemotherapy. Thus, Axl might be implicated in development of chemoresistance in AML. Analyses of BM sections revealed that expression of Axl`s ligand Gas6 was low in AML cells, similar to healthy hematopoietic cells while it was abundantly expressed in AML BM stromal cells with fibroblastic/mesenchymal morphology (referred to as BMDSCs). Gas6 expression was considerably lower in control BMDSCs (86±14% vs. 20±20%; n=5/7; P<0.05) thus suggesting a possible paracrine interaction between AML cells and BMDSCs leading to Gas6 upregulation in the stroma compartment. In order to test this hypothesis we utilized co-cultures of (murine) BM stroma cells (primary, OP9, S17) with Mv4-11 and OCI-AML5 leukemia cell lines. These experiments revealed specific upregulation of murine (m)Gas6 in BMDSCs mediated by leukemia-cell derived IL-10 and M-CSF. Co-cultures with antibodies blocking hIL-10 and hM-CSF could abrogate stromal Gas6 upregulation. Protective effect of stroma cells towards cytarabine-induced cytotoxicity could be eliminated by shAxl, sAxl or by BGB324. Thus, interaction between stroma-derived Gas6 and Axl^+ leukemia cells forms a chemoprotective niche for leukemia cells. In line with these findings Axl blockade

chemosensitizes Mv4-11 cells for treatment with doxorubicine *in vivo.*
Summary / Conclusion: Axl represents a therapeutic target in AML and Axl inhibition by BGB324 holds potential to treat chemosensitive and –resistant AML.

P028

RECURRING FLT3 N676K MUTATIONS IN CORE BINDING FACTOR LEUKEMIA ACTIVATE MAPK-SIGNALING AND CONFER FACTOR INDEPENDENT GROWTH OF BA/F3 CELLS

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Background: The t(8;21) and inv(16)/t(16;16) rearrangements affecting the core-binding factors, RUNX1 and CBFB, respectively, are found in 15-20% of adult *de novo* AML cases and are associated with a favourable prognosis. Since expression of CBFB/MYH11 or RUNX1/RUNX1T1 on their own is not sufficient to cause leukemia it is likely that additional mutations are required for malignant transformation.
Aims: Identification of mutations which may collaborate with CBFB/MYH11 during leukemogenesis.
Methods: We performed exome sequencing of an AML sample with an inv(16). The sample was selected based on availability and absence of known additional genetic alterations. By comparing the AML exome sequence with the exome sequence of a remission sample from the same patient we were able to identify leukemia-specific sequence variants as described previously (Greif *et al*., 2012, Blood).
Results: By exome sequencing of a an AML patient with inv(16) we found an N676K mutation in the ATP-binding domain (TKD1) of the *fms-related tyrosine kinase 3* (*FLT*3) gene. Mutations affecting N676 resulting in variable amino acid changes (N676D or N676S) were initially discovered in a screen for resistance to tyrosine kinase inhibitors (TKI) in *FLT3* internal tandem duplication (ITD) expressing Ba/F3 cells (Cools *et al*., 2004, Cancer Res). An N676K point mutation has been reported in a cytogenetically normal (CN) AML patient with *FLT3*-ITD and TKI-resistance (Heidel *et al*., 2006, Blood). In contrast, our patient with inv(16) and the *FLT3* N676K did not carry an additional *FLT3*-ITD. In a cohort of 84 *de novo* AML patients with a CBFB/MYH11 rearrangement and in 36 patients with a RUNX1/RUNX1T1 rearrangement, the FLT3 N676K mutation was identified in 5 and 1 patients, respectively (5/84, 6%; 1/36, 3%). None of the CBF AML patients with *FLT3* N676K mutation had an additional *FLT3*-ITD. In 90 CN-AML patients we detected only a single *FLT3* N676K mutation and the affected patient had a concurrent *FLT3*-ITD. In addition we analyzed mutational hotspots of several commonly mutated genes in our CBFB/MYH11 positive cohort: FLT3, KRAS, NRAS, KIT, WT1, MLL and NPM1 (Figure 1).

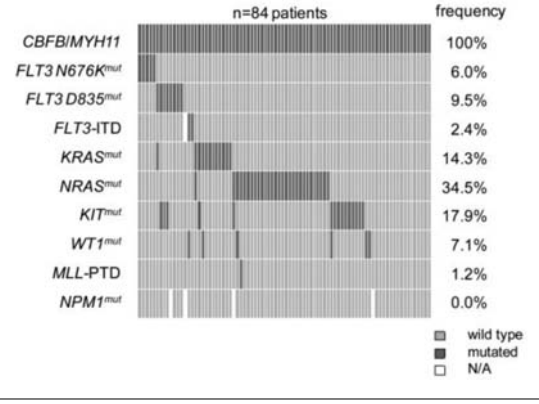

Figure 1. Frequency of additional mutations in CBFB/MYH11 positive AML.

Clinical parameters of 56 CBFB/MYH11 rearranged patients enrolled in the AMLCG-99 trial were correlated with FLT3 N676 status: The *FLT3* N676K mutation was significantly associated with higher leukocyte counts (P=.02), elevated LDH (P=.02) and male sex (P=.02). There was no significant difference in survival of patients with a FLT3 N676K (n=4) compared to patients with FLT3 N676 wild type (n=47). However, there was a trend towards reduced complete remission rates associated with FLT3 N676K mutations.

To test the transforming potential we expressed the FLT3 N676K mutant in Ba/F3 cells. As controls we expressed FLT3 wild type (WT), FLT3 mutants D835Y or ITD in parallel. Cell surface expression of N676K was similar to WT, but increased compared to D835Y and ITD. Cell proliferation assays were done in presence and absence of IL-3 or FLT3 ligand (FL). FLT3 N676K leads to IL-3 and FL independent cell growth reaching 25% of IL-3 mediated growth. FLT3 inhibition by AC220 or PKC412 abrogates this proliferation, but N676K is slightly more resistant to inhibition than ITD. In contrast to ITD expression that results in STAT5 phosphorylation, N676K expression leads to phosphorylation of MAPK and AKT.
Summary / Conclusion: Our findings point towards a specific association of activating *FLT3* N676K mutations with CBF leukemia. Although *FLT3* is a well known mutational target in AML, it appears that the spectrum of *FLT3* mutations is still not fully understood. Unbiased mutation screening by exome sequencing allows the detection of novel sequence variations even in extensively studied genes.

P029

THE EFFECT OF ARA-C TREATMENT ON HEMATOPOIETIC STEM CELL EXPANSION AND LEUKEMOGENESIS IN A MOUSE MODEL OF CEBPA MUTANT ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is the most common acute leukemia in adults. Acquired mutations in the *CEBPA* gene are found in 11-12% of all AML cases and include N- and C-terminal mutations that are frequently found within the same patient on separate alleles. We have recently demonstrated that combining N- and C-mutations in mice resulted in loss of hematopoietic stem cells (HSC) quiescence and expansion of premalignant pool of cells, associated with accelerated AML. Therefore, pharmacologic targeting of the pre-leukemic HSCs has emerged as another critical step to combat tumor progression particularly relevant to prevent tumor relapse. Increased cycling of mutant pre-leukemic HSCs suggests that they could be susceptible to the action of anti-proliferative agents used in chemotherapy.
Aims: We will address the impact of cytosine arabinoside (Ara-C) treatment on proliferating mutant HSCs survival, as well long-term tumor development in a mouse model of *CEBPA* mutant AML. We will evaluate whether 1) Ara-C treatment leads to a selective mutant HSCs apoptosis and a consequent drop in mutant HCS number; 2) reduction in mutant HSC number affects tumor development.
Methods: We performed Ara-C treatment experiments in radiation chimeras generated by transplanting E14.5 fetal liver cells (CD45.2 allotype) of wild type or *CEBPA* N/C combined mutant genotype along with wild type competitor bone marrow cells (CD45.1 allotype). Apoptosis in wild type (CD45.1+) and mutant (CD45.2+) long term-HSCs (defined as Lin-c-kit+Sca1+CD150+) was measured by flow cytometry method using Annexin V/SytoxBlue staining. Frequency and total number of wild type and *CEBPA* mutant HSCs was calculated at 24 and 72 hours after a single injection of 150 mg/kg of Ara-C. In addition, cohorts of mice treated or not with Ara-C for 1 week were monitored overtime for: i) CD45.2/CD45.1 ratio, ii) percentange of Mac1+ cells, iii) white and red blood cells counts in the peripheral blood and mice survival.
Results: Here we demonstrate that Ara-C efficiently and selectively induced apoptosis in mutant HSCs and downregulated their frequency and total number. However it did not lead to their complete elimination. Interestingly, we found that mice treated with Ara-C at early stages of AML progression showed a greater accumulation of Mac-1+ cells and a reduction in the frequency of B and T cells in the peripheral blood at 4 and 6 months after treatment, as compared to untreated mice. Moreover mice treated with Ara-C showed a statistically significant increase in the number of white blood cells, a reduction in the number of red blood cells, hemoglobin and hematocrit at 6 months following the Ara-C treatment, suggesting an earlier onset of leukemic blasts accumulation and leukemia-associated anemia in these mice.
Summary / Conclusion: These data demonstrate that Ara-C treatment induces preleukemic HSC apoptosis, but does not lead to complete mutant cell clearance, revealing that Ara-C resistant mutant HSC population exists and initiates leukemia. Moreover, our results demonstrate that Ara-C-mediated HSC reduction does not lead to delay in leukemia progression. To the contrary, several parameters show even negative long-term effects of Ara-C treatment on AML progression, suggesting that either Ara-C directly modifies HSCs or post-treatment bone marrow provides environmental cues favoring further HSC expansion and leukemia progression. Caution therefore has to be taken in evaluating of the presence of residual mutant HSC in patients' bone marrow after chemotherapy. This study points to a critical role of therapy-resistant HSCs in leukemia progression or relapse and warrants further studies on their better characterization.

P030

COMBINED ANALYSES OF ROS, CELL CYCLE AND IMMUNOPHENOTYPE SHOWS THAT NORMAL HEMATOPOIETIC PROGENITOR SUBSETS HAVE A DIFFERENTIAL ROS PROFILE THAT IS LOST IN ACUTE MYELOID LEUKEMIA

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Background: There has been recent interest in the role of reactive oxygen species (ROS) in myeloid malignancies, driving efforts to target redox state as a therapeutic strategy. Leukemia stem cells (LSC) in acute myeloid leukemia (AML) appear to maintain low levels of ROS, which confers drug-resistance. However certain AML genetic abnormalities such as FLT-3/ITD are associated with increased ROS. Redox state may vary in the heterogenous leukemic subpopulations that are enriched for LSC. This may impact on redox-targeting drug sensitivities. It is thus important to understand if ROS are differentially modulated in normal CD34+ hematopoietic stem cells (HSC) and downstream progenitor subsets and how this may be altered in AML.
Aims: To compare ROS/cell cycle profile of normal CD34+ stem/progenitor with immunophenotypically equivalent AML subsets.
Methods: We performed 8-colour flow cytometric analysis of normal bone marrow (BM) and AML presentation BM and peripheral blood (PB) samples. This combined the ROS indicator dye, dichloro-dihydro-fluorescin diacetate (DCF), with monoclonal antibodies (mAb) specific for human stem/progenitor cell markers. Intracellular ki67 staining of DCF/mAb labelled cells allowed cell cycle analysis. Colony Forming Unit (CFU) assays were performed on purified cells to verify lineage potential of selected cell subsets.

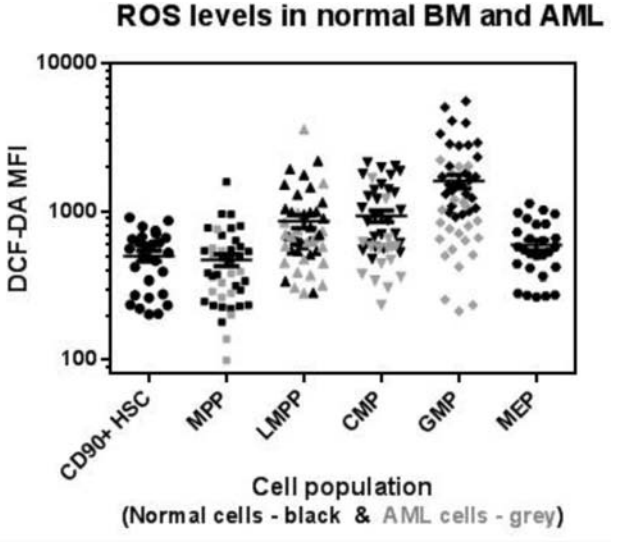

Figure 1.

Results: Total CD34+38hi cells had higher DCF staining than total CD34+38lo cells in normal BM (n=27) but within these two immunophenotypic compartments there was differential DCF staining (Figure 1). In CD38lo cells, lymphoid-primed multi-potent progenitors (LMPP; CD90-CD45RA+) had the highest DCF staining (mean 877 arbitrary units - calculated from MFI of 27 samples) while both HSC (CD90+CD45RA-) and multipotent progenitor cells (MPP; CD90-CD45RA-) were DCF-low (means 501 and 516 respectively). In the CD38hi subset, common myeloid progenitors (CMP; CD123+CD45RA-) were DCF-int (mean 1131), with ROS levels upregulated further (DCF-high - mean 2179) in granulocyte-macrophage progenitors (GMP; CD123+CD45RA+). However megakaryocyte-erythrocyte progenitors (MEP; CD123-CD45RA-) were mainly DCF-low (mean 596), akin to HSC and MPP. Purified CD34+38hi ROS-hi cells exclusively generated granulocyte (CFU-G) and macrophage (CFU-M) or mixed GM colonies (CFU-GM) in CFU assays, and had lost CFU-E potential. Sorting of CD45RA- cells into ROSlo/ROS-int/ROShi fractions revealed that CFU-E potential was limited to the ROS-low fraction and that increasing ROS correlated with higher CFU-M potential. CD38hi cells were also more actively cycling (40-90% ki67+) than CD38low cells (5-23% ki67+) consistent with an association between quiescence and low ROS. However since GMP (ROS-hi), CMP (ROS-int) and MEP (ROS-lo) were all mainly ki67+ (means 57.5%, 60%, 65% respectively), lower ROS could not be correlated with quiescence in CD34+CD38hi cells. CD34+ AML presentation samples (n=29) were most frequently composed of abnormally expanded GMP-/LMPP-like populations, although MPP-/CMP-like populations were also observed. ROS levels varied but were significantly lower in both immunophenotypically immature (MPP- and LMPP-like) and mature (CMP- and GMP-like) subsets (Figure 1 grey symbols) compared to normal subsets, with no difference between BM and PB blasts. Blasts with lowest ROS were enriched for quiescent cells.
Summary / Conclusion: ROS levels are maintained at different levels in normal stem/progenitor subsets; GMP cells are ROS-hi, while MEP cells are ROS-low similar to HSC. However both immature and more mature CD34+AML blasts appear to have downregulated ROS levels, suggesting a shared redox adaptation in different types of AML cells. These results provide a platform to investigate whether leukemic progenitor ROS levels correlate with treatment resistance and can be used to detect residual LSC.

P031

PROGNOSTIC IMPACT OF EXPRESSION LEVELS OF CELL SURFACE PROTEINS COMMONLY EXPRESSED BY BLASTS AND HEMATOPOIETIC PRECURSOR CELLS IN DE NOVO ACUTE MYELOID LEUKEMIA: A REPORT FROM THE SPANISH CETLAM GROUP

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Background: The prognostic impact of immunophenotypic markers in AML has been long controversial. Few studies have analyzed the prognostic value of the intensity of surface membrane antigen expression commonly expressed on AML blasts.
Aims: The prognostic impact of CD34, CD117, CD7, and CD123 levels of expression was analyzed on AML blasts and normal hematopoietic precursors by Multiparameter Flow Cytometry (MPFC) in *de novo* AML patients (pts). We also determined the correlation with disease characteristics and clinical outcome.
Methods: Five hundred ninety-two bone marrow samples from adult *de novo* AML pts excluding APL and diagnosed between 12/2003 to 08/2011 were included. All cases were diagnosed according to the WHO criteria and they were immunophenotyped using an extensive 4-color panel of monoclonal antibodies by MPFC. Patients were treated according to the multicenter CETLAM AML-03 protocol. Blast cells were gated after excluding all other cell populations, as well-delineated clusters of $SSC^{low/int}/CD45^{dim}$ events ("blast gate") using the merge function of the Infinicyt software (Cytognos SL, Salamanca, Spain). For each case, the reactivity for the following markers was evaluated in terms of mean fluorescence intensity (MFI; arbitrary units): CD123, CD117, CD34 and CD7. Normal residual bone marrow lymphocytes were used as reference for internal quality control purposes. Cytogenetic and molecular risk stratifications were based on the European LeukemiaNet (ELN) criteria. Overall survival (OS), leukemia-free survival (LFS) and relapse incidence (CIR) were measured by the Kaplan–Meier method and curves were compared with the log-rank test. Multivariate analysis was performed using the Cox regression model. P-value ≤0.05 was considered to be statistically significant.
Results: Median age was 52 years (range: 16-70) and M/F ratio was 324/268. Seventy-two pts (12%) had favourable cytogenetics, 380 (64%) intermediate, and 90 (15%) adverse according to MRC classification; 29 (5%) pts had no metaphases and 21 (4%) were unknown. NPM1mut was detected in 152 pts (34%), 136 pts (23%) harboured a FLT3-ITDmut, 23 (5%) pts carried CEBPA-mut, and 29 (5%) had MLL-PTD. In the overall series, the median follow-up of survivors was 17 months (range 3-100) and the OS, LFS and RI at 5 years were 39±2%, 42±3% and 47±7%, respectively. Independent prognostic variables were, age (P<.001), cytogenetics (P<.001), NPM1/FLT3-ITD status (P=.001),

molecular category (NPM1mut or CEBPAmut/FLT3-ITD neg vs. other; P<.001) and MLL-PTD rearrangement (P=.05) for OS. Univariate analysis of prognostic factors showed an association between higher MFI CD117 expression (>284.01; P=.019) and a higher MFI CD34 (>143.39; P=.004) expression with both a shorter OS and DFS and a higher RI. In addition, higher MFI CD7 expression (>15.61; P=.017) was associated with a shorter DFS and a higher RI. In multivariate analysis, a higher CD34 MFI retained its value as an independent predictor of a shorter OS (P=.005; HR=1.51, 95% CI=1.13-2.01) and DFS (P=.002; HR=1.66, 95% CI=1.20-2.29), and a higher RI (P=.016; HR=1.62, 95% CI=1.09-2.41). High MFI CD117 levels were an independent predictor of shorter DFS (P=.019; HR=1.52, 95% CI=1.07–2.16). Moreover, higher levels of MFI CD7 expression were an independent risk factor for RI (P=.017; HR=1.63, 95% CI=1.09–2.43).
Summary / Conclusion: In this study we show that high expression levels of immunophenotypic markers associated with immature myeloid precursors (CD34, CD117, and CD7) as assessed by MPFC, may have some prognostic value in *de novo* AML.

P032

C/EBPA REGULATES CXCR4 EXPRESSION IN ACUTE MYELOID LEUKEMIA

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Background: CCAAT/enhancer binding protein α (C/EBPα) is a transcription factor, whose expression plays an essential role in granulopoiesis. It blocks cell cycle progression and induce terminal maturation of hematopoietic cells. Mutations in one or both alleles of *CEBPA* are reported in about 7% to 15% of patients with acute myeloid leukemia (AML). These mutations can be divided into two types: the N-terminal mutations that result in the expression of the p30 isoform and the C-terminal mutations that disrupt the bZip region, which is responsible for DNA binding. Retrospective studies showed that patients with *CEBPA* mutant AML have an improved prognosis. SDF-1/CXCR4 signaling is involved in tumor progression, metastasis, and cell survival. In mouse models, the expression of CXCR4 in AML cells induces leukemia cell chemotaxis and migration beneath marrow stromal cells. Those leukemic cells show cell cycle arrest and reduced numbers of cell divisions, providing a potential mechanism of leukemia cell evasion from chemotherapy. AML patients with higher expression of CXCR4 have poorer prognosis than those with lower expression.
Aims: By searching the publicly available microarray database, we found that *CXCR4* transcription can be induced by CEBPα activation in murine 32D-CEBPα-ER cells. In addition, AML patients with *CEBPA* mutations showed lower *CXCR4* mRNA expression when compared to those with wild type *CEBPA*. Sequence analysis also revealed that the *CXCR4* promoter contains several conserved C/EBPα binding motifs. These clues compelled us to investigate the role of C/EBPα in the regulation of CXCR4-mediated chemotaxis in AML cells and evaluate whether *CEBPA* mutation would influence this effect.
Methods: C/EBPα wild type (p42) or mutants were ectopically expressed in 293T and K562 cells, which do not express C/EBPα. The RNA and protein levels of CXCR4 were measured by using quantitative RT-PCR and Western blot analysis. C/EBPα-mediated transcriptional activation of *CXCR4* was studied using luciferase reporter, gel-shift and chromatin-immunoprecipitation (ChIP) assays. Finally, RNA interference methods in combination with *in vitro* cell migration assays were used to assess the role of C/EBPα in regulation of CXCR4-mediated chemotaxis.
Results: Wild-type C/EBPα overexpression both in 293T or K562 cells increased endogenous *CXCR4* expression. In contrast, the expression of p30 diminished *CXCR4* transcription. Similarly, p42 but not p30 increased the *CXCR4* promoter activity. Furthermore, p42 no longer activated the truncated *CXCR4* promoter which does not contain any C/EBPα binding site. These data showed C/EBPα as an activator of *CXCR4*. Next, we defined the site at -231 to -246 on the *CXCR4* promoter that can be occupied by C/EBPα using the gel-shift assay and detected the binding of p42 to the *CXCR4* promoter *in vivo* by the ChIP assay. Finally, we demonstrated that the induction of p42 in the inducible K562-CEBPα cell lines increased the chemotactic migration, whereas the expression of mutants did not. Moreover, decreased expression of C/EBPα by RNA interference decreased levels of CXCR4 protein expression in U937 cells, thereby abrogating CXCR4-mediated chemotaxis.
Summary / Conclusion: Our results provide the first evidence that C/EBPα indeed regulates the activation of *CXCR4*, which is critical for the homing and engraftment of AML cells. Thus, CXCR4 blockage in AML cells may disrupt their interaction with the BM niche and sensitize them to chemotherapy.

P033

COMBINED INHIBITION OF C-KIT AND PI3K/MTOR SIGNALLING HAS SYNERGISTIC ACTIVITY IN C-KIT MUTANT AML

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Background: Activating mutations of the c-KIT tyrosine kinase (TK) receptor are found in AML and mast cell leukaemias. In AML c-KIT is frequently mutated (~28%) in core binding factor leukaemias (CBFLs). CBFL are defined by the presence of t(8;21)(q22;q22) or inv(16)(p13;q22) which generate the fusion proteins RUNX1-RUNX1T1 and CBFβ-MYH11 respectively. This subset of patients have a favourable outcome although ~40% will relapse with current therapy. c-KIT is highly expressed in most CBFLs and is mutated via point mutations in exon 17 or insertion/deletions in exon 8. The presence of c-KIT mutations is associated with an increased risk of relapse. Dasatinib, an inhibitor of wild-type and mutant c-KIT isoforms, has been shown to inhibit c-KIT phosphorylation and induce apoptosis in cell lines expressing c-KIT mutations. Mutant c-KIT can activate downstream proteins including PI3K, mTOR, MEK and AKT. However, therapy with single agents targeting individual cell signalling molecules has had only modest activity in early clinical trials in AML and other malignancies.
Aims: Therefore this study examined the effect of combining Dasatinib with selective inhibitors of AKT (AZD5363), MEK (PD184352), mTOR (WYE-354), PI3K (ZSTK-474) and PI3K + mTOR (BEZ-235) on cell survival and signalling. Model cell lines including the CBFL Kasumi-1 (c-KIT N822K) and the mast cell leukaemia HMC1.2 (V560G/D816V c-KIT) were used to evaluate drug combination efficacy.
Results: In both Kasumi-1 and HMC1.2 cells combined inhibition of c-KIT (Dasatinib) and PI3K (ZSTK-474), mTOR (WYE354) or PI3K + mTOR (BEZ-235) led to strong synergistic cell killing (<0.3 Combination Index (CI) by Chou-Talalay). The most potent effects were seen combining Dasatinib with the dual PI3K/mTOR inhibitor BEZ-235 (e.g. Kasumi-1 cell line: average live cell fraction±SEM as % control; Dasatinib alone (20 nM) 69%±1.25, BEZ-235 alone (0.25 μM) 66.2%±4.4, Dasatinib (20 nM) + BEZ-235 (0.25 μm) 38%±4.6) No significant effect was seen when combining Dasatinib with MEK (PD184352) blockade in either cell line. In HMC1.2 cells Dasatinib inhibited c-KIT autophosphorylation fully at 313nM. However, there was still residual MEK, AKT, PI3K and mTOR signalling as shown by western blotting. In Kasumi-1 cells 40nM of Dasatinib inhibited c-KIT autophosphorylation and MEK fully but there was residual PI3K/mTOR signalling. Combining Dasatinib and BEZ-235 eliminated c-KIT auto-phosphorylation and markedly reduced residual MEK, AKT, PI3K and mTOR signalling compared with just Dasatinib treatment in both cell lines. Cell death was apoptotic shown by an increase in PARP cleavage and a reduction in pro-caspases 3 and 9. Screening of the Bcl-2 family of apoptosis regulators showed combined c-KIT and PI3K/mTOR inhibition led to a decrease in Mcl-1, Bcl-2 and Bcl-x. As a control for Dasatinib specificity, MV4-11 & MOLM-13 (FLT3-ITD AML cell lines) showed no synergistic effect when treated with a combination of Dasatinib and BEZ-235. However, combined FLT3 inhibition (AC220) and BEZ-235 demonstrated synergistic cell killing suggesting a broader application for combining TK inhibition with PI3K/mTOR blockade.
Summary / Conclusion: The combination of Dasatinib and PI3K/mTOR inhibition was synergistic at cell killing in CBFLs. Similar results were observed with AC220 + PI3K/mTOR inhibition in FLT3-ITD AML cell lines suggesting that a combination of TK and PI3K/mTOR blockade may provide a novel approach to treating c-KIT mutant CBFLs as well as FLT3 mutant AML.

P034

THE FUSION GENE NUP98-HOXA9 AS A MODEL FOR LEUKEMOGENESIS: EXPLORING ITS MOLECULAR MECHANISMS AND FUNCTIONAL EFFECTS

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Background: The chromosomal translocation t(7; 11)(p15, p15), that results in the oncogenic fusion protein *Nup98-Hoxa9*, appears in 1% of patients with Acute Myeloid Leukemia and it is associated with poor prognosis and low degree of overall survival. *Nup98-Hoxa9* seems to regulate the transcription of target genes involved in leukemogenesis and it appears that not only the homeodomain of the partner gene *HOXA9* is necessary in gene regulation, but also the region of *NUP98* present in the fusion could play a role in its oncogenic effect. Still, there is a long way to have a good understanding of the molecular mechanisms supporting the malignancy of this fusion protein. In fact, recent studies in mouse models have suggested a possible interaction between *Nup98* and *p300*, a molecular relationship that remains to be explored in the context of our fusion gene model.
Aims: We are aimed to investigate the molecular mechanisms by which the fusion protein *Nup98-Hoxa9* may be responsible of the leukemic transforma-

tion; mostly studying its binding sites in the DNA and its interactions with other transcription factors.
Methods: - Development of human cellular models that constitutively expresses *Nup98-Hoxa9* (human hematopoietic precursors (hHSC) and HEK293FT cells).
- Chromatin Immunoprecipitation Sequencing (*ChIP-Seq*), which will offer insight into the genomic binding regions of *Nup98-Hoxa9*.
- Validation of target gene regions by *qRT-PCR* and *luciferase assays*.
- Co-Immunoprecipitation assays for knowing which proteins interact with each other in the activator complex.
Results: We have cloned the cDNA of *NUP98-HOXA9* fused to FLAG-tag into a lentiviral vector and we have efficiently transduced hHSC and HEK293FT cells. Transcription of the fusion mRNA and the expression of the fusion protein have been demonstrated by conventional methods. Preliminary functional studies included the analysis of long-term cultures of hHSC and shown that the expression of *NUP98-HOXA9* conferred an advantage in proliferation, supporting so far the fitness of the model for further studies. We have optimized the *ChIP-seq* protocol in *NUP98-HOXA9* transduced human cells, customizing the *qRT-PCR* design to analyze the enrichment in some of the target genes of the fusion previously described. This is relevant since no previous data of the binding DNA sequences within the target genes of *NUP98-HOXA9* has been documented. Our data showed, for the analyzed genes, an enrichment of the binding sites of the fusion protein that was evenly scattered along the 15 Kb region upstream of the transcription start site.
Summary / Conclusion: Our results provided the first evidence of the specific binding of *Nup98-Hoxa9* to the DNA, describing its topography, and clarifying the role of this fusion protein as a transcription factor. This work provides a general methodology for the establishment of hHSC models that will allow the analysis of the effect of this and other leukemic fusion genes. Although the preliminary results are very promising, it is necessary to complete the planned experiments to fully understand the molecular mechanisms of *Nup98-Hoxa9*. Finally, the importance of this project lies in two major areas. By one hand, it will provide a better knowledge about the role of this fusion protein in the development of such as an aggressive leukemia. Secondly, it has an intrinsic biological interest since it will allow to study how a structural protein, such as the nucleoporin *Nup98*, upon a chromosome rearrangement, is able to function as a transcription factor, merely by changing its subcellular localization.

P035

ACID CERAMIDASE PROMOTES ACUTE MYELOID LEUKEMIA SURVIVAL THROUGH MCL-1 UPREGULATION

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Background: Acute Myeloid Leukemia (AML) is a heterogeneous disease affecting the differentiation and survival of myeloid precursors characterized by the CD34+ marker. As a result, patients have elevated immature leukemic cells known as blasts. Current therapeutics in AML exhibit limited success, and only approximately 25% of patients will remain disease free after achieving complete remission. Sphingolipids have recently emerged as a class of bioactive molecules, where the balance between pro-apoptotic ceramide and pro-survival sphingosine-1-phosphate (S1P) has been shown to play an important role in cancer cell fate. Ceramidases are hydrolases acting within the sphingolipid pathway that metabolize ceramide into sphingosine and free fatty acid. Sphingosine can then undergo sphingosine kinase-mediated phosphorylation to form S1P. Hence, elevated ceramidase activity increases S1P levels while reducing ceramide levels, thereby shifting the sphingolipid balance to a pro-survival state. Lysosomal acid ceramidase (AC) is highly expressed in solid tumors isolated from prostate, melanoma and breast cancers, as well as T-cell large granular lymphocyte (T-LGL) leukemia. Moreover, targeting AC induces programmed cell death and increases sensitivity to cytotoxic agents.
Aims: Our aims were to determine whether AC is elevated in AML, is essential in AML survival, and to identify the mechanism through which AC maintains blast survival in AML.
Methods: We used microarray and fluorogenic AC substrate to quantify AC expression and activity levels in primary cells. AC inhibitor LCL 204 and AC shRNA were used to assess AC's role in survival of patient samples and cell lines. We created a stably-overexpressing AC cell line for functional studies into AC-mediated survival. We used ESI-MS/MS to perform lipidomics analysis with AC inhibition and overexpression. For *in vivo* studies, we used a murine model to determine efficacy of AC inhibition to increase overall survival of mice engrafted with leukemic cells.
Results: Here, we report that AC is an important enzyme in AML blast survival. Our microarray data showed that AC, but not neutral or alkaline ceramidases, exhibited significantly elevated expression in AML patient mRNA samples compared to normal donor CD34+ cells. We confirmed this finding with an AC activity screen of 66 AML patients' samples and 12 normal donor CD34+ cells. These results showed elevated AC activity in each prognostic group (good, intermediate and poor) compared to normal controls. Treatment of patient samples and seven human AML cell lines with LCL 204 reduced viability and induced apoptosis in a dose-dependent manner through caspase-3 activation. Furthermore, AC overexpression in HL-60 cell line increased proliferation rate and expression of pro-survival Mcl-1. This is of significance as Mcl-1 has been identified as the predominant Bcl-2 family member responsible for survival in AML. Conversely, AC knockdown or inhibition reduced Mcl-1 levels. AC overexpression also induced a pro-survival lipid profile, with S1P levels significantly increased while ceramide species C_{16}, C_{24} and total ceramide decreased. Interestingly, AC overexpression was shown to induce NF- κB activation, possibly through the increase of endogenous S1P. Our *in vivo* study using murine AML cell line (C1498) engraftment model showed AC inhibition significantly increased overall survival.
Summary / Conclusion: Collectively, these studies demonstrate that AC is important in AML survival through its regulation of Mcl-1 and should be further explored as a potential therapeutic target in AML.

P036

ANTILEUKEMIC EFFECT OF PI3K-MTOR INHIBITORS IN ACUTE MYELOID LEUKEMIA- GENE EXPRESSION PROFILES REVEAL CDC25B EXPRESSION AS DETERMINATE OF PHARMACOLOGICAL EFFECT

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Background: Acute myeloid leukemia (AML) is a heterogeneous malignancy with an overall leukemia-free survival of only 40-50% even after intensive therapy. Intracellular signaling through the phosphatidylinositol 3-kinase (PI3K)-Akt-mammalian target of rapamycin (mTOR) pathway is important for regulation of cellular growth and metabolism and aberrant signaling through this pathway has been implicated in AML. PI3K and mTOR inhibitors have currently entered clinical trials for AML, however despite their theoretical potential as antileukemic agents, they effects seem limited with potential effect in only subset of patients. The reasons of their lack of affectivity remain elusive.
Aims: The aims of the present study were to further analyze effects of different inhibitors of the PI3K-Akt-mTOR pathway in primary human AML cells, and especially to search for differences in gene expression which can define differences in pharmacological effect among AML patients.
Methods: The study included consecutive adult patients with a diagnosis of AML and with high peripheral blood blast counts. Cells were isolated from peripheral blood by density gradient separation. AML cells were cultured under highly standardized *in vitro* conditions, and pharmacological effects of two mTOR inhibitors (rapamycin and temsirolimus), and two PI3K inhibitors (GDC-0941 and 3-methyladenin (3-MA)) were evaluated. We analyzed cytokine dependent proliferation by seven days culturing (^{3}H-thymidine incorporation). Results were compared with microarray experiments performed using the Illumina iScan Reader. Real time polymerase chain reaction (rt-PCR) for control was performed.
Results: A larger experiment of antiproliferative effect of the four PI3K-mTOR inhibitors at four different concentrations including 56 consecutive AML patients using the ^{3}H-thymidine incorporation assay was performed. Heterogeneous effects among patients were observed, with patients usually responding similar to the different agents. The results were used to perform an unsupervised hierarchical clustering analysis base on differences in pharmacological responses, dividing patients in two major patient subset, were then identified; one group with a minimal effect of the pharmacological intervention (23 of 56 patients, 41%), and a second group with a relatively strong antiproliferative effect for all drugs and drug concentrations (36 of 56 patients, 59 %). The differences observed in antiproliferative effects had no correlation to cytogenetic, *FLT-3* or *NPM-1* mutation status. For 48 of the 56 patients described for antiproliferative effect of mTOR-PI3K inhibitors gene expression data were obtained. By dividing the patient population in sensitive and resistant cases we used the significance analysis of microarrays (SAM) algorithm to detect differently expressed genes between the two groups. Only five genes were found highly different expressed between the two subset (false discovery rate (FDR)<0.01). The only gene detected know to be involved in cell proliferation was *CDC25B*, encoding the protein cell division cycle 25B, a phosphatase which are a key regulator of the cell cycle. The finding was confirmed by correlation to RT-PCR for CDC25B.
Summary / Conclusion: Their lack of antileukemic effect of mTOR-PI3K inhibitors has been elusive. Here we demonstrate a distinct gene profile associated with resistance to these agents, special characterized with high expression of *CDC25B*. Further targeting of this pathway remains potential in AML.

P037

L-ASPARAGINASE ALTERS TRANSCRIPTION AND TRANSLATION OF GENES INVOLVED IN METABOLISM IN MYELOID LEUKEMIA

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Background: L-asparaginase is an enzyme that has been a part of the standard components of acute lymphocytic leukemia therapy for decades. The enzyme catalyzes the degradation of extracellular asparagine and glutamine. Cancer cells are vulnerable to the amino acid depletion as cells need excess amino acids for rapid biomass and protein synthesis for proliferation and cell survival. Amino acid degradation, in particular glutamine, may be useful in targeting cancer cell metabolism in addition to inhibiting protein synthesis.
Aims: To characterize the cellular response to L-asparaginase in myeloid leukemic cells and identify biological pathways affected by L-asparaginase treatment, and to assess the potential use of L-asparaginase in acute myeloid leukemia (AML) treatment.
Methods: To see the effect of amino acid depletion by L-asparaginase, we assayed proliferation and colony forming ability in a panel of myeloid leukemia cell lines and primary AML cells. Primary AML cells were transplanted in mice to study CD45+ cell engraftment *in vivo*. We selected two cell lines - K562 and HL60 – as representative resistant and sensitive lines respectively, to compare responses to L-asparaginase treatment by polysome profile analysis. Total RNA and efficiently translated RNA from polysomal fractions were isolated to evaluate and compare changes in gene transcription and translation. Geneset enrichment analysis of microarray data was utilized to identify enriched biological pathways in differentially expressed genes after L-asparaginase treatment.
Results: We observed inhibition of cell growth in cell lines treated with L-asparaginase. L-asparaginase also inhibited clonogenic survival of cell lines and primary AML cells. Results from the *in vivo* experiment demonstrate the average levels of CD45+ cell engraftment in the bone marrow was markedly decreased in treated mice.
Polysome profile analysis revealed a decrease in the amount of polysome-bound ribosomal RNA in both cell lines after treatment, indicating global RNA translation inhibition. Microarray analysis of total and polysomal mRNA provided information on enriched biological pathways altered by L-asparaginase treatment in K562 and HL60. As predicted, metabolic pathways were identified in addition to other cellular processes. In the resistant cell line K562, metabolic pathways such as aminoacyl-tRNA biosynthesis, amino acid metabolism, TCA cycle, pyruvate metabolism, and nucleotide metabolism were identified. Interestingly, it appears that K562 is capable of down-regulating TCA cycle. In HL60, amino acid metabolism pathways were also enriched as well as other carbohydrate metabolism pathways. For most genes, changes in total RNA levels (transcription) correlated with changes in polysomal mRNA (efficiently translated). However, we identified a short list of genes that are predicted to be translated differently through post-transcription regulation as a response to compensate for nutrient starvation.
Summary / Conclusion: L-asparaginase has cytotoxic and cytostatic effects in myeloid leukemia and inhibits global mRNA translation. It also induces a metabolic response in cell lines, demonstrating the potential of L-aspraginase as an effective drug with multiple targets including cancer metabolism, a role that was not previously recognized. This study discusses the off-label use of L-asparaginase for acute myeloid leukemia.

P038

THE TYROSINE KINASE CSK ASSOCIATES WITH FLT3 AND KIT RECEPTORS AND REGULATES DOWNSTREAM SIGNALING IN ACUTE MYELOID LEUKEMIA

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Background: The type III receptor tyrosine kinases (RTKs), FLT3 and KIT are widely expressed in acute myeloid leukemia (AML) and a number of AML patients carry an oncogenic mutant of FLT3 or KIT. In normal cells these receptors play important roles in a variety of cellular processes. A number of SH2-domain containing proteins interact with FLT3 and KIT regulating downstream signaling. The tyrosine kinase Csk is mainly studied in context of regulating Src family kinases. Here we present an additional role of this SH2-domain containing non-receptor protein tyrosine kinase in RTK signaling.
Aims: We aimed to identify the role of Csk in type III RTKs, FLT3 and KIT signaling in the context of AML.
Methods: We used Ba/F3 cells as a model system to analyze downstream signaling of FLT3 and KIT. Different Csk mutants were used to detect binding sites and localization patterns. Csk mRNA expression data from patient samples and corresponding healthy donors were used to compare Csk expression in AML. Both selective Csk inhibitor and Csk siRNA were used to study downstream signaling and biological events.
Results: We show that Csk interacts with FLT3 and KIT in a phosphorylation dependent manner. This interaction is facilitated through the SH2-domain of Csk. Under basal condition Csk is mainly localized throughout the cytosolic compartment but upon ligand stimulation it is recruited to the inner side of cell membrane. Csk association did not alter receptor ubiquitination or phosphorylation but disrupted downstream signaling. Selective depletion of Csk using siRNA, or inhibition with the Csk inhibitor, led to an increased phosphorylation of Akt and Erk but not of p38 upon FLT3-ligand stimulation. KIT-ligand-mediated Akt and Erk phosphorylation was also elevated by Csk inhibition. However, siRNA mediated Csk knockdown increased KIT-ligand stimulated Akt phosphorylation but decreased Erk phosphorylation. Akt activation was mediated through phosphorylation of SHC, Gab2 and SHP2. Furthermore, Csk depletion contributed to oncogenic FLT3- and KIT-mediated cell proliferation, but not to survival. A significant decrease in Csk expression was also detected in AML patients.
Summary / Conclusion: The results indicate that Csk association with type III RTKs, FLT3 and KIT can have differential impact in receptor downstream signaling in AML.

P039

WHOLE GENOME AND TARGETED BISULFITE SEQUENCING REVEALS 3 DNA METHYLATION CLUSTERS AND NEW BIOLOGICALLY RELEVANT HYPERMETHYLATED GENES IN AML PATIENTS

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Background: In acute myeloid leukemia (AML), aberrant DNA methylation has been linked to the pathogenesis and progression of the disease. Changes in DNA methylation of promoters, or other regions, are studied primarily with respect to which pathways are involved in tumor transformation and their impact on prognosis.
Aims: The aim was to profile DNA methylation changes in AML patients using methods of next-generation sequencing and to correlate these changes with gene expression for discovering biologically relevant genes affected by hypermethylation.
Methods: Whole genome (3 AML, 2 controls) and targeted (14 AML, 1 control) bisulfite libraries were run on a HiSeqTM2000 sequencer (Illumina) using 105 bp paired-end sequencing reads. Validation of acquired methylation data was performed using 454 pyrosequencing and HumanMethylation27 Infinium arrays (Illumina). HumanHT-12 v4 Expression BeadChip (Illumina) was utilized for whole genome expression profiling. Expression data of selected genes were validated and extended to a larger number of examined patients by TaqMan real-time PCR.
Results: Unsupervised hierarchical clustering of CpG methylation outside and/or inside CpG islands revealed three DNA methylation clusters. From the clinical and molecular characteristics, only *CBF*/*MYH11*+ patients clustered together. None of the other molecular abnormalities (i.e. *DNMT3A* mutations, *MLL* translocation, *NPM1* mutations *FTL3*/ITD and *CEPBα* mutation) formed clusters and we did not observe an effect of clinical status of AML (*de novo*, secondary, AML with dysplastic changes or relapsed AML). A correlation between methylation and expression data enabled us to distinguish between aberrant DNA methylation with no effect on expression of downstream located genes (probably tissue-specific methylation) and biologically relevant DNA methylation (accompanied with changes of gene expression). *ELAVL2* and *CACNA1E* had enormous differences in DNA methylation between AML samples and controls; however they are very probably genes displaying tissue-specific methylation changes, because we did not detect their expression even in healthy precursor cells. On the other hand, there were genes - most notably *CHFR* and *PBX3*, where DNA methylation changes were accompanied with a change in their expression. We measured expression of *CHRF* and *PBX3* genes in 123 AML samples at diagnosis. 20% of AML had down- and 22% up-regulated *PBX3* expression, 5% of AML down- and 7% up-regulated *CHFR* expression. 454 pyrosequencing confirmed the role of DNA methylation in down- and up-regulation of PBX3 gene, hypomethylation (median methylation level 0.25, range 0.15 – 0.36) of a regulatory region located downstream of an annotated CpG island were connected with elevated levels of *PBX3*, whereas hypermethylation (median methylation level 0.51, range 0.31 – 0.98) with decreased levels of expression. Control samples displayed intermediate levels of methylation (median 0.35, range 0.19 – 0.51). Furthermore we observed correlation between *PBX3* and *HOXA9* expression, which is consistent with recently published data that *PBX3* is an important cofactor of *HOXA9* in leukemogenesis.
Summary / Conclusion: In summary, we found new and biologically important genes that are influenced by differential DNA methylation and were able to distinguish 3 major DNA methylation clusters in AML patients.
This study was a part of the COST Action BM0801 (EuGESMA) and was supported by the Czech Ministry of Education, Youth and Sports OC10042 grant and institutional funding grant IHBT00023736.

P040

EXPRESSION OF THE SPLICE VARIANT NPM1-R2 HAS STRONGER PROGNOSTIC VALUE THAN NPM1 MUTATIONAL STATUS IN ACUTE MYELOID LEUKEMIA

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Background: Recently, next generation sequencing technology has identified many new gene mutations in acute myeloid leukemia (AML) that provide novel insights into the mechanisms of leukemogenesis and that further unravel the molecular heterogeneity, in particular within the group of cytogenetically normal (CN) AML. In addition to genomic aberrations, aberrant expression levels of several genes have been identified as prognostic markers and deregulated gene expression is also involved in the pathogenesis of CN-AML. In this group of patients mutations of the nucleophosmin-1 (*NPM1*) gene identify a group with favorable prognosis in the absence of a FMS-like tyrosine kinase 3 internal tandem duplication(*FLT3*-ITD). Notably, wildtype *NPM1* encodes for three alternatively spliced isoforms R1 (B23.1), R2 (B23.2), and R3 (B23.3) that might also impact cellular function.
Aims: Since splicing variants play an important role in cellular functioning and splicing factor mutations have been reported in myeloid tumors including AML, the current study focuses on the characterization of *NPM1*-R2 splicing variant expression as well as its impact in AML patients.
Methods: For 201 samples (105 CN-AML and 96 samples with cytogenetic aberrations) qRT-PCR was performed. Expression level of *NPM1*-R2 was assessed. The existence of *NPM1*-R2 at the protein level was evaluated with the use of Western Blot technique.
Results: We found that the expression of R2 splicing variant was significantly higher in all AML patients compared to HVs with a median expression of 1.64 vs 0.33 (P=0.009). We have found no differences between groups of AML patients with and without *NPM1* mutations (1.21 vs 0.82, P=0.13). Based on the high coincidence of high expression R2 levels and *FLT3*-ITD mutations we analyzed the relevance of R2 high expression and *FLT3*-ITD mutations by comparing R2high/no*FLT3*-ITD, R2low/no*FLT3*-ITD, R2low/*FLT3*-ITD, R2high/*FLT3*-ITD. As shown in Figure1, overall survival (OS) was longer in R2high/no*FLT3*-ITD than in the rest of groups (P<0.001).
Summary / Conclusion: In our study we found that the expression level of *NPM1*-R2 was elevated compared to HVs suggesting that not only *NPM1* mutation but also its splice variant expression might play some role in the process of the tumorigenesis. As the R2 splicing variant represents a truncated form of *NPM1* gene due to the of the lack of exons 11 and 12 (coding for the domain responsible for nucleolar localization of the protein), this isoform mostly localizes in the nucleoplasm, and thus might also have a biological impact in the malignant cells. Most importantly, in our cohort of cases survival differences seen between the established ELN groups according to a *NPM1*/*FLT3*-ITD stratification were less impressive than between groups stratified according to R2 expression combined with *FLT3*-ITD mutational status. In summary, the expression of *NPM1*-R2 might be of biological importance for CN-AML patients. Moreover, R2 splice variant provides prognostic value for CN-AML patients and might information in addition the *NPM1* mutational status.

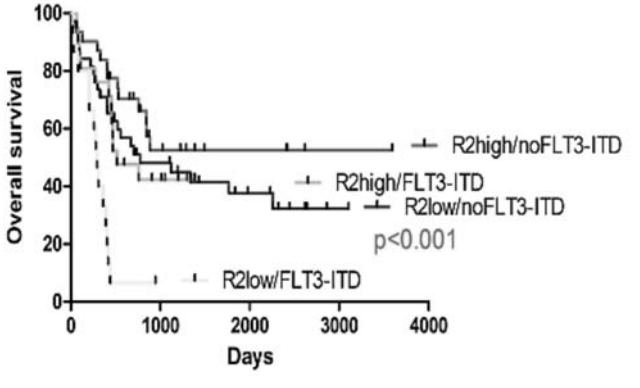

Figure 1.

P041

LEUKEMOGENIC FUNCTION OF TIM-3, A LEUKEMIA STEM CELL MARKER, IN ACUTE MYELOGENOUS LEUKEMIA AND MYELODYSPLASTIC SYNDROMES

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Background: Acute myeloid leukemia (AML) originates from self-renewing leukemic stem cells (LSCs), an ultimate therapeutic target for AML. We have reported that the T-cell immunoglobulin mucin-3 (TIM-3) is expressed on LSCs in most types of AML but not on normal hematopoietic stem cells (HSCs). TIM-3$^+$ AML cells reconstituted human AML in immunodeficient mice, whereas TIM3$^-$ AML cells did not, suggesting that the TIM-3$^+$ population contains all functional LSCs. We established an anti-human TIM-3 mouse IgG2a antibody having complement-dependent and antibody-dependent cellular cytotoxic activities. This antibody did not harm reconstitution of normal human HSCs, but blocked engraftment of AML after xenotransplantation. Furthermore, when it is administered into mice grafted with human AML, this treatment dramatically diminished their leukemic burden, and eliminated LSCs capable of reconstituting human AML in secondary recipients (Kikushige *et al*, Cell Stem Cell, 2010).
Aims: The aim of this study is to clarify the expression and function of TIM-3 in various types of human hematological malignancies.
Methods: We analyzed bone marrow samples from primary MDS patients by multicolor FACS, and also performed the gene expression analysis of primary AML samples as we previously reported(Kikushige *et al*, Cell Stem Cell, 2010).
Results: We extended the analysis of TIM-3 expression into various types of human hematological malignancies, and found that human TIM-3 is expressed in the vast majority of CD34$^+$CD38$^-$ LSCs of human myeloid malignancies including chronic myeloid leukemia, chronic myelomonocytic leukemia and myelodysplastic syndromes (MDS). Although TIM-3 was not expressed in CD34$^+$CD38$^-$ stem cell fraction in normal bone marrow cells, TIM-3 was progressively up-regulated in this population of MDS, along with disease progression into leukemia: The average percentages of TIM-3$^+$ cells in the CD34$^+$CD38$^-$ population was 7.8% in RCMD (n=10), 19.2% in RAEB-1 (n=10), 84.0% in RAEB-2 (n=10) and 92.2% in overt AML (n=10). Thus, TIM-3 might be useful to isolate malignant stem cells responsible for progression into AML in MDS patients. The close association of TIM-3 expression with transformation into AML led us to hypothesize that TIM-3 itself has a function in AML stem cell development. TIM-3 is type 1 cell-surface glycoprotein and has a structure that includes an N-terminal immunoglobulin variable domain followed by a mucin domain, a transmembrane domain and a cytoplasmic tail. Tyrosine residues are clustered in the cytoplasmic tail, suggesting that TIM-3 can induce signal transduction in TIM-3$^+$ AML cells. Previous reports have shown that galectin-9 and HMGB-1 are the ligand of TIM-3 in lymphocytes and dendritic cells. TIM-3 is reported to signal differently in lymphocytes and myeloid cells, because TIM-3 ligation results in different patterns of tyrosine phosphorylation in these cell types, suggesting that TIM-3 has lineage- or cellular context-dependent signal transduction pathways or functions. Therefore, we considered that it should be critical to identify the function of TIM-3 in primary AML cells. We cultured TIM-3$^+$ AML cells in the presence or absence of galectin-9 or HMGB-1, and performed cDNA microarray analysis to find genes activated in response to TIM-3 ligation. Interestingly, pro-apoptotic genes such as BAX and SIVA were significantly down-regulated in the presence of ligands, suggesting that TIM-3 signaling could promote survival of TIM-3-expressing LSCs.
Summary / Conclusion: These data suggest that TIM-3 is a surface marker useful to track malignant LSCs in progression from MDS to AML, and TIM-3 may function for maintenance of LSC through inducing survival-promoting signaling.

P042

LARGE SCALE ANTIBODY ARRAY ANALYSIS OF PROTEIN MACHINERIES IN LEUKAEMIA

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Background: Leukaemia classification is currently based on morphology, surface markers, cytogenetics and molecular genetics. Cellular behaviour is regulated by proteins that act in networks. Knowledge of these networks is relevant to understand intracellular pathways involved in leukemogenesis.
In this study, we applied a bead-based antibody array to detect up to 1725 proteins in each sample of mononuclear cells (MNC). MNC's were procured before treatment from 34 leukaemia patients. (23 AML, 6 ALL, 4 CLL and 1 T-PLL). Informed consent was obtained.
Aims: The overall aim of the study was to determine whether our bead based antibody array could be applied to study protein expression in leukaemic cells.

Subsequently, we wanted to investigate lineage-specific protein expression in subgroups of leukaemias.
Methods: *Sample preparation:* Proteins from cytosol, organelles, membranes and nuclei were extracted using a newly developed chemical method (Figure ure 1A). Proteins were biotinylated and separated by size exclusion chromatography (SEC) (Figure 1B and Figure 1C). A total of 96 sample fractions were incubated with arrays of antibodies bound to colour-coded latex beads. (Figure 1D). Captured proteins were labelled with fluorochrome-conjugated streptavidin and detected by flow cytometry. (Figure 1E and Figure1 F). *Data Analysis:* Flow cytometry files were processed using a custom-made software that automatically identifies populations of colour-coded beads and exports data for the median streptavidin fluorescence for each subsets. (Figure 1F). The output text files were imported into excel spreadsheets. Excel was used to generate line plots and calculate peak integrals.
Results: Many antibodies captured more than one target. Knowledge of the subcellular distribution of the intended target was highly useful to discriminate specific binding from cross-reactivity. Antibodies to nuclear proteins including RUNX1, PARP, DNMT1 and RB were found to capture well defined targets from the nuclear fraction. Cell surface proteins (CD antigens) were detected as reactivity peaks in the membrane fraction with elution profiles typical for micelle-associated proteins. Caspases, CDK`s and STAT`s were expressed in the cytosolic fraction. Markers of cytoplasmic organelles were found in a separate fraction obtained by treatment of digitonin-permeabilized cells with the detergent Tween 20. The size distribution profiles showed that several of the proteins occur in multi-molecular complexes. Results obtained with antibodies against hematopoietic lineage markers were in good accordance with immunophenotypes obtained by diagnostic flow cytometry. CD33 was consistently found in AML and CD14 was expressed in acute monocytic leukaemia. CD10 was recognized in ALL patients. (Figure 2).Lineage-specific markers included CD2, CD3E, CD4 and CD8 in T-ALL and CD40 and CD 72 in B-ALL. PAX5 was regularly expressed in CLL and B-ALL patients. (Figure 2)
Summary / Conclusion: The results show that it is feasible to perform parallel detection with thousands of antibodies. Combination of subcellular fractionation and SEC provides the resolution needed to ensure specificity. The ability to classify leukaemias on the basis of lineage-restricted surface proteins demonstrates that the technology has diagnostic potential. Compared to standard flow cytometry, antibody array analysis allows detection of more intracellular proteins.

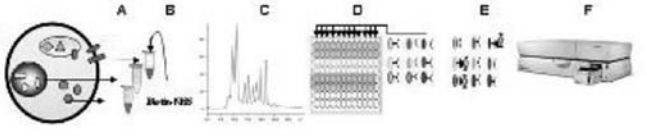

Figure 1.

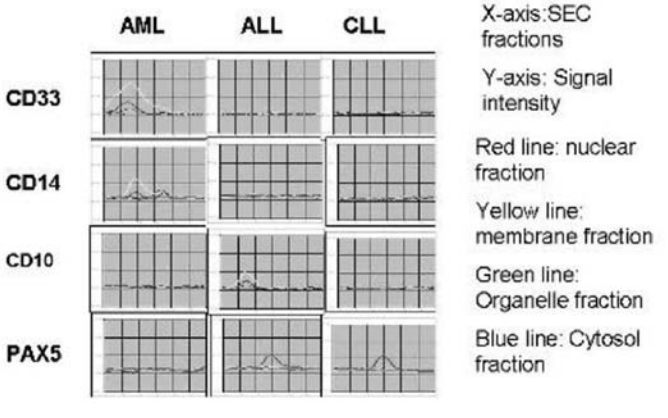

Figure 2.

Acute myeloid leukemia - Clinical 1

P043

HIGH RESPONSE RATE AND BRIDGING TO HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH QUIZARTINIB (AC220) IN PATIENTS WITH FLT3-ITD–POSITIVE RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA (AML)

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Background: FMS-like tyrosine kinase 3 internal tandem duplications (FLT3-ITD) in acute myeloid leukemia (AML) are associated with early relapse after standard chemotherapy and poor survival. Quizartinib (AC220) is an oral FLT3 receptor tyrosine kinase inhibitor that is active against both ITD mutant and wild type FLT3.
Aims: To assess the efficacy of quizartinib monotherapy in FLT3-ITD–positive patients with AML relapsed or refractory to second-line, salvage chemotherapy or relapsed after hematopoietic stem cell transplantation (HSCT).
Methods: A phase 2 study was conducted to assess the efficacy and safety of quizartinib monotherapy in FLT3-ITD–positive and FLT3-ITD–negative patients (N=333 in 2 cohorts). We present here final data on the 136 FLT3-ITD–positive patients, comprising patients aged ≥18 years with AML relapsed or refractory to second-line, salvage chemotherapy or relapsed after HSCT. Patients were tested by a central laboratory for the FLT3-ITD mutation with a >10% ratio of ITD to total FLT3 defined as positive. Quizartinib was administered once daily as an oral solution during 28-day treatment cycles. The initial starting dose administered to the first 17 patients was 200 mg/day, but because of the occurrence of QT interval prolongation, the dose was reduced to 135 mg/day for men and 90 mg/day for women. Composite complete remission (CRc) consisted of complete remission (CR) plus CR with incomplete platelet recovery (CRp) plus CR with incomplete hematologic recovery (Cri).
Results: The CRc rate was 46% (5 CR, 2 CRp, and 55 CRi), with a median duration of CRc of 10.6 weeks and median overall survival of 24.0 weeks. Of those refractory to their last AML therapy, 47% achieved a CRc with quizartinib. Quizartinib was discontinued for HSCT in 47 of 136 patients (35%); 44 of the 47 patients (94%) had at least a partial response (PR) with 2 CRp, 24 CRi, and 18 PR on quizartinib. 8 of 47 patients (17%) had received previous HSCT and 32 of 39 patients (82%) who did not receive previous HSCT were refractory to second line therapy. Median overall survival (OS) was 41.5 weeks for patients who achieved a CRc (n=26) prior to HSCT and 29 weeks for patients with PR (n=18). The 1 year survival rate was 39% for both response groups. Patients with a CRc (n=36) or PR (n=20), but no HSCT, had a median OS of 24.5 weeks and 20.9 weeks, respectively, and 1-year survival rates of 25% and 5%, respectively. Of 27 patients with OS >52 weeks, 17 (63%) had HSCT. The group comprising responders going to HSCT compared to responders not receiving HSCT, were similar with median age of 44.5 vs. 47 years, 70% vs. 54% refractory to last chemotherapy and 5% and 4% prior MDS. Responders receiving HSCT were less likely to have had a prior transplant (25% vs. 46% previous HSCT).
Summary / Conclusion: The prognosis of relapsed/refractory FLT3-ITD–positive patients, including those relapsing after HSCT, is remarkably poor. In this context, our results demonstrate notable activity of quizartinib therapy and very promising survival, particularly for responding patients who subsequently received HSCT.

P044

PROGNOSTIC IMPACT OF THE FERRITIN LEVEL AT DIAGNOSIS IN ACUTE MYELOID LEUKEMIA PATIENTS WITH INTERMEDIATE CYTOGENETIC RISK

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Background: Elevated ferritin level (FL) has been described as an unfavorable prognostic factor in myelodysplastic syndromes. Little is known in acute myeloid leukemia (AML) although the pre-allogeneic stem cell transplantation (alloSCT) high FL has been shown to influence outcome.
Aims: In this study, we evaluated the prognostic impact of the FL at diagnosis in *de novo* AML patients with intermediate cytogenetic risk treated by intensive chemotherapy.
Methods: Since 2007, quantification of the FL performed by spectrophotometry is included in the initial workup of AML. Between May 2007 and December 2011, 164 patients with *de novo* WHO defined-AML were treated by 3+7-like chemotherapy in Toulouse University Hospital Center. The relevant ferritin threshold was calculated using ROC curves analysis. Briefly, median age of these patients was 59 years (16-81y); complete remission (CR) was achieved in 85% (140/164); 5-years disease free (DFS) and overall survival (OS) were 35% and 38%. Median follow-up for patients alive at the date of last contact was 30 months. 43 patients (26%) had a FL at diagnosis superior to 4N. Those patients had a higher white blood cell (WBC) count (P=0.003) and a higher rate of good mutational profile (NPM1c^{pos}FLT3-ITD^{neg}, P=0.04). Then, results were validated in an independent cohort of 66 patients treated between 2007 and 2011 in Amiens University Hospital Center.
Results: FL, WBC count and NPM1c^{pos}FLT3-ITD^{neg} had no impact on achievement of CR whereas age had a significant impact. By logistic regression, age lower than 60 years and WBC count retained significance for CR achievement. For the 140 complete responders, WBC count had no impact on disease-free survival (DFS). Interestingly, patients with FL<4N and FL>4N had median DFS of 26.9 and 9.2 months, respectively (P=0.033, Figure 1A). NPM1c^{pos}FLT3-ITD^{neg}, age and alloSCT were also significantly associated with DFS. In multivariate analysis, FL>4N was significantly associated with shorter DFS (P=0.001, HR: 2.39, 95%CI 1.4-4.0). FL, age, WBC count, NPM1c^{pos}FLT3-ITD^{neg} and alloSCT significantly influenced OS. Median OS was 39 and 11.6 months for patients respectively with FL<4N and those with FL>4N (P=0.0015, Figure 1B). Multivariate analysis for OS retains 4 significant factors: WBC count (HR: 1.01, 95%CI, 1.00-1.01, P=0.004), alloSCT (HR: 0.36, 95%CI, 0.2-0.7, P=0.0009), NPM1c^{pos}FLT3-ITD^{neg} (HR: 0.24, 95%CI, 0.12-0.47, P<0.0001), and FL>4N (HR: 2.64, 95%CI, 1.6-4.4, P=0.0002). Among the 35 patients with NPM1c^{pos}FLT3-ITD^{neg}, FL>4N was predictive of shorter DFS (median: 10.5 months vs not reached, P=0.005) and OS (median: 21.4 months vs not reached, P=0.01). The impact of the FL at the threshold of 4N, was also significantly correlated with OS in an independent cohort of *de novo* AML patients with intermediate cytogenetic-risk (n=66). Median OS was 27.6 and 7.7 months for patients with FL<4N and with FL>4N, respectively (P=0.01).
Summary / Conclusion: This study emphasizes the prognosis impact of the FL at diagnosis in AML patients. If validated on a larger prospective cohort of patients enrolled in multicenter clinical trials, the FL could represent a new prognosis marker easily translated into the clinical practice. Furthermore, the heterogeneity of FL at diagnosis in a "non-iron overload" setting and its impact on leukemic relapse suggest a link between inflammatory response, oxidative stress, metabolic syndrome and chemoresistance.

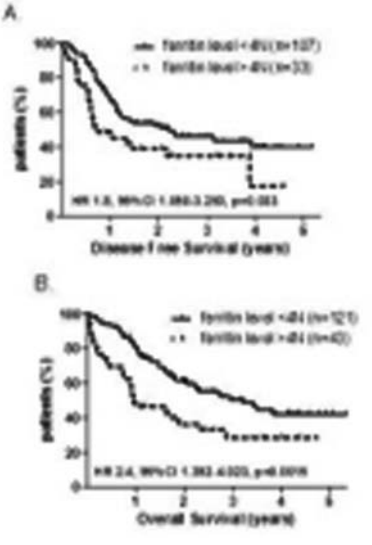

Figure 1. Impact of FL on outcome of patients with *de novo* AML. A. DFS of 140/164 complete responder patients. B. OS of 164 patients treated by 3+7 chemotherapy.

P045

PROGNOSTIC RE-CLASSIFICATION OF ADULT ACUTE MYELOID LEUKEMIA BASED ON THE COMPREHENSIVE GENETIC ANALYSIS

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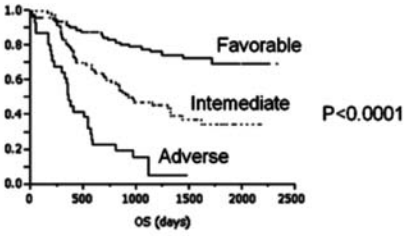
Background: Several genetic alterations, which are involved in the development and progression of acute myeloid leukemia (AML), have been identified. Although their prognostic relevance are not fully clarified, the European LeukemiaNet (ELN) recently recommended a novel risk classification system based on the genetic status in addition to the cytogenetic risk. However, several groups suggested that the combination with another genetic status would stratify each ELN risk group into further risk groups.
Aims: To establish more precise risk classification system, we comprehensively analyzed mutations in 51 genes as well as the karyotype, and evaluated their prognostic impacts in AML.
Methods: The study population included 197 *de novo* adult AML patients who were registered to the Japan Adult Leukemia Study Group (JALSG) AML201 study. Bone marrow samples were collected from them at initial diagnosis. Informed consent was obtained from all patients to use their samples for banking and molecular analysis, and approval was obtained from the ethics committee of all participating institutes. We screened mutations in 51 genes using the high throughput sequencing system, and confirmed each mutation by the Sanger sequence method.
Results: Mutations were identified in 44 genes. We identified mutations of Class I in 117 patients (59%), those of Class II in 84 (42%), those of epigenetic regulators in 91 (46%), those of BCOR family in 17 (9%), those of NCOR family in 18 (9%), those of NOTCH1/2 in 19 (10%), those of the cohesion complex in 22 (11%), and those of splicing factors in 9 (5%). *FLT3*, *NPM1*, *CEBPA*, *DNMT3A* and *IDH2* mutations were predominantly identified in cytogenetically normal (CN)-AML, while *KIT* and *TP53* mutations were frequent in core binding factor (CBF)-AML and AML with complex karyotype, respectively. A multivariate logistic-regression analysis showed that *TP53* mutation, wild-type *NPM1* and cytogenetic findings other than good risk were independent unfavorable factors for achieving complete remission (CR). Univariate analysis showed that *FLT3*-ITD, *DNMT3A*, *TP53*, *RUNX1*, and *MLL*-PTD mutations were the poor prognostic factors for overall survival (OS). When patients were stratified into the ELN risk groups, their prognoses were clearly distinguished. However, *DNMT3A*, *GATA2*, *MLL*-PTD and *TP53* mutations were identified to be further poor prognostic factors in the favorable genetic risk, intermediate risk I, intermediate risk II and adverse risk groups of the ELN classification, respectively. Based on these findings, we could stratify AML patients into 3 risk groups; Favorable: CBF-AML or CN-AML harboring *CEBPA* or *NPM1* mutation without *FLT3*-ITD and *DNMT3A* mutation (n=83, OS at 4 year: 73%, event free survival (EFS) at 4 year: 48%, CR rate: 93%), Intermediate: *DNMT3A* mutation, *FLT3*-ITD, adverse-risk karyotype and none of the mutation or cytogenetic abnormality leading to assignment into Favorable and Adverse groups (n=83, OS at 4 year: 37%, EFS at 4 year: 21%, CR rate: 80%), Adverse: *RUNX1*, *MLL*-PTD, *GATA2* or *TP53* mutations (n=31, OS at 4 year: 5%, EFS at 4 year: 0, CR rate: 58%).
Summary / Conclusion: We demonstrated a modified risk classification system for adult AML patients based on the genetic and cytogenetic status by the

comprehensive mutation analysis. However, the frequencies of most mutations were less than 5%. Further large-scale studies are required to confirm the prognostic impact of each mutation.



Risk group	Subsets	4yr OS
Favorable (n=83)	CBF-AML Normal karyotype CEPBA Mt. or NPM1 Mt. without FLT3-ITD and DNMT3A Mt.	73%
Intermediate (n=64)	FLT3-ITD or DNMT3A Mt. Adverse karyotype not classified to favorable or adverse	37%
Adverse (n=50)	TP53 Mt., MLL-PTD, RUNX1 Mt. or GATA2 Mt.	5%

Figure 1. Overall survival according to risk groups.

P046

LYSYLOXIDASE IS ASSOCIATED WITH INFERIOR OUTCOME AND EXTRAMEDULLARY DISEASE IN ACUTE MYELOID LEUKEMIA

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Background: Lysyloxidase (LOX) has been described as necessary for premetastatic niche formation in epithelium-derived malignancies and its expression level correlates with distant metastasis free- and overall survival (OS).
Aims: We were interested to investigate whether the LOX-concentration in AML plasma samples is of prognostic relevance and whether it is associated with certain characteristics such as extramedullary disease.
Methods: Plasma samples of 683 patients with AML (age 17 – 60 years) who were treated within the prospective AML2003 trial (NCT00180102) of the SAL study group were analyzed for LOX concentration using the Amplite Fluorimetric LOX Assay Kit (AAT Bioquest, Sunnyvale, CA, USA). All fluorescence reads were performed in triplicate with recombinant human LOXL2 (R&D Systems, Minneapolis, MN, USA) for standard curve estimation. Signals were read by a fluorescence microplate reader at Ex/Em 540/590 nm. Supernatant of the *NPM1* mutated AML cell line OCI/AML3 served as internal control. The method of Kaplan-Meier was used to estimate OS and event-free survival (EFS). Survival distributions were compared using the log-rank test. Prognostic parameters were tested in a Cox regression model for OS and event-free survival (EFS).
Results: The range of LOX concentration for all patients was 0 – 2184 ng/mL. Inspection of the Martingale residuals of a Cox model testing the influence of LOX on OS revealed that a cut-off model is the most appropriate. The optimal cut-off LOX value was determined using a minimal-p-value method resulting in a logarithmic logLOX = 2.0403 (109 ng/mL) which identified dichotomizing all patients into a LOX-high group (>109 ng/mL, n=272, 40%) and a LOX-low group (≤109 ng/mL, n=411, 60%). Comparing LOX-high and LOX-low patients revealed a 3-year OS of 47% (95% CI: 40 – 53%) and 53% (95% CI: 48 – 58%, P=.022), and 3-year EFS of 27% (95% CI: 21 – 32%) and 35% (95% CI: 31 – 40%, P=.005), respectively. In the Lox-high group significantly more patients had reported extramedullary AML compared to the Lox-low group, P=0.037. Indeed in the multivariate analysis the LOX-extramedullary interaction term for OS and EFS was significant (P=.025 and P=.006, respectively). Therefore, in patients with extramedullary disease the LOX level predicted survival. Patients within the LOX-low group had an OS of 43% (95% CI: 23 – 63%) and EFS of 36% (95% CI: 17 – 54%) as compared to the LOX-high group with an OS of 13% (95% CI: 1 – 25%) and EFS of 6% (95% CI: 0 – 15%), P=.002 and P=.008, respectively.
Summary / Conclusion: High LOX levels are associated with statistically significant worse OS and EFS in AML patients. The positive correlation between high LOX levels and extramedullary AML suggests a potentially pathophysiological relevant mechanism involved in extramedullary homing and growth of AML and may offer further insights into AML biology. Furthermore, the prognostic heterogeneous group of AML patients with extramedullary disease can be separated in those with superior and those with inferior survival by applying the plasma LOX level at diagnosis. Future prospective clinical trials will have to confirm these data and experimental studies will need to address the functional modalities of how LOX is regulated and how it contributes to migratory and adhesion properties in AML.

P047

REAL-WORLD OUTCOMES AMONG AML PATIENTS TREATED WITH DECITABINE OR AZACITIDINE

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Background: Both decitabine (DEC) and azacitidine (AZA) are indicated for treatment of elderly AML patients with 20%>30% BM blasts in the EU.
Both agents have shown an improvement in survival, however these studies were limited: DEC demonstrated an OS benefit (2.1 month gain; P=0.5735) in an unplanned statistical analysis (DACO-016 clinical study) and AZA demonstrated a 9 months (P=0.001) survival benefit (the AZA-001 trial) in a study of high grade MDS patients that also included elderly AML patients with 20-30% blasts.
Aims: The purpose of this study is to compare overall survival (OS) and other clinical benefits of DEC and AZA using real world data.
Methods: US health plan data were assessed between 01 January 2006 and 31 April 2012 to identify enrollees initiating DEC or AZA for treatment of AML (ICD-9-CM: 205.0x). Comparisons between DEC and AZA patients made via survival models to account for variable length of follow up. Similarly, the number of hospitalizations was measured and calculated as 'hospitalizations per person-year.' A Cox proportional hazards model was used to examine the relationship between choice of demethylating agent and OS, controlling for age, gender, comorbidity score, prior MDS diagnosis, prior red blood cell transfusion, prior hospitalizations, and insurance type.
Results: 487 AML patients were identified as initiating therapy during the study period (n=199 DEC and n=288 AZA). The two cohorts were similar in terms of age, gender, prior MDS diagnosis, prior red blood cell transfusion needs, and insurance coverage, but patients receiving DEC were more likely to have had a hospitalization in the prior six months (70% vs 62%; P=0.032) and more likely to have Charlson comorbidity score of 3 or greater (59% vs 50%; P=0.036). Median OS (6.9 vs. 10.1 mos.; P=0.007) and time to hospitalization (3.9 vs. 6.6 mos.; P=0.015) were significantly longer among AZA treated patients. After controlling for demographic and clinical characteristics, AZA treated patients continue to show better OS (hazard ratio=0.721; P=0.008) and a greater time to hospitalization (hazard ratio=0.787; P=0.020). DEC patients averaged more hospitalization (3.15 per person-year) than AZA patients (2.72 per person-year).
Summary / Conclusion: Although AZA and DEC have not been formally compared head to head in elderly patients with AML, it is generally accepted that both agents have clinical activity in such patients. This analysis suggests that AML patients treated with AZA do better clinically than those patients who physicians elect to treat with DEC based on statistically significant longer OS and fewer hospitalizations.

P048

OUTCOME OF PERSONALIZED THERAPY BASED ON HIGH-THROUGHPUT *EX VIVO* DRUG SCREENING IN PATIENTS WITH RELAPSED, CHEMOREFRACTORY ACUTE MYELOID LEUKEMIA (AML)

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Background: Recent genomic studies have provided new insight on mutations contributing to AML onset and progression. However, the aberrations observed are often complex and rarely directly actionable in selecting patient therapies. To rapidly identify novel patient-specific therapies, we developed a high-throughput drug sensitivity and resistance testing (DSRT) platform. This assay allows us to determine the sensitivity of patient AML cells *ex vivo* against a pharmacopeia-wide panel of oncology drugs, as well as emerging inhibitors to discover novel therapeutic options for individual patients.
Aims: Here we present drug screening results for the first 16 AML patients tested, and report the clinical outcome of DSRT-guided therapies.
Methods: Fresh mononuclear cells from bone marrow aspirates of 16 relapsed or refractory AML patients (>50% blast count) and 5 healthy donors were screened against a collection of all clinically available cytotoxic chemotherapy agents (n=103) and targeted preclinical and clinical drugs (n=100-170). The drugs were tested over a 10,000-fold concentration range for constructing dose-response curves for each compound and sample. A leukemia-specific drug sensitivity score (sDSS) was derived from the area under each dose response curve in relation to the total area, and relating results from leukemia samples to normal bone marrow. The turnaround time for the DSRT assay was 4 days. When enough cells were available, the samples also underwent exome and transcriptome sequencing to define clonal evolution and mechanisms of

response and drug resistance from consecutive sampling of the patients.
Results: DSRT-profiles were unique to each AML patient and sample (Figure 1 for an example). Drugs which commonly showed selective responses *ex vivo* were dasatinib, temsirolimus, MEK-inhibitors and sunitinib. DSRT results were considered clinically implementable in a patient if a distinct leukemia-selective response pattern was observed and drugs with the most leukemic-selective responses were clinically available (as compassionate or on/off-label use). DSRT-guided therapy was possible in 13/16 (81%) of the patients. Non-guided chemotherapy was chosen for 6 patients (treatment protocol). Of the remaining 7 patients, 2 patients received 2 courses of DSRT-guided personalized treatment based on two separate DSRTs from different relapse time points. Of the 9 therapies, 3 patients had a response to therapy (ELN criteria, table): 1 complete response (CRi), 2 with a morphologic leukemia free state. Three remaining patients showed meaningful clinical responses not meeting the ELN criteria. Patient 560 showed a rapid clearance of blasts in peripheral blood after five days of treatment (dasatinib, sunitinib) after which therapy was discontinued due to gastrointestinal toxicity. Patient 252 had an 8 week progression-free period during dasatinib monotherapy (bone marrow blasts 65-40-70%). Patient 784 achieved a transient response with bone marrow blasts decreasing from 70 to 35%. Analysis of exome/transcriptome indicated mechanistic clues to patient responses/relapses and suggested individual biomarkers for response.
Summary / Conclusion: Our drug-response phenotype-based assay provided actionable drug candidates for most patients with relapsed, chemorefractory AML. Promising clinical responses suggest good predictive power of the *ex vivo* testing platform. DSRT is a powerful tool for drug repurpositioning and devising personalized therapies. When combined with next-gen sequencing, DSRT will provide unique insight into the pathogenesis of relapsed AML and may facilitate introduction of new treatments to otherwise refractory patients.
Off-label use: Many of the drugs used in DSRT-guided therapies are not indicated for AML.

Pt #	DSRT-guided treatment	Disease state at treatment start	Treatment response (ELN)
252	DAS	Relapsed, resistant	SD 8 weeks
393	DAS	Relapsed, PR	Not evaluable
560_1	DAS, TEM	Relapsed, CR	RD
560_2	DAS, SUN	Relapsed	Clearance of blasts (blood)
600	DAS, TEM, SUN	Relapsed, resistant	CRi
718	CLO, SOR	Relapsed, resistant	Morphologic leukemia-free state
784_1	DAS, SUN, TEM	Resistant	RD, transient response
784_2	CYT, ETO, SUN, VBL	Resistant	RD, transient response
800	CLO, DAS, VBL	Resistant	Morphologic leukemia-free state

DAS=Dasatinib, SUN=Sunitinib, TEM=temsirolimus, SOR=sorafenib, CLO=clofarabine, CYT=Cytarabine, ETO=etoposide

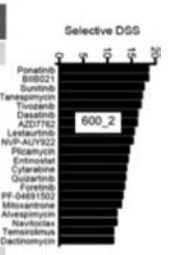

Figure 1.

P049

***EX VIVO* PHARMACOLOGICAL EVALUATION OF 16 DRUGS IN 60+ ACUTE MYELOID LEUKEMIA PATIENTS USING WHOLE BONE MARROW SAMPLES ANALYZED BY AUTOMATED FLOW CYTOMETRY**

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Background: To aid in the identification of effective treatments for individual patients, *ex vivo* assays for detecting cell death inducible by drugs for hematological malignancies have been in development for over 20 years. We have developed a novel automated flow cytometry-based platform (ExviTech).
Aims: The purpose of this study is to examine the *ex vivo* pharmacology of single drugs used to treat AML against the malignant cell population in bone marrow samples from 80 AML patients.
Methods: Bone-marrow samples from patients diagnosed with AML were sent to Vivia from 24 hospitals across Spain within 24 hrs, in collaboration with PETHEMA. The whole sample was plated into 96-well assay plates containing 8 concentrations of each drug. The plates were incubated for 48-hours, and then prepared for analysis by our flow cytometry-based ExviTech© platform. All processes have been automated and multiple controls are used that greatly increase the accuracy of the analysis. The percentage of leukemic cell death was determined via labeling with monoclonal antibodies and AnnexinV-FITC. A survival index is computed for each drug, the lower the survival index, the more effective the drug. Dose-response curves of cytarabine, idarubicin, daunorubicine, etoposide, mitoxantrone, fludarabine, decitabine, 5-azacitidine, clofarabine, panobinostat, sorafenib, melphalan, cyclophosphamide, lenalidomide, busulfan and 6-thioguanine were measured in 64-99 patient samples.
Results: There is a large range of interpatient variability in the response to a single drug. These two results are depicted in Figure 1. The red line is the average patient response to fludarabine, while the light grey lines are the individual results from 94 patients, representing wide interpatient variability. The dark blue lines are average dose response curves for the other 15 drugs referenced above, demonstrating the range of effect of these drugs *ex vivo*. Interestingly, panobinostat (far left blue line), was the most potent and effective drug tested, suggesting that for a subset of patients it could potentially be a useful treatment. The anthracyclines, idarubicin, daunorubicin and mitoxantrone show a similar average response. Although anthracyclines are stronger drugs than fludarabine *on average*, certain fludarabine patient curves actually overlap with Dauno and Mito average curves. This means personalizing treatment may be as important as average drug strength. Clofarabine presented the widest variability of all of the drugs tested, with some patients responding very well while others were totally resistant. Epigenetic drug 5-azacytidine, which clinically requires several cycles to work at low doses, shows depletion dose responses at 48 h similar to Cytarabine. This likely reflects its cytotoxic mechanism at high doses, but still most sensitive patients identified here may also be sensitive for the hypomethylation mechanism. Interestingly, the related epigenetic drug Decitabine acting on the same target is very inefficient in this assay.
Summary / Conclusion: By testing the drugs used in the treatment protocols for AML directly on patient samples, a pharmacological based model could be developed to infer drug resistance or sensitivity, patient by patient. Idarubicin, daunorubicin and mitoxantrone are commonly used in combination with cytarabine, and testing may be able to determine which would be better for each individual patient. Similarity, testing could be used as a companion diagnostic to identify subsets of patients for which treatments such as clofarabine or panobinostat would be effective.

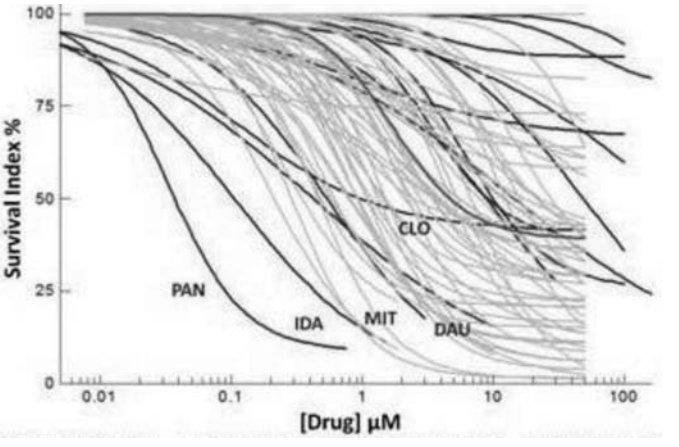

Figure 1. Red line, average curve of fludarabine; gray lines, individial curves of fludarabine; black lines, average curves of other drugs tested with panobinostat (PAN), idarabucin (IDA), mitoxantrone (MIT), daunorubicin (SDAU) and clofarabine (CLO) identified.

P050

SYSTEMATIC REVIEW AND META-ANALYSIS OF ANTI-CD33 ANTIBODY TREATMENT IN AML – GEMTUZUMAB OZOGAMACIN HAS ANTI-LEUKAEMIC EFFICACY

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Background: Immunotherapeutic strategies represent a major advance in the treatment of haematological malignancies. Conventional chemotherapy is ineffective in the majority of patients with Acute Myeloid Leukaemia (AML). Monoclonal antibodies (gemtuzumab ozogamacin (GO) and lintuzumab) which recognise the CD33 antigen expressed on myeloid progenitors) have been reported to improve outcome in some randomised controlled trials (RCTs). However, reports of associated excess toxicity have resulted in GO's licence being withdrawn. As a result, the role of these agents in the management of AML remains unclear.
Aims: Our aim was to assess the effectiveness and safety of anti-CD33 antibody treatment in patients with AML.
Methods: Standard systematic review methods were employed. We searched Medline (1946-2012), Embase (1974-2012), Cochrane Library (up-to 2012) and major conference proceedings for RCTs, where one arm included anti-CD33 antibody therapy. Fixed effects meta-analysis methods were used, involving calculation of observed minus expected number of events, and variance for each endpoint in each trial, with the overall treatment effect expressed as Petos' odds ratio with 95% confidence interval.
Results: Fourteen RCTs met the inclusion criteria, 12 with GO, 2 lintuzumab. Meta-analysis of 12 RCTs with 14 randomisations involving GO was undertaken. GO did not improve complete remission (CR) rates (OR=1.03, 95% CI=0.77-1.37, P=0.9). In the setting of induction treatment (total 1961 patients), 13 cases of VOD were reported, but there was no significant adverse impact of GO on induction death. However, a clear reduction in resistant disease (P=0.02) can be seen. There was an impact of GO on cumulative incidence of relapse (CIR) (HR=0.88, 95% CI=0.79-0.97, P=0.01), largely related to the use of GO in induction (HR=0.83, 95% CI=0.73-0.93, P=0.002). There was no effect of GO on death in CR (P=0.7). The reduction in CIR resulted in an improvement in relapse-free survival (RFS) with GO (HR=0.88, 95% CI=0.81-0.96, P=0.003). Subgroup analyses investigated the optimal delivery of GO and whether some types of patient may benefit more. A benefit on RFS was clearly seen when GO was used as part of induction therapy (HR=0.85, 95%CI=0.77-0.94, P=0.001). There was no evidence that the effect size for RFS differed depending on the dose of GO (less than or more than 9 mg/m^2) or with patient age (less than or greater than 60). Based on data from a limited number of trials, the benefit of GO appears greatest in patients with favourable genetics, with no evidence of benefit in patients with adverse genetics, for overall survival (test for trend: P=0.009).
Summary / Conclusion: GO has a clinically important anti-leukaemic effect, as demonstrated by reductions in relapse and resistant disease. The suggestion of increased toxicity of GO is not confirmed by this meta-analysis. Further trials are needed to clarify whether certain types of patients benefit more than others and what is the optimal dose and schedule of GO.

P051

RISK-ADAPTED TRANSPLANT SELECTION ATTENUATES THE NEGATIVE PROGNOSTIC ROLE OF MINIMAL RESIDUAL DISEASE AND GENETIC HIGH RISK FEATURES

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Background: The outcome of adult AML still remains unsatisfactory due to two main reasons: 1) the risk-category allocation based on the sole definition of pre-treatment biological features may fail to distinguish some high-risk patients who, therefore, might necessitate to be timely addressed to allogeneic SCT (ASCT); 2) the delivery of ASCT is often hampered by the paucity of candidates (25-30%) with a full matched family donor. We have reported (Buccisano *et al*, Blood, 116:2295-303, 2010) that a proper combination of upfront genetics/cytogenetics and minimal residual disease (MRD) represents a powerful tool to predict the risk of relapse on an individual basis.
Aims: We have designed a risk-adapted strategy in which high-risk patients (those with an adverse karyotype, FLT3-ITD mutations, or MRD positivity after consolidation therapy) should receive ASCT.
Methods: Definition of MRD positivity required ≥ 3.5x10^{-4} residual leukemic to be counted in the bone marrow and once a given patients was declared to be at high-risk, he received ASCT whatever the source. For comparison, we analyzed the outcome of a matched historical cohort of high-risk patients who were submitted to ASCT only in the case a full matched family donor was available or, alternatively, to autologous stem cell transplantation (AuSCT).
Results: The prospective and retrospective cohort included 34 (1 MRD+, 3 favorable-K/MRD+, 16 intermediate-K/MRD+, 7 adverse-K and 7 FLT3-ITD) and 77 patients (2 MRD+, 9 favorable-K/MRD+, 50 intermediate-K/MRD+, 3 adverse-K and 13 FLT3-ITD), respectively. Sixteen of 77 (21%) in the retrospective cohort received ASCT whereas in the prospective cohort 25 of 34 (74%) did the same (8 from a matched family donor, 17 from alternative sources) (P<0.001). After a median follow-up of 33 and 50 months, respectively, survival of high risk patients in the prospective cohort was significantly longer than the one of high risk patients in the retrospective one (61% vs 21%) and similar to that of a historical group of patients categorized as low risk (58%) (P=0.006) (Figure n.1). Relapse rate before transplant was similar in the 2 cohorts (26% vs 22%, respectively, P=NS).
Summary / Conclusion: We conclude that in adult patients with AML, a therapeutic approach based on the risk-driven "transplant *versus* no transplant" rather than "donor *versus* no donor" strategy is feasible and may favorably impacts on the negative prognostic role of MRD positivity and adverse genetics/cytogenetics.

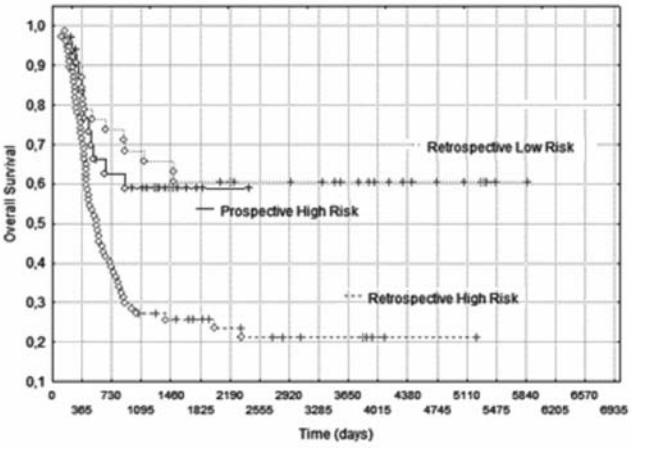

Figure 1.

P052

CHARACTERISTICS AND PROGNOSIS OF ACUTE ERYTHROLEUKEMIA (AEL) - RESULTS OF A SUBGROUP ANALYSIS OF THE STUDY ALLIANCE LEUKEMIA (SAL) AML TRIALS

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Background: Acute erythroleukemia (AEL) represents a rare type of acute myeloid leukemia accounting for less than 5% of all cases. So far, according to WHO classification this AML entity is thought to have a poor prognosis in itself.
Aims: We assessed the influence of relevant clinical and demographic parameters, FLT3-ITD, NPM1 status and cytogenetics on complete remission rates (CR), overall survival (OS) and event free survival (EFS) separately in AEL and non-AEL patients.
Methods: 3267 patients with newly diagnosed AML were treated according to the protocols of the AML96, AML2003 or AML60+ studies of the Study Alliance Leukemia (SAL). Informed consent was obtained by all patients. 116 of these patients had acute erythroleukemia (AEL). In only 78 of these cases slides for systematic morphologic review were available. Only cases with morphological review were included into subgroup analysis.
Results: After morphological review, three diagnostic groups could be separated according to WHO 2008: acute erythroid/myeloid leukemia (n=54; AML M6a according to FAB), pure erythroid leukemia (n=4; AML M6b according to FAB) and acute myeloid leukemia with multilineage dysplasia (n=20). No significant differences between these distinct groups could be elucidated, but all BM harborded severe dysplastic features and mostly multilineage dysplasia. Compared to non-AEL AML, NPM1 and FLT3-ITD mutations were found in 13.3% and 6.3% of the patients with AEL and in 32.1% and 20.7% of the patients with non-AEL AML (P=0.002, P=0.008), respectively. All patients with AEL and NPM1 mutations were *de novo* AMLs and had normal cytogenetics. Several cytogenetic aberrations, most of them associated with poor prognosis, are found more often in the AEL cohort than in the non-AEL AML cohort (Trisomie 8: 16.3% vs.

9%, P=0.026; del(5q): 12.5% vs. 6.6%, P=0.038; -7: 12.5% vs. 5%, P=0.003; complex aberrant: 27.5% vs. 13.3%, P<0.001; abnl 17p: 11.3% vs. 4.2%, P=0.003; other monosomies: 16.3% vs. 5%, P<0.001). Despite these differences, no significant differences in CR rates, OS and EFS were found between both groups. This finding was confirmed in a multivariate analysis including cytogenetics, molecular markers and clinical parameters (LDH, WBC, blast count, platelet count and ECOG). According to the analysis, AEL morphology was not an independent prognostic factor for OS and EFS. Within the AEL group, patients with monosomy 7 (n=10), complex aberrant karyotype (n=22) and abnl 17p had a shorter median OS (5.7, 5.2, 4.3 months) compared to patients with AEL and not such aberrancies (20.4, 22.3, 18.9 months) (P=0.001, <0.001, 0.015), respectively. A complex aberrant karyotype was found more often in patients with secondary AEL than in patients with *de novo* AEL (P=0.024).
Summary / Conclusion: According to our data, the characteristic morphological features of acute erythroleukemia do not confer an unfavorable prognosis in itself. Compared to non-AEL AML, cytogenetic aberrations associated with poor prognosis are found more often in patients with AEL, but it confers only within the AEL group to a significant worse OS. NPM1 and FLT3-ITD mutations were much less common in patients with AEL.

P053

ELACYTARABINE / IDARUBICIN IN PATIENTS WITH ACUTE MYELOID LEUKEMIA WHO FAILED CYTARABINE BASED INDUCTION, AND EVALUATION OF THE IMPACT OF THE TRANSPORTER HENT1 ON RESPONSE; RESULTS OF A PHASE II STUDY
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Background: Elacytarabine is a novel, patented anti-cancer drug in development (phase III) for treatment of acute myeloid leukemia (AML). Elacytarabine has demonstrated longer plasma elimination half-life, prolonged intracellular retention compared to cytarabine and activity independence of membrane nucleoside transporters. The human equilibrative nucleoside transporter 1 (hENT1) in particular has been reported as an important modulator of resistance to cytarabine (Hubeek *et al*., 2005). A phase I study established elacytarabine 1000 mg/m^2/d continuous infusion (CIV) d 1 – 5 as the recommended dose given in combination with idarubicin 12 mg/m^2/d IV d 1-3 (Giles *et al*, 2012).
Aims: To determine the efficacy and safety of elacytarabine in combination with idarubicin in adult patients with AML who did not respond to a first cytarabine-anthracycline remission induction course. To explore the relationship between the hENT1 status in AML cells and response to elacytarabine and to cytarabine.
Methods: A bone marrow (BM) sample was collected at diagnosis and analysed by immunocytochemistry (ICC) for hENT1 protein expression. Patients who had not attained blast clearance after the first remission induction course, entered the study and received elacytarabine + idarubicin at doses/schedules as described above. The hENT1 expression level in BM was again analysed before elacytarabine+idarubicin treatment. Safety and efficacy were assessed. Patients are followed for duration of response and survival for at least 12 months.
Results: The study recruited 51 patients [28 male, 23 female, median age 61 years (range 18-78), ECOG PS 0-2]. 35% of the patients were diagnosed with secondary leukaemia. The median time from start of cytarabine induction to study entry was 37 days (range 18-78). After treatment with elacytarabine and idarubicin the overall response rate (ORR) is 45%, with 21 out of 47 evaluable patients in CR or CRi. Median time to remission was 41.5 days (range 21 to 169). The incidence of low-level hENT1 protein expression both at time of initial AML diagnosis as well as at study entry was approximately 45%. The response to elacytarabine was similar in both groups with 10 out of 23 patients responding in the hENT1 high expression group and 8 out of 19 patients in the hENT1 low expression group. The response to cytarabine was lower for the hENT1 low expression group (9 out of 18 patients) compared to the hENT1 high expression group (15 out of 22 patients). The most frequently reported non haematological adverse events, CTCAE grade ≥3, were infections/sepsis, hypokalemia, hypoalbuminaemia, fatigue and hypoxia. Five patients died within 30 days after start of treatment, all due to sepsis/infections. Four patients died within 60 days after start of treatment either due to progressive disease (three patients) or complications after stem cell transplantation (one patient). Seven of nine patients who died early were initially diagnosed with secondary leukaemia.
Summary / Conclusion: Elacytarabine in combination with idarubicin shows promising clinical activity with a CR/CRi rate of approximately 45% in patients in whom an initial and single cytarabine based induction course has failed. As hypothesized, the hENT1 expression level demonstrated no impact on the efficacy of elacytarabine while it influenced response to cytarabine. The safety profile is manageable and is as expected for a combination cytotoxic therapy. Updated event free survival and survival data will be presented.

P054

RISK STRATIFICATION OF 444 PATIENTS WITH DE NOVO NON-M3 ACUTE MYELOID LEUKEMIA BY INTEGRATING MOLECULAR GENE MUTATIONS WITH CYTOGENETICS
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Background: Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy with great variability in the pathogenesis, clinical features and treatment outcomes. The goal of risk stratification is to explore personalized therapy, thereby reduce the risk of relapse and treatment-related side effects.
Aims: Extensive mutational analysis can better discriminate AML patients into various prognostic groups.
Methods: We investigated a comprehensive analysis of cytogenetic change and 17 molecular alterations, includingClass I mutations, such as *FLT3*-ITD and *FLT3*-TKD*, N-RAS, K-RAS, JAK2, KIT and PTPN11* mutations and Class II mutations, such as *MLL*-PTD*, CEBPA* and *AML1/RUNX1* mutations, as well as *NPM1, WT1,* and epigenetic alterations, such as *ASXL1, IDH1, IDH2,TET2* and *DNMT3A* mutationsin a large cohort of 444 *de novo* non-M3 AML patients in Taiwan.
Results: Genetic alterationsoccurred more frequently in patients with intermediate-risk cytogenetics (92.1%) than in those withfavorablekaryotype (68.9%) or unfavorable cytogenetics (50.8%, P<0.0001).We found that mutations of *AML1/RUNX1, WT1, ASXL1* and *DNMT3A* were associated with poorer overall survival (OS) while *NPM1*$^+$/*FLT3*-ITD$^-$, *CEBPA*$^{double\text{-}mutation}$ and *IDH2* mutation predicted better survival. Further refinement of intermediate-risk cytogenetic group can be achieved through molecular screening of these eight relevant genes. Patients with mutant *NPM1, IDH2* and *CEBPA*$^{double\text{-}mutation}$ in absence of *FLT3*-ITD belong to a favorable molecular genotype, an unfavorable molecular genotype including mutant *AML1/RUNX1, WT1, ASXL1*, and *DNMT3A* while the remaining genotypes was part of intermediate genotype. The probability of achieving a CR for favorable-risk, intermediate-risk and unfavorable-risk profile was 90.2%, 76.9% and 64.1%, respectively (P<0.001). The relapse rate for the three risk groups was 40.6%, 63.3% and 67.1%, respectively (P=0.0006). The median OS for favorable-risk, intermediate-risk and unfavorable-risk profile was not reached, 25 months and 12.3 months, respectively and five-year survival rate was 59.6%, 38.6% and 18.2% in these three risk groups (both P<0.001). Intriguingly, allogeneic HSCT may improve OS among patients with unfavorable molecular genotype.
Summary / Conclusion: These findings suggest the proposed risk stratification using integration of both cytogenetic and mutational profiles can further discriminate patients into different prognostic groups, especially in those with intermediate-risk cytogenetics. Further, allogeneic HSCT may provide survival benefits for patients with unfavourable mutational profile.

P055

ACUTE MYELOID LEUKEMIA (AML) PATIENTS HARBOURING BOTH, FLT3-ITD AND NPM1 MUTATION, MAY BENEFIT FROM A MINIMAL RESIDUAL DISEASES (MRD) GUIDED TRANSPLANT DECISION
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Background: Minimal residual disease (MRD) monitoring in patients with acute myeloid leukemia (AML) can predict relapse clearly in advance and therefore allows early therapeutic intervention. Moreover, recent studies have highlighted the significance of personalized treatment on the basis of MRD status for improving outcome in AML.
Aims: The *FLT3* internal tandem duplication (*FLT3*-ITD) occures in 15-35% of all AML. Clinically, *FLT3*-ITDs have been strongly associated with poor outcome. However, due to the high sequence variability of individual *FLT3*-ITD commonly a universal PCR approach is applied which has a low sensitivity (approx. 1: 5x10^2).
Methods: To develop a novel cDNA-based, highly sensitive quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) assay for the detection of the *FLT3*-ITD mutation level. On the basis of invidual *FLT3*-ITD, mutation-specific primers were designed. The expression of *FLT3*-ITD was determined using complementary DNA samples at different points in time diagnosis and subsequent treatment.
Results: From a total of 394 newly diagnosed AML patients 55 (14%) were *FLT3*/ITD positive. Retrospectively we analyzed ITD mutation of *FLT3* in 39 available cases. The length of ITD ranged from 3 to 144 base pairs (median

46). Nine patients had extra insertions of 2-38 base pairs between two repeats. For the *FLT3*-ITD quantification we developed patient-specific qRT-PCR for 29 individuals with mutation-specific forward primers and studied 83 peripheral blood and 61 bone marrow samples. Three cases with *WT1*, fifteen with *NPM1*, three with *MLL*-PTD and five with *PML*/*RARα* fusion genes expression levels were compared with *FLT3*-ITD expression levels. 26 of 29 assays (90%) were highly specific ($1:10^4$ - $1:10^5$) and yielded similar results when compared to other high sensitive assays for molecular markers like *NPM1* or *PML*/*RARα*. In three cases (10%) a co-amplification of the wild-type could not be avoided resulting in lower sensitivity ($1:10^3$). We could show that *FLT3*-ITD positivity reliably predicted relapse up to 10 months in advance. 92% patients, who achieved *FLT3*-ITD negativity with our assay, did not relapse. Furthermore we compared paired PB and BM samples at diagnosis and after induction therapy in 5 cases. The difference in *FLT3*-ITD expression were not statistically significant (P=0.8) which is in line with recent studies.

Summary / Conclusion: We conclude that highly sensitive detection of individual *FLT3*-ITD posses equal prognostic power in AML like established molecular MRD markers. Using this approach MRD guided treatment decisions appear to be justified and should be incorporated in future studies.

P056

PATTERNS OF RELAPSE IN 169 PATIENTS TREATED WITH ORAL ARSENIC TRIOXIDE DURING INDUCTION AND/OR MAINTENANCE: A 12-YEAR EXPERIENCE

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Background: Despite excellent response rates and long-term survivals with current treatment strategies, multiple relapses and central nervous system (CNS) disease remain major causes of treatment failure and mortality in patients with acute promyelocytic leukaemia (APL).

Aims: In this study, 169 patients with APL treated with oral arsenic trioxide (As_2O_3) during maintenance at first complete remission (CR1), or as re-induction and subsequent maintenance, were prospectively investigated. We aimed at describing different patterns of relapse, identifying risk factors for relapse and CNS disease, and prognostic factors for survival.

Methods: This was a 12-year prospective follow-up study of 169 patients treated with oral arsenic trioxide during induction and/or maintenance. Clinicopathologic features, treatment characteristics and outcome of relapsed APL were reviewed. Prognostic factors for relapse and CNS involvement were analyzed using logistic regression. Survivals were analyzed using Kaplan-Meier method and Cox-proportional hazard regression.

Results: ***Risk factors for relapse***. A total of 79 patients had one or more relapses, including 14 patients with CNS involvement. Maintenance regimens without arsenic trioxide at CR1 and high peak white blood cell (WBC) count were independently associated with increased risk of relapse (P<0.001 and P=0.008 respectively). By Cox-proportional hazard regression, patients who received maintenance regimens without arsenic trioxide had significantly inferior relapse-free survival (hazard ratio=12.97, P<0.001, 95% confidence interval:6.61-12.05).

Patterns of relapse. Three different patterns of relapse were compared. There were 65 patients with isolated medullary relapse, 8 patients with concurrent bone marrow and CNS involvement at relapse, and 6 patients with isolated CNS relapse. Risk factors associated with isolated CNS relapses included high WBC count on presentation (P=0.007); relapses beyond second complete remission (P=0.003); and relapse while on maintenance regimens containing oral arsenic trioxide (P=0.001). Risk factor for concurrent medullary and CNS relapse was relapses beyond second complete remission (P=0.003). Patients relapsing while on oral arsenic trioxide maintenance have more frequent Fms-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) at the time of relapse (odds ratio=4.61, P=0.03, 95% confidence interval:1.13-18.92). Isolated positive molecular testing for *PML-RARA* fusion gene during oral arsenic trioxide maintenance predicted a subsequent morphologic relapse (odds ratio=6.18, P=0.024, 95% confidence interval:1.27-29.94).

Survivals. The 5-year overall survival of our cohort of patients was 85.9%. Three factors were associated with significantly inferior survivals: relapse while on oral arsenic trioxide (hazard ratio=9.43, P<0.001, 95% confidence interval:4.01-21.32); relapses beyond third complete remission (hazard ratio=16.45, P<0.001, 95% confidence interval:4.49-60.34); and concurrent bone marrow and CNS relapse (hazard ratio= 22.84, P<0.001, 95% confidence interval:5.88 - 88.74).

Summary / Conclusion: Advanced relapses, high WBC count and relapse while on oral arsenic trioxide were associated with a higher risk of CNS involvement and in turn inferior overall survivals. Identification of such risk factors is important in formulating effective treatment and prophylactic protocols for high-risk APL patients.

P057

SURVIVAL IMPACT OF COMPLEX AND MONOSOMAL KARYOTYPE AND LOSS OF CHROMOSOMES5, 7 AND 17 IN OLDER PATIENTS WITH AML. FIRST EVALUATION OF CYTOGENETIC DATA FROM THE POPULATION BASED SWEDISH AML REGISTRY

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Background: The incidence and impact of chromosomal abnormalities (abns) in AML have mostly been evaluated in younger and/or highly selected patients (pts) treated within clinical trials, whereas data from large population-based studies are lacking. The Swedish National Acute Leukemia Registry contains data on 98% of all Swedish AML pts diagnosed 1997-2006 (Blood 2009;113:4179). During this period, karyotyping was recommended at diagnosis for all pts eligible for intensive treatment, but molecular analyses were not routinely performed. We have now retrospectively added cytogenetic data from the original medical reports to the database.

Aims: We analyzed the impact on survival of the number of chromosomal abns, those involving 5q-/-5, 7q-/-7, and/or 17p-/-17, and monosomal karyotype in pts treated with intensive chemotherapy. Pts with APL were excluded in this analysis. Survival was updated in May 2012. Data was analyzed by the R-program software (http://www.r-project.org), and p-values were computed from a Cox proportional hazards model.

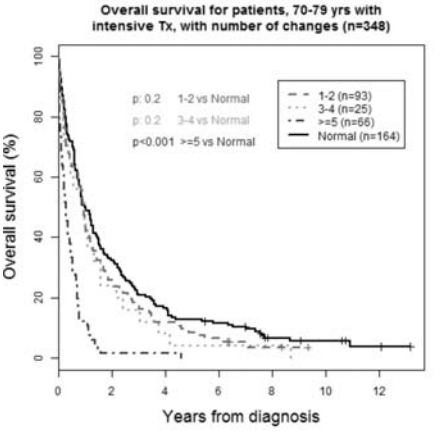

Figure 1.

Results: Evaluable karyotypes were found in 1904/3293 registered patients, i.e., in 150 (78%) of patients <40 years, 492 (78%) in ages 40-60 yrs, 467 (71%) between 60-69 yrs, 591 (58%) in patients 70-79 yrs, and 199 (25%) of patients over the age of 80. Karyotypes were missing due to several reasons, e.g., not performed, not indicated, report not available. Pats with ≥5 chromosomal abns had significantly worse overall survival than those with 3-4 vs 1-2 abns or normal karyotype in all age groups (Figure 1), whereas the difference between 3-4 vs fewer abns were less. Monosomal karyotype did not separate survival curves within the groups with 3-4 vs≥5 abns in any age group; e.g., 2 yrs OS with 3-4 abn:s: MK+ 48% vs MK- 44%; and with ≥5 abn:s MK+15% vs MK-, 16%, (P=NS). Abns involving 5/7/17 were strongly correlated and often occurred together in monosomal karyotypes; pats with all three of those abns had very poor outcome.

Summary / Conclusion: We now have population-based data on cytogenetics in the largest age cohort of AML, i.e., 60-79 years. There is a significant overlap between complex karyotypes, and abns involving chromosomes 5/7/17, and monosomal karyotype, in all age cohorts. Five or more chromosomal abns were clearly worse than 3-4 abns, especially with combined chromosome abns involving 5/7/17.

P058

PATIENTS WITH LATE RELAPSE OF ACUTE MYELOID LEUKEMIA HAVE A HIGH RATE OF DURABLE SECOND COMPLETE REMISSIONS WHEN TREATED WITH CHEMOTHERAPY ONLY

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Background: Complete remission (CR) rates are high among patient with acute myeloid leukemia (AML) but long-term survivors are few, because eventually more than half of the patients relapse. A relapse usually occurs within two years of achieving CR, while late relapses, defined as a recurrence of AML after more than five years from achieving CR, are considered rare events. The prognosis is generally poor when there is a relapse. However, the remission rate is higher among patients with a long duration of the first complete remission (>18 months) and with favorable cytogenetics at diagnosis. Allogeneic stem cell transplantation (SCT) is often recommended to sustain the second CR although a number of patients, particularly those with favorable cytogenetics, can achieve durable second remissions when treated with chemotherapy alone.
Aims: The aim was to assess the number of patients with late relapse in a population-based cohort of long term AML survivors and to collect detailed information on clinical and laboratory features.
Methods: All patients diagnosed with AML in the Stockholm, Uppsala, and Örebro regions between January 1st 1973 and December 31st 2003 were identified in the Swedish Cancer Registry. Patients who died within five years of their AML diagnosis were identified through the Cause of Death Registry and excluded from the study. Thus, only patients surviving five years or more were included. In 228 long-term survivors clinical information was available. Detailed information on laboratory variables including cytogenetics and immunophenotype at diagnosis and relapse, treatment (type of chemotherapy regimen, number of courses, SCT), myelodysplastic syndrome (MDS) preceding the relapse and cause of death was collected from medical records.
Results: Among 228 patients surviving for at least 5 years after their AML diagnosis, 33 patients had a recurrence of their disease 36 months or more after first CR. Results of cytogenetic analysis at diagnosis were available in 23 patients (70%); thirteen had a normal karyotype, three patients had unfavorable karyotypes while there were no patients with core binding factor (CBF) leukemia. Cytogenetic analyses were available in 13 patients (39%) at relapse; five patients had a normal karyotype. In 11 patients cytogenetic information was available at both diagnosis and relapse, with a change in karyotype occurring in four patients. Seven patients were diagnosed with MDS (n=6) or chronic myelomonocytic leukemi (CMML; n=1) preceding their AML relapse. In two of these patients, MDS related karyotypes were identified at relapse but not at diagnosis. Results of treatment will be presented here in detail only for the group of patients who relapsed five years or more after AML diagnosis because the inclusion criteria excluded patients relapsing prior to this date if they did not survive at least two years from their relapse. There were 23 patients (10%) out of 228 patients with a relapse after more than five years of achieving first CR (median age at relapse 66 years; range 32-88). Seventeen were treated with intensive chemotherapy and 12 (71%) achieved a second CR. Intensively treated patients had a median survival of 3.8 years (Figure 1). Six patients are alive (one underwent allogeneic SCT) with a median follow-up time after relapse of 7 years. The cause of death was AML in 18 patients, one patient died from graft versus host disease, and one patient died of pneumonia while in MDS phase. In three patients the cause of death was not associated with AML and in the remaining the cause was unknown.
Summary / Conclusion: Late relapse of AML is probably more common than we like to think. However, in the event of a late relapse the outcome of treatment is favorable, also among elderly patients, unless MDS features are present. Therefore, this group of patients may not necessarily need SCT to remain in a second CR.

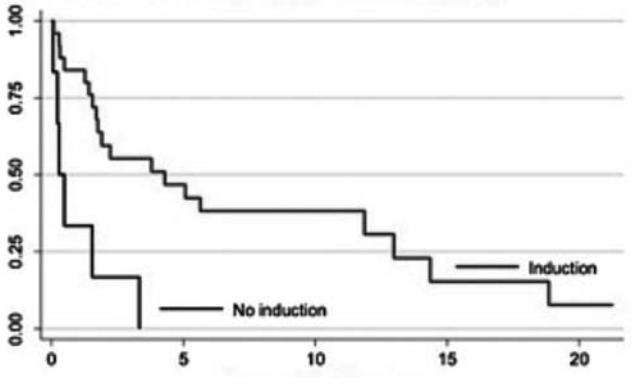

Figure 1. Overall survival (years) among patients with first five or more years after diagnosis, stratified by treatment.

Acute myeloid leukemia - Clinical 2

P059

SURVIVAL IN PATIENTS WITH ACUTE MYELOBLASTIC LEUKEMIA IN GERMANY AND THE UNITED STATES: UNEXPLAINED DIFFERENCES IN SURVIVAL IN YOUNG ADULTS

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Background: The prognosis for patients with acute myeloblastic leukemia (AML) varies by age, with older patients usually showing poorer prognosis. However, previous population based studies in the United States (US), found lower survival in young adults compared to middle-aged adults, with adults age 15-24 having a lower survival than adults age 25-34 at 47.2% and 53.5%, respectively, in 2001-2005 (Pulte D, Gondos A, Brenner H. Expected long-term survival of patients diagnosed with acute myeloblastic leukemia during 2006-2010. Ann Oncol. 2010;21(2):335-4). Here, we compared survival for patients with AML in the US with patients in Germany. If the unexpectedly low survival in young adults in the US is due to biological factors, a similar pattern is likely to be observed in Germany. If, however, the pattern is due to socioeconomic issues such as poverty, compliance, or insurance a consistent decrease with age, rather than a deep decrease in early adulthood with a better survival in middle age, is more likely to be observed in Germany.
Aims: To determine survival of patients with AML diagnosed in Germany in the early 21st century and compare these results to patients diagnosed in the same time period in the US
Methods: Data were extracted from the Surveillance, Epidemiology, and End Results database in the US and 11 cancer registries in Germany. Patients diagnosed with AML and age <65 were included in the analysis. Cases reported to the cancer registry by death certificate only were excluded. Period analysis was used to estimate 5-year relative survival (RS) for the period 2002-06. Because acute promyelocytic leukemia (APL) has a better prognosis and different treatment options compared with other forms of AML, an analysis excluding APL was also performed, to rule out the possibility that differences observed were due to differences in the frequency of APL.
Results: Overall, age-adjusted RS for patients age 15-64 was higher in Germany at 40.4% than in the US at 32.8% (Table 1). Estimates of 5-year RS in Germany were higher than in the US for each age group, with differences ranging from +16.3 percentage points (age 18-24) to +5.4 percentage points (ages 35-44 and 60-64). In Germany, survival decreased with age, with the highest RS observed for patients age 18-24 at 60.2%, compared with 43.9% for patients of the same age in the US. In contrast, in the US the highest survival was seen in patients aged 35-44 at 50.4%. When APL was excluded from the analysis, survival decreased for both countries, but the differential in survival increased, with overall RS for Germany and the US being 39.0% and 27.9%, respectively.
Summary / Conclusion: Five year relative survival for younger and middle-aged patients with AML is higher in Germany than in the US. Survival for patients with AML in the US is lower in young adults than in middle-aged adults. This pattern is not observed in Germany such that there is an especially large disparity for ages 18-24.

Table 1.

	Germany			US				
Age	N	5-year RS	SE	N	5-year RS	SE	Diff	P-value (Model)
18-24	134	60.2	5.8	364	43.9	3.8	+16.3	0.0309
15-24	193	58.2	4.8	507	45.0	3.1	+13.2	0.0145
25-34	261	56.0	4.3	652	49.5	2.8	+6.5	0.1073
35-44	546	55.4	3.0	908	50.4	2.5	+5.4	0.1537
45-54	642	47.1	2.7	1410	34.0	1.9	+13.1	<.0001
55-59	526	30.3	2.9	897	24.3	2.2	+6	0.0936
60-64	834	22.9	2.1	950	17.5	1.9	+5.4	0.0081
Overall*	3002	40.4	1.2	5324	32.8	0.9	+7.6	<.0001

P060

TREATMENT WITH FLAG-IDA OR FLAGO-IDA REGIMEN IN ADULT PATIENTS WITH RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA. RETROSPECTIVE ANALYSIS OF THE PETHEMA AML REGISTRY

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Background: Patients with acute myeloid leukemia (AML) who fail to achieve a complete remission (CR) after the first cycle of induction therapy and those with relapsed disease have a bleak prognosis. The FLAG-IDA regimen (fludarabine plus idarubicine and cytarabine) has been frequently used to achieve a second CR in refractory/relapsed patients and may be a bridge towards stem cell transplant (SCT). In an attempt to improve the CR rates in relapsed/refractory patients, the Programa Español de Tratamientos en Hematología (PETHEMA) group recommended between 2007 and 2010 the addition of gentuzumab ozogamizin (GO) to the FLAG-IDA regimen (FLAGO-IDA).
Aims: To analyse the results (short- and long-term outcomes) of salvage therapy with FLAG-IDA or FLAGO-IDA regimen in a large series of patients with relapsed/refractory AML treated with first-line PETHEMA LMA protocols.
Methods: This retrospective and multicenter study was performed in patients treated with first-line PETHEMA LMA protocols (LMA99, LMA2007, and LMA2010) consisting of 3+7 regimens (daunorubicin or idarubicin plus cytarabine) followed by consolidation with anthracycline/cytarabine combination with or without SCT in first CR. In patients who achieved a partial remission (PR) after first induction cycle an identical second cycle of 3+7 was recommended in the PETHEMA LMA99 and LMA2010 protocols, while FLAG-IDA or FLAGO-IDA administration was recommended in the LMA2007 protocol. In all protocols, FLAG-IDA or FLAGO-IDA was routinely administered in patients showing absolute resistance after first cycle or relapse. The inclusion of GO to second-line induction therapy depended on the resources of each centre. We perform a univariate analysis to establish the factors associated to CR rates and overall survival (OS) estimates using the Kaplan-Meir method. A multivariate analysis for OS risk factors is also reported.
Results: Between May 1999 and January 2012, 157 patients with refractory/relapsed AML who received second line therapy with FLAG-IDA or FLAGO-IDA were identified in the PETHEMA AML registry (82 males, 75 females, median age 50 years [range 14-76], 40 patients older than 60 years). Study protocol distribution was: 48 patients in the LMA99, 89 in the LMA2007, 20 in the LMA2010. FLAGO-IDA/FLAG-IDA was administered in 85 refractory patients and in 72 relapsed patients; 122 patients received FLAG-IDA and 35 patients received FLAGO-IDA. FLT3-ITD was present in 21% of patients, favourable karyotype in 12 patients (9%), intermediate in 86 (62%), unfavourable in 40 (29%), and no available results in 19 patients (12%). The median follow-up of the patients still alive was 25 months. The median OS was 16.5 months (21% OS at 5 years). The CR rate was 46% in relapsed patients (33 out 72) and 34% in refractory/PR patients (30 out 85). Stem-cell transplantation (SCT) was performed in 43 patients (39 allogenic, 4 autologous). The univariate analyses showe significant differences between patients relapsed after 12 months, before 12 months, and primary refractory patients (median OS: 104, 13.8, and 9.3 months, respectively; P<0.0001), cytogenetic risk (median OS: favourable 34.7, intermediate 19.2, and unfavourable 7.8 months; P=0.0002), FLT3-ITD vs. no FLT3-ITD (median OS: 9 vs. 22.3; P=0.004), and SCT vs. no SCT (median OS: 45.8 vs. 11.4 months; P<0.0001). No significant differences were observed between FLAGO-IDA and FLAG-IDA (median survival 11.3 vs. 18.7 months; P=0.26). Age >60 years was almost significant associated with lower OS (median 10 vs. 19, P=0.052). In multivariate analyses unfavourable karyotype, no SCT, FLT3-ITD and primary refractory/early relapse were associated with lower OS.
Summary / Conclusion: FLAG-IDA or FLAGO-IDA may induce a CR2 in an acceptable percentage of patients with relapsed/refractory AML We could identify several risk factors predicting worse OS after second-line therapy (unfavourable karyotype, FLT3-ITD, refractory disease/early relapse). Patients with favourable genetic and molecular characteristics presenting late relapse (>12 months after CR1) have a good prognosis after salvage therapy with FLAGO-IDA or FLAG-IDA, especially when CR is followed by an allo-SCT. Alternative salvage strategies to improve outcomes could be recommended in the remaining subsets.

P061

LOW- DOSE CHEMOTHERAPY AND DIFFERENTIATING AGENTS AS POST- REMISSION MAINTENANCE PROLONGED DISEASE- FREE AND OVERALL SURVIVAL IN A CASE-CONTROL RETROSPECTIVE STUDY ON POOR PROGNOSIS AML/MDS PATIENTS

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Background: Patients aged over 60 or with poor prognosis AML or MDS have an unfavourable outcome despite of the achievement of a complete response (CR) with aggressive treatments. In fact, only about 10% of these patients become long-term survivors. It is therefore evident that standard post-remission chemotherapy should be integrated by somewhat different strategies to improve the outcome of this group of patients. Indeed, some studies have already shown an advantage in relapse free survival with low dose cytarabine based maintenance treatment (Büchner *et al* 2003, Krug *et al* 2010).
Aims: To evaluate the impact of a maintenance therapy with low-dose chemotherapy plus differentiating agents on survival and remission duration in a cohort of poor prognosis AML/MDS patients in CR after standard induction chemotherapy.
Methods: We included 92 patients treated from 1997 to 2012. AML patients (82) had poor prognosis for either age over 60, and/or therapy-related AML, AML secondary to MDS, or second CR. MDS patients (10) were at intermediate 2/high risk according to the IPSS.
All patients have been in stable CR for at least 2 months after induction +/- consolidation therapy and were ineligible to allogeneic stem cell transplantation at CR achieving. Forty-two patients received a maintenance therapy and were compared to a matched historic population of 51 patients that had not received further therapy after consolidation. Maintenance treatment consisted on two alternated schedules: the first one included 6-thioguanine 40 mg daily for 3 weeks and 13-cis retinoic acid 40 mg daily + (OH)2 vitamin D3 (D3) 1 ug daily for 5 weeks; the second one contained cytarabine 8 mg/mq bis daily by subcutaneous injection for 2 weeks and all-trans retinoic acid 30 mg bis daily + D3 0,5 ug bis daily for 5 weeks. This therapy was started after a median time of 3 months from CR achievement and was continued for 4 years or until relapse.

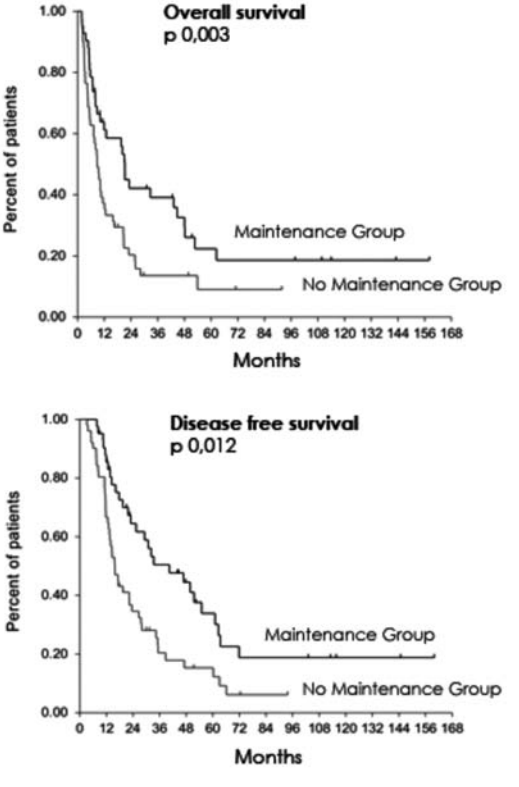

Figure 1.

Results: The two groups of patients were balanced with regard to both patient and disease characteristics, in particular median age (63 years) and prevalence of unfavourable karyotype (24% versus 28%). Relapse incidence (RI) was significantly lower in the maintenance group than in the control group: 53,6% versus 83,3% at 3 years and 67,5% versus 88,5% at 5 years, respectively (P

0,004). Disease free survival (DFS) too was better in the maintenance group with a median of 21 months (ms) vs 9 ms in the control group (P 0,012). The reduction in the relapse rate led to an overall survival (OS) advantage too, with a median OS of 40 ms and survival rates at 3 and 5 years of 51.4% and 34.5%, respectively, in the maintenance group compared to a median OS of 16 ms and 21.4% and 12.8% survival at 3 and 5 years, respectively, in the control group (P 0,003). The presence of unfavourable karyotype negatively affected OS, but in multivariate analysis maintenance therapy remained an independent outcome predictor.

Summary / Conclusion: Our maintenance therapy significantly prolonged patients survival by reducing the relapse incidence. The treatment schedule was safe and well tolerated. Therefore, our results suggest that a different strategy of post-remission therapy based on the association of differentiating agents and low dose chemotherapy might improve the outcome of poor risk AML/MDS patients. These data, however, need to be confirmed in a larger prospective study.

P062

A STUDY OF ELACYTARABINE IN RELAPSED OR REFRACTORY AML EVALUATING CARDIAC SAFETY AND PHARMACOKINETIC PROPERTIES

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Background: Elacytarabine, an elaidic acid ester derivative of cytarabine, has pharmacokinetic (PK) and pharmacodynamic properties that may lead to improved clinical outcome (Adema *et al* 2011) compared to cytarabine (ara-C). Elacytarabine is metabolized to active cytosine arabinoside 5`-triphosphate (ara-CTP) and inactive ara-U. The compound has reached phase III of clinical development for the treatment of patients with relapsed or refractory acute myeloid leukaemia (AML).

Aims: The on-going study was designed to evaluate the cardiac safety and PK of elacytarabine. In addition the clinical efficacy and safety were evaluated.

Methods: Elacytarabine for infusion 7.5 mg/mL was administered at 2000 mg/m^2/day as a continuous intravenous infusion (CIV) for 120h to adult patients with relapsed or refractory AML. Blood samples for PK analyses were collected and electrocardiography (ECG) was assessed pre-treatment, during treatment and after treatment at specified time-points. The PK was characterised by compartmental as well as non-compartmental analyses. The ECG was centrally monitored. Safety and efficacy were assessed. Patients are followed for relapse and survival for at least six months.

Results: Forty-three patients [26 males, 17 females, median age 64 years (range 18-77), ECOG PS 0-1] were treated with elacytarabine. All patients had previously been treated with ara-C, and had relapsed or were refractory following previous treatment. Of these 43, 23 had blood sampled using a rich-sampling protocol for PK characterization and 30 had ECGs taken with matching blood sample collection for determination of plasma concentration of elacytarabine and its metabolites. Thirty-six patients were evaluated for response and the overall response rate was 42% with nine patients achieving a complete remission (CR) and six patients a complete remission but with incomplete blood count recovery (CRi) following study treatment. The most frequently reported non-haematological adverse events (CTCAE grade ≥3) were infections, febrile neutropenia, hyponatremia, hypokalaemia and increased alanine aminotransferase. The ECG assessments showed that 12 of 30 patients had a change in the QTcF interval from baseline to peak by >30 msec, in addition three patients had a change in the QTcF interval from baseline to peak by >60 msec. This increase seemed to be unrelated to the plasma concentrations of elacytarabine, ara-C and ara-U and there were no concomitant torsades de pointes. No patient had a peak QTcF above 500 msec. The most frequently reported cardiac adverse events was tachycardia (six patients). One patient experienced a grade 3event of; all others were grade 1 or 2. The plasma concentrations of elacytarabine and ara-C declined rapidly once the infusion was stopped. Using non-compartmental analysis, the median terminal half-life and total clearance for elacytarabine were 12.8 h and 4.6 L/h/m^2. For ara-C and ara-U the median terminal half-lives were 2.6 and 8.9 h, respectively.

Summary / Conclusion: Elacytarabine is a novel anti-leukemic agent in development for treatment of patients with advanced AML. Its efficacy, tolerability, safety and PK characteristics make it a promising single agent treatment for patients with relapsed or refractory AML. Finale results will be presented at the meeting.

P063

MULTI-LINEAGE DYSPLASIA AS ASSESSED BY IMMUNO-PHENOTYPE HAS NO IMPACT ON OUTCOME OF ACUTE MYELOID LEUKEMIA WITH MUTATED NUCLEOPHOSMIN (NPM1)

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Background: World Health Organization (WHO) classification of myeloid neoplasms considers AML with multi-lineage dysplasia (MLD) as a separate subset. Morphologic assessment of residual hemopoiesis at AML diagnosis is the standard criteria for defining MLD. The prognostic value of MLD is still under debate due to technical and biologic reasons. Technical reasons include: i) residual non-blast cells are few at AML diagnosis; ii) morphology is an operator-dependant technique. Moreover, MLD-related poor prognosis is supposed to rely on neoplastic progression: MLD would imply a pre-existing myelodysplastic syndrome (MDS). On the other hand, MLD might merely be the result of pathologic differentiation/maturation by the leukemic clone. A major controversy concerns the significance of MLD in NPM1-mutated (NPM1+) AML. This issue has major implications since NPM1+ status correlates with a relatively good prognosis (especially when FLT3-wt). Falini *et al* (Blood 2010) showed that morphologic MLD has no impact on biologic and prognostic features of NPM1+ AML.

Aims: To investigate the prognostic significance of MLD in NPM1+ AML by a technique alternative to morphology. Flow cytometry (FC) is emerging as a useful method to study dysplasia, mainly by investigating the expression of key antigens along with maturation. The application of FC to maturing cell compartments at AML diagnosis can provide some advantages compared to morphology: i) the amount of studied cells is much larger; ii) phenotypic parameters can be quantified and referred to controls and thus reliably standardized.

Methods: Patients: 70 pts with NPM1+ AML were included and characterized according to standard criteria (morphology, karyotype, molecular genetics). Flow cytometry: FACSCanto II (BD) and Infinicyt software (Cytognos) were used for data acquisition and analysis, respectively. Some major bone marrow compartments (blast cells; maturing granulocytic, monocytic and erythroid compartments) were identified on the basis of light scatter and reactivity for CD45 and CD34. We adapted to AML an approach previously described for MDS (Matarraz *et al*, Leukemia 2008): MLD was appraised through an immuno-phenotypic score (IPS) including 18 parameters (14 for granulocytic and 4 for erythroid lines).

Results: MLD was analyzed in 70 intensively treated NPM1+ AML cases. Median age was 57 (24-70). Median white blood count (WBC) was 48.0 x 10^9/L (1.2-260.0). Karyotype was normal in 62 (88.6%) pts. FLT3-ITD occurred in 27 pts (38.6%). MLD was assessable by morphology in 66 cases; according to WHO criteria, 24 (36.4%) showed MLD. IPS was calculated in all 70 pts. Median IPS value was 6.25 (range 0.5-18.5). Pts were categorised according to IPS higher (IPS+) or lower-equal (IPS-) than the median. Age, WBC and incidence of morphologic MLD were not different in IPS groups. IPS+ group showed lower incidence of FLT3-ITD (25.7% vs 51.4%; P=0.048); interestingly, Falini *et al* (Blood 2010) have reported analogue results with morphologic analysis. CR rate was not different in IPS- (82.9%) and IPS+ (74.3%; P=0.56) groups. IPS did not affect disease-free survival and overall survival, as displayed in Figure 1 A-B. FLT3 status confirmed its prognostic value in our cohort (Figure 1 C-D).

Summary / Conclusion: This study provides evidence that MLD, as assessed by flow cytometry, does not influence clinical characteristics and outcome of NPM1+ AML. These findings further support NPM1+ AML to be considered as a separate entity and its prognostic assessment should not be based upon MLD.

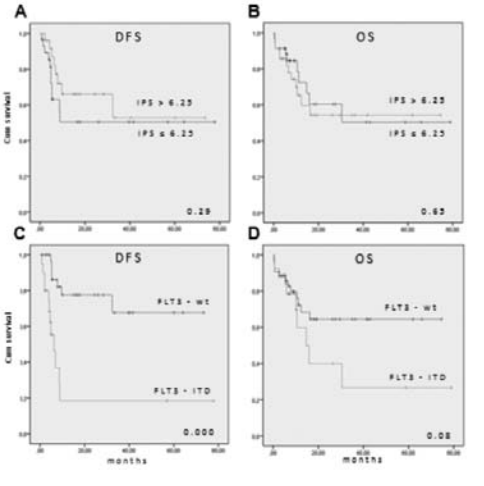

Figure 1.

P064

COMPREHENSIVE GENE MUTATION ANALYSES IN ACUTE MYELOID LEUKEMIA: OVERLAP OF THE GENE MUTATIONS ARE IMPORTANT FOR THE PROGNOSIS OF THE INTERMEDIATE RISK KARYOTYPE GROUP

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Background: Molecular abnormalities in acute myeloid leukemia (AML) have been revealed to be important.
Aims: To clarify their importance, we analyzed *de novo* AML patients with the intermediate risk karyotype for molecular abnormalities in *C/EBPα, NPM1, MLL-PTD, FLT3, N/K-RAS, DNMT3a, IDH1/2* and *TET2* genes.
Methods: We analyzed samples from 142 AML patients with the intermediate risk karyotype diagnosed at Nippon Medical School Hospital from 2000 to 2012. Bone marrow or peripheral blood samples containing 20% or more blast cells were used for analyses. Mutation analyses were performed using PCR amplification for *FLT3*-ITD and *MLL*-PTD, and direct sequence for *NPM1*, *C/EBPα*, *DNMT3a*, *IDH1/2*, *TET2 and N/K-RAS*.
Results: The *NPM1, DNMT3a, FLT3*-ITD*, IDH1/2, TET2, C/EBPα, N/K-RAS* and *MLL*-PTD mutations were detected in 40.1%, 26.8%, 24.6%, 18.3%, 17.6%, 14.8%, 13.4% and 6.3% of our cohort, respectively. Many gene mutations were found to be overlapped, but mutations of *TET2* and *IDH1/2*, *FLT3*-ITD and *N/K-RAS* mutation were mutually exclusive. When we performed prognostic analyses for mutations of these genes, *DNMT3* mutation and *FLT3*-ITD were isolated as a poor prognostic factor in overall survival (OS) as previously reported (*DNMT3a* mutation: P=0.0056) (*FLT3*-ITD: P=0.0077). Moreover, in the *FLT3*-ITD positive cases, OS of patients with *DNMT3a* R882 mutation was significantly shorter than those without R882 mutation (P=0.0256). On the other hand, *NPM1* and *C/EBPα* mutation known as a favorable prognostic factor in the *FLT3*-ITD negative cases did not affect the prognosis. Patients with *DNMT3a, FLT3*-ITD*, NPM1, IDH1/2, TET2* and *C/EBPα*, mutations frequently had overlapping gene mutations in 92.1%, 82.9%, 77.2%, 76.9%, 72.0% and 38.1%, respectively. Furthermore, the prognosis of these AML patients could be classified by the number of overlapping mutations of these genes except no mutation group (Figure 1, P<0.0001), and their prognostic importance was also shown after excluding *FLT3*-ITD positive cases (P=0.0034). Additionally, in patients with multiple gene mutations, *DNMT3a* mutations, especially of R882 mutations, were found highly frequently (*DNMT3a*: P<0.0001) (*DNMT3a* R882: P<0.0001).
Summary / Conclusion: Our study revealed that the gene mutations appeared to be overlapped, and the number of these gene mutations significantly affected the prognosis. Intriguingly the *DNMT3a* mutation may contribute to an occurrence of other gene mutation by giving genetic instability to acute myeloid leukemia cells.

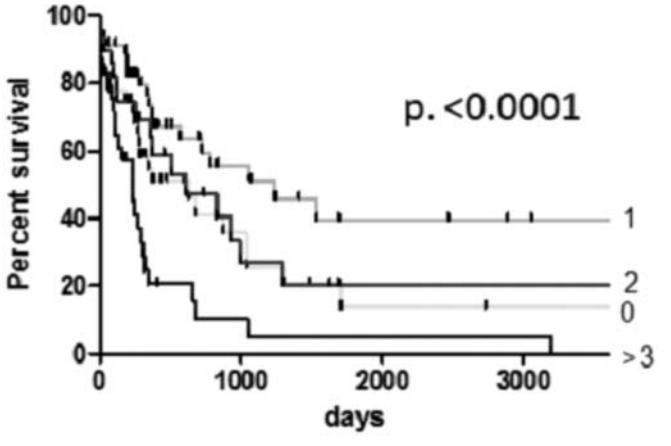

Figure 1.

P065

MEDIUM-SIZED FLT3 INTERNAL TANDEM DUPLICATIONS CONFER WORSE PROGNOSIS, THAN SHORT AND LONG DUPLICATIONS

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Background: The internal tandem duplication (ITD) of the fms-like tyrosine kinase 3 (FLT3) gene occurs in about 25% of adult acute myeloid leukemia (AML) patients. Several previous studies attempted to characterize the impact of the size and the load of FLT3-ITD. While high mutant-to-wild type ratio was repeatedly shown to be associated with adverse prognosis, different conclusions emerged about how the length of the duplication may influence the outcome.
Aims: To investigate the frequency of FLT3-ITD mutations and the possible correlation between FLT3-ITD insertion size and clinical associations and prognosis in AML patients.
Methods: 388 AML patients (184 males/204 females; median age: 51; range: 16-93 years) diagnosed and followed between 2001-2009 were enrolled in the study. Remission and relapse rates and survival were analyzed for 324 patients younger than 60 years and treated with curative intention. FLT3-ITD mutations were studied using PCR followed by capillary electrophoresis.
Results: FLT3-ITD mutations were detected in 82/388 (21.1%) cases. The presence of ITD was related to *de novo* AML etiology (P<0.001). It associated more frequently with M5 [34% (25/74) ITD+ vs. 15% (40/273) ITD-; P=0.0004]. ITD+ patients presented with significantly higher WBC counts and LDH at diagnosis (both P<0.001). ITD+ associated with intermediate risk cytogenetics [86% (65/76) ITD+ vs. 50% (147/292) ITD-; P<0.001] and NPM1 mutation [59% (47/80) ITD+ vs. 16% (50/304) ITD-; P<0.001]. In our entire patient cohort, ITD+ itself did not associate with adverse prognosis, only relapse rate seemed to be increased [65% (33/51) in ITD+ vs. 51% (88/174) ITD-; P=0.08]. The FLT3 ITD- and NPM+ subgroup showed better overall and disease free survival (OS and DFS) in the intermediate and normal karyotype subgroups. In contrast, FLT3-ITD mutants with insertion length shorter or longer than 48bp, showed similar clinical characteristics at diagnosis, but profoundly different treatment outcome in the entire AML group. Longer ITDs were associated with higher early death ratio [17% (5/30) ITD>48bp vs. 0% (0/38) ITD<48bp; P=0.01]; a tendency toward higher relapse rate [84% (16/19) ITD>48bp vs. 55% (17/31) ITD<48bp; P=0.06]; resulting in worse OS (P=0.02) and DFS (P=0.005). Patients with ITD>48bp had worse OS and DFS compared to ITD negative patients (n=255; P=0.04; P=0.013 respectively). Interestingly individuals with longer than 60bp insertions (ITD>60bp; n=15) showed better OS and DFS compared to patients with insertion between 48 and 60bp (ITD48-60bp; n=15, P=0.014; P=0.019 respectively). Median survival at 24 months was 0% in ITD48-60bp, 40±3% in ITD negative, 45±8% in ITD<48bp and 33%±12% in ITD>60bp AML groups.
Summary / Conclusion: Our results suggest that the length of ITD mutations may influence disease outcome, in a way that medium-sized FLT3 ITDs (48-60 bp) may confer worse prognosis than shorter or longer mutations. As a possible explanation, ITD was reported to lead to the constitutive activation of the kinase domain by disrupting the autoinhibitory interaction between the juxtamembrane domain and activation loop. In line with this hypothesis, longer ITDs could lead to more profound destabilization of the inactive kinase. On the other hand, longer ITDs were reported to be inserted more C-terminally in the FLT3 protein, which may cause increased interference with the kinase activity, leading to a reduced transforming capacity.

P066

CHARACTERIZATION OF FLT3 AND NPM1 MUTATIONS IN 17,783 AML PATIENTS

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Background: A recent series of publications confirms that *FLT3* internal tandem duplications (ITD) mutations are driver mutations for acute myeloid leukemia (e.g., Smith *et al.*, *Nature*. 2012. 485:260-3). From 2008 through February 2013 our laboratories assayed bone marrow specimens and peripheral blood 17,783 AML patients to identify and characterize ITD, *FLT3* tyrosine kinase domain (TKD) and *NPM1* mutations.
Aims: A systematic review of the data derived from testing 17,783 patient samples for ITD, TKD, and 2743 patients for *NPM1* mutations.
Methods: The ITD and TKD containing regions of the *FLT3* gene, and exon 12 of *NPM1* gene were amplified using PCR. The size of the ITD and *NPM1* amplicon products were determined by capillary electrophoresis. The TKD amplicon product was first digested with the EcoRV restriction enzyme and presence of the mutation was determined by capillary electrophoresis. We determined the rates of mutation for age/gender groups, and probed the dataset for trends for co-incident evidence of mutations. Patients older than 100 were allocated to the 90-100 years age group. The mutations in ITD positive patients (n=3,240) were assessed and characterized by ITD insertion size, number of insertions and the presence of a concurrent TKD or *NPM1* exon 12 mutation.
Results: For all mutations, the highest mutation rates were found in samples in the middle age range and were lower in younger and older patients (e.g. 10-20 years ITD 19.4%, TKD 4.1%, *NPM1* 22.1%; cf 30-40 years ITD 21.9%, TKD 7.6%, *NPM1* 22.1%; cf 80-90 years ITD 12.7%, TKD 4.5%, *NPM1* 5.1%). The positive rate in females was higher compared to male subjects (14% higher for all ages, but 48% higher in the 50-60 years age group). For all patients who tested positive for an ITD mutation, there is an increased chance that they will test positive for the TKD mutation. In the 20-50 years age group, this rate is 15%;

between 80-90 years the co-mutation rate is 55% increased in a TKD positive result compared to the chances of being positive over all. Similar trends are seen when comparing other combinations. ITD insertions varied from 3bp to 382bp with over 50% of insertions less that 57bp. Approximately 9% harbour the TKD mutation and >29% contained more than one ITD insertion. Of patients (n=304) also tested for NPM1 48% had a detectable exon 12 mutation. Although the majority of AML patients harbouring ITD mutations have a single mutation and an ITD that is <57bp, more than 9% of patients had ITD mutations that were more than 100bp in size.
Summary / Conclusion: The rate of *FLT3* ITD and TKD mutations seen in this patient population (22.5%, n=17783) is similar to the 23% (n~53000) reported by the Wellcome Trust Sanger COSMIC DB (www.sanger.ac.uk/genetics/CGP/cosmic/) for all mutations in *FLT3*. Forty eight percent of FLT3 ITD positive patients also had a NPM1 mutation.

P067

THERAPY-RELATED MYELOID NEOPLASMS: REPORT OF THE ITALIAN NETWORK ON SECONDARY LEUKEMIAS

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Background: In 2001, the World Health Organization (WHO) recognized therapy-related myeloid neoplasms (t-MN) as a distinct entity including acute myeloid leukemia (AML) and myelodisplastic syndromes (MDS). At present, about 10% of all AML patients have a previous history of exposure to chemotherapy and/or radiation for a primary malignancy or autoimmune disease.
Aims: In 2009, we initiated through a Web-database an epidemiological registry, with the purpose of collecting t-MN observed at Italian Hematological or Oncological Divisions. Demographic and clinical information on individuals with t-MN were included in the database whose access was restricted to selected users and was password-protected.
Methods: Between May 2009 and December 2012 a total of 275 patients observed at 21 Italian Centers [119 males and 154 females; median age 64 years (range 24-88 years)] with secondary leukemia were registered in the web-database. Patients were diagnosed with a t-MN between 1999 and 2012, with 246 cases arising after chemo or radiotherapy for a primary malignancy or immunosuppressive therapy for an autoimmune disease, while in 29 cases leukemia represented a second cancer in patients treated for a primary malignancy with surgery alone.
Results: The primary malignancy (PM) was a hematological neoplasm in 115 cases (42%), a solid tumor in 152 cases (55%), an autoimmune disease in 8 patients (3%). Thirteen patients (5%) had a history of two or more previous cancers. Among hematological malignancies, the most frequent PM were lymphoproliferative diseases (83/115 cases), while breast cancer (62/152 cases) was the most frequent primary solid tumor. In particular, hematological PM were: 83 lymphoprolipherative diseases (62 Non Hodgkin and 17 Hodgkin lymphoma, 4 chronic lymphocytic leukemia); 12 Multiple myeloma; 1 Acute lymphoblastic leukemia; 4 Acute myeloid leukemia (acute promielocytic leukemia in 2 cases). There were also 15 patients with a previous history of myeloproliferative neoplasms (10 Myelofibrosis; 3 polycitemia vera; 2 essential thrombocythemia). Sites of primary solid tumors were: 62 Breast; 37 Urogenital (17 prostate; 7 bladder; 1 kidney; 7 uterus; 5 ovarium) ; 17 Colon-rectal; 11 Lung; 7 Thyroid; 6 CNS; PM were localized at sites uncommon for t-MN in 11 patients (1 stomach, 4 skin, 4 oropharynx; 2 sarcoma); 1 unknown. Eigth patients had previously received immunosuppressive therapy for a rheumatologic disease (5 with mitoxantrone and 3 with methotrexate). Two-hundred-eight patients had previously received chemotherapy for their primary malignancy, associated to radiotherapy in 79 cases. RT represented the only primary treatment in 38 cases. Median latency between PM and t-MN was 6.6 years (range 0.2-48). No differences were observed in age of patients (P=0.09) or in the median latency (P=0.20) between t-MN after lymphoprolipherative diseases or after breast cancer. According to morphology, t-MN were classified as 172 AML, 97 MDS and 6 ALL. Kariotype was available for 188 patients only and was unfavorable in 65 patients (complex in 51 patients including del(7) in 36 cases; 14 cases with isolated del(7)]. A recurrent chromosomal translocation was present in 12 patients only [3 t(8;21), 8 t(15;17) and 1 inv(16)]. One-hundred-forty-eight patients received chemotherapy for t-MN, while the hypomethylating drug Azacitidine was administered to 54 patients. Fifty-four patients underwent bone marrow transplantation (39 allogeneic and 15 autologous). Median OS from the t-MN diagnosis was 9.4 months (range 0.2-128+).
Summary / Conclusion: The incidence of t-MN is rising as a result of the increasing number of cancer survivors. Lymphoprolipherative diseases and breast cancer result as the most common primary malignancies at risk of developing this disorder.

P068

AZACITIDINE FOR RELAPSE TREATMENT IN MYELOID MALIGNANCIES FOLLOWING ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Relapse after allo-HSCT for acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) remains the main cause of treatment failure and is associated with poor prognosis and short survival. Pharmacological immune-modulatio, chemotherapy with or without DLI(Donor Lymphocyte Infusion) or second allo-HSCT are classically used as slavage therapy, but usually yield disappointing results with a relatively high toxicity, highlighting the need for novel salvage therapies in this situation.
Aims: We aimed to assert the safety and efficacy of the hypomethylating agent 5-Aza with a standard dosing (75 mg/m^2/d for 7 days) as a salvage regimen at relapse after Allo-SCT for myeloid malignancy, associated or not with DLI.
Methods: Between Sept. 2006 and Sept. 2012, thirty-one pts (median age, 57 y (range, 14-69) with *de novo* AML, n=13; secondary AML, n=6; MDS, n=11; MPN, n=1, were treated in our institution with AZA for relapse occurring at a median of 3.7 months (range, 1.7-37.6) after allo-HSCT.
Results: AZA was given SC at 75 mg/m^2/d for 7 days per cylce. Grade III-IV toxicities were observed in 32% of cases. AZA had to be discontinued due to toxicity in 8 pts. 35% of the pts had to be readmitted to hospital due to complications of AZA. No *de novo* GVHD was reported during AZA therapy. Eleven pts achieved a response: 7 partial responses, 4 complete responses, with a median time to best response of 92 d (range, 35-247). The median overall survival (OS) time from treatment with AZA for the whole group was 153 days (range, 39-928) without any difference in survival between patients receiving or not subsequent DLI or other additional treatments. In univariate analysis, achieving a response to 5-Aza improved the OS (308d vs 108d) whereas having a monosomic or complex caryotype impaired the OS (118 vs 188, 105 vs 198, respectively), there was no interaction between rate of response to 5-aza and caryotype characteristics, except for a trend between having a complex caryotype and non-response to 5-Aza (OR=0,16 [P=0,11]). With multivariate analysis, only the response to 5-Aza and the monosomic caryotype did still have a significant level to impact the OS. Of note, the DLI had no significative benefit, even for people obtaining a response (31months with DLI vs 13m without) in our small cohort.
Summary / Conclusion: These findings suggest that AZA might be a potentially effective strategy for relapse of high risk myeloid malignancies post allo-HSCT, without unexpected toxicities or *de novo* GVHD. Prospective studies, but also preemptive strategies using AZA are needed in such high risk diseases.

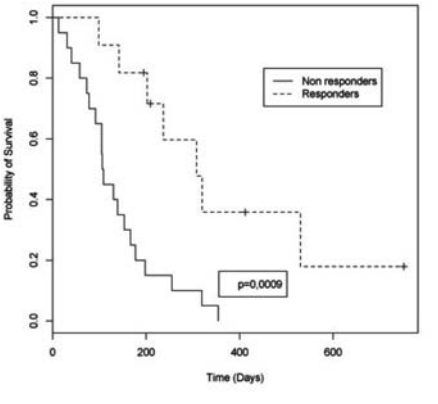

Figure 1.

P069

MONITORING MINIMAL RESIDUAL DISEASE IN NPM-1 MUTATED ACUTE MYELOID LEUKEMIA. PRELIMINARY RESULTS AT A SINGLE INSTITUTION

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Background: The measure of minimal residual disease (MRD) can be a reliable prognostic tool in patients (pts) with acute myeloid leukemia (AML), even in those with better prognosis because of NPM-1 mutation.
Aims: To identify pts at higher risk of relapse westudied MRD by real-time quantitative PCR of NPM-1 (Gorello, 2006) at different time points, compared with WT-1 expression quantification (Cilloni, JCO 2009) and with identification of aberrant leukemia-associatedimmunophenothypes (LAIPs) by six-color multiparametric flow cytometry (MFC).
Methods: From June 2010 to January 2013, we observed 31 *de novo*, consecutive NPM-1 mutated pts (1 B-type, 30 A-type) [M/F 15/16; median age 60y (range 30-75y)], treated according to GIMEMA AML17 and NILG AML00 protocols. At diagnosis, 26/31(83.8%) had normal karyotype and 5 had chromosomal abnormalities; FLT3-ITD was detected in 10/30 (33,3%) cases. Peripheral blood (PB) and bone marrow (BM) samples were collected at diagnosis, after induction, consolidation and at the end of therapy. The cut-off value used for MFC detection of MRD was 0.1% after induction (Al-Mawali, 2009) and 0.035% after consolidation (Buccisano, 2010).
Results: Complete remission (CR) was achieved in all pts after one course of chemotherapy (ICE or MICE or FLAG). Relapse occurred in 6 (19.3%) at a median of 12 months (range 6-17) from the achievement CR and 1,5 months (range 1-7) from the end of therapy. Median follow-up was 12 months (1-41). MRD data at different time points were evaluated as predictors of relapse risk. There weren't differences in the pretreatment parameters of CCR vs relapsed pts, particularly median age (60 vs 63 y), FLT3-ITD mutation (36% vs 20%) and chromosomal aberrations (19% vs 20%), respectively. The median level of NPM-1/ABL x 10^4 was significantly lower in CCR then in the relapsed pts [50054 (range 380-97280) vs 96175 (range 843-132390), (P 0.039 Student's T test)]. At CR achievement and after consolidation, the negativity of NPM-1, considering all the series, was achieved in 4/29 (13,8%) and in 10/27 (37%) respectively, without difference between the 2 groups of pts. The decrease of NPM-1 level compared to pretreatment levels was greater in CCR then in relapsed pts (40,6% vs 20%>P 0.3 Fisher's test). At the end of therapy NPM-1 was undetectable in 11/19 pts (57,9%), with significant difference between CCR and relapsed pts (11/13 vs 0/6: p0.001 Fisher's exact test). The WT-1 was overexpressed in PB and in BM in all pts, without differences between PB vs BM and CCR vs relapsed pts. Significantly more pts who obtained the WT-1 normalization levels (<213/abl x10^4) in BM after CR did not relpase(P 0,009-Fisher's test). LAIPs were identified in 21/25 (84%) at diagnosis; 9/19 (47.3%) and 4/18 (22,2%) had MRD negative in MFC in the post induction and after consolidation, respectively, without differences in 2 groups. The overall survival between 2 groups was significantly different (P 0.017).
Summary / Conclusion: In AML patients with NPM-1 mutation, only NPM median levels among pretreatment parameters including FLT3-ITD status, age and additional chromosomal aberrations, significantly influenced prognosis. Achieving WT-1 negativity post induction and, more strongly, NPM-1's negativity at the end of therapy, as reported also by Kronke (JCO, 2011), significantly predicted continuous CR. The role of NPM-1 clearance during treatment and of LAIPs MRD levels need further studies.

P070

THE PROGNOSTIC IMPACT OF ADDITIONAL KARYOTYPIC ABNORMALITIES IN T(8;21) ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) with translocation (8;21)(q22;q22) is associated with a favourable prognosis with standard treatment for AML with an overall survival of 50-60% based on large databases. However, significant heterogeneity appears to exist amongst the patients diagnosed with t(8;21) AML, with some patients experiencing early relapse and subsequently refractory disease. In addition, a few Asian studies also showed that t(8;21) was not found to be associated with a better survival compared with normal risk AML, suggesting that unidentified genetic polymorphism might be at play.
Aims: We aim to perform a retrospective analysis of patients with t(8;21) AML from registry databases in the National University Hospital (NUH) and the Singapore General Hospital (SGH) looking at prognostic factors that may influence outcomes of these patients.
Methods: Patients diagnosed with t(8;21) AML identified from the registry were included in the review. Those who did not receive induction chemotherapy were excluded. Univariate survival and outcomes data were analysed according to demographic and cytogenetic subgroups using the Kaplan Meier method. Multivariate analysis of the different subgroups was performed using cox-regression.
Results: 61 patients were available for analysis. The median age was 38 years old (interquartile range (IQR): 25-51 years), 31.1% of patients were younger than the age of 30. 47.5% of the patients were female. The median total white count was 11.65x10^9/L (IQR: 5.75-25.68) and median bone marrow blast was 60% (IQR: 40-77%).
5 patients were excluded from analysis of overall survival and progression survival as they did not receive intensive chemotherapy with curative intent. The median follow up time was 81.3 months (range: 1.4 to 178 months). 3 patients (5.4%) of the patients did not attain a complete remission after induction and required a second induction. Overall survival at 8 years was 61.9% and relapse free survival was 50.6% Most of the patients relapsed within 15 months after initiation of induction treatment. In univariate analysis, the age of > 30, > 1 additional structural cytogenetic abnormality and the presence of additional chromosomal abnormalities other than sex chromosome or 9q deletion appears to be associated with a worse overall survival. In particular, t(8;21) AML associated with additional chromosomal abnormalities other than sex chromosome or 9q deletion is associated with only 22.7% 8 year survival compared with 82.2% 8 year survival in those with an additional sex chromosome or 9q deletion (P=0.001). This remained significant after multivariate analysis, HR 4.14 (95% CI: 1.02-16.95). C-Kit mutation analysis was performed on 42(68%) patients, D816 mutation was found in 8 (19%) of the patients. The presence of D816 mutation showed a trend towards worse relapse free survival (P=0.059) but not overall survival (P=0.32).
Summary / Conclusion: There is significant heterogeneity amongst the t(8;21) AML cohort, those with complex cytogenetics or additional chromosomal abnormalities other than sex chromosome or 9q deletion appear to be associated with a worse prognosis. Further analysis using whole exome sequencing is underway to further identify partner mutations that may affect the prognosis of this cohort of patients.

P071

BAALC GENE EXPRESSION REDUCTION DURING INDUCTION PREDICTS OUTCOME IN ACUTE MYELOID LEUKEMIA

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Background: Age, cytogenetics and secondary *versus de novo* disease are the most important factors predicting CR in AML patients. These factors provide a pre-treatment stratification in risk groups with different probabilities to obtain CR but they are not sufficient to predict the individual response to induction treatment that is variable. Moreover, to collect the necessary information in time to allow stratification at diagnosis is not generally possible. Recently, a variety of novel molecular markers have refined the risk stratification of intermediate-risk AML; in addition altered genes expression have been proposed as important prognostic marker. BAALC (Brain And Acute Leukemia, Cytoplasmic) is a gene located on chromosome 8q22.3, it encodes a protein with unknown function. BAALC expression was found mainly in CD34+ cells but is restricted to the compartment of progenitor cells, while no expression was detected in mature bone marrow or circulating white blood cells. High BAALC expression levels were shown to be associated with treatment outcome in AML.
Aims: In our previous studies we displayed that early peripheral blast clearance (PBC), as assessed by flow cytometry and by WT1 copy reduction, well correlates with first course response and CR attainment in AML. We investigated whether assessment of BAALC transcript levels in peripheral blood the first days during standard induction therapy could provide information on the chemosensitivity of leukemic blasts and predict for clinically relevant end points.
Methods: We explored the kinetics of BAALC transcript to estimate PBC in peripheral blood samples collected on day 1 (immediately before starting therapy) and on day 5 (the fifth day after start of treatment, immediately before cytarabine infusion) in 57 adult patients with AML. Written informed consent was obtained in accordance with the Declaration of Helsinki. Quantification of BAALC gene expression was carried out by real time quantitative PCR using BAALC ProfileQuant Kit from Ipsogen. We calculated BAALC ratio as the ratio of copy number measured on day 1 and on day 5. We used median BAALC ratio as the cut-off to divide the patients into two groups.
Results: Main characteristics of 57 examined patients were: median age 48 years (18-65), median WBC count/microL 13400 (1240-286000), low intermediate and high cytogenetic risk group patients number was7, 32, 15 respectively, FLT-3-ITD occurred in 14 patients (24%). The median BAALC ratio in overall cohort was 5.82 (range 0.83-373.11). The median BAALC ratio was greater in patients attaining CR as compared to non responders (10.7 vs 2.04; P=0.0006). Of 29 patients with a BAALC ratio >5.82, 24 (82%) achieved CR compared to 11 of 28 patients (41.4%) with a ratio ≤5.82 (P=0.002). We found that among the 35 patients attaining CR after the "3+7" course, the DFS (Figure 1A) and OS (Figure 1B) were significantly longer in those patients displaying a BAALC ratio >5.82 compared to patients with a BAALC ratio ≤5.82 (P=0.024 and P<0.001, respectively).
Summary / Conclusion: These data confirmed that PBC assessment by

BAALC kinetics is an early predictor of disease outcome and it allows accurate stratification of patients since the very first days of therapy; as such, it entails potential implications for the management of AML, specifically in order to customize treatment since the outset.

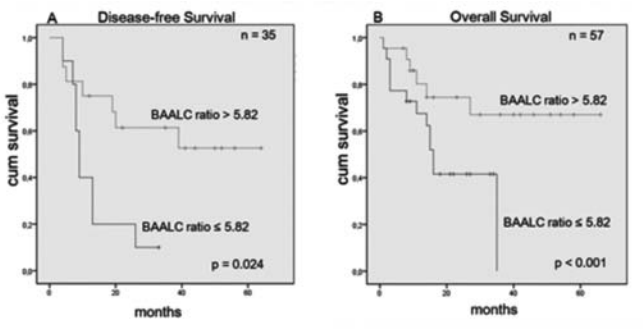

Figure 1.

P072

MIDOSTAURIN (MIDO) DEMONSTRATES A FAVORABLE SAFETY PROFILE IN OLDER PATIENTS (PTS) WITH ACUTE MYELOID LEUKEMIA (AML) OR MYELODYSPLASTIC SYNDROME (MDS)

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Background: The majority of pts with AML are aged ≥ 60 years (y) and have therapeutic challenges related to host factors (such as age or comorbidities) and to intrinsic resistance to available therapies. Fms-like tyrosine kinase receptor 3 internal tandem duplications (FLT3-ITD) are observed in 25-30% of older pts with AML and correlate with poor prognosis. The multitargeted FLT3 tyrosine kinase inhibitor (TKI) mido (PKC412) has demonstrated activity and tolerable safety as a single agent in pts with systemic mastocytosis (Gotlib *et al. Blood*. 2012) as well as in a phase 2 study in AML or high-risk MDS (Fischer *et al. J Clin Oncol*. 2010). The current subset analysis represents full safety data from the latter study to better define the safety of mido in older (60-74 y and ≥ 75 y) pts with AML or MDS.
Methods: Pts with relapsed/refractory (r/r) AML or *de novo* AML not on standard chemotherapy (n=85) or MDS (n=10) were included. Pts received mido 50 mg (n=51) or 100 mg (n=44) twice daily (BID) in 28-d cycles. All pts received at least 1 dose of study drug and were evaluable for safety.

Table 1. Most frequent studydrug related-AEs (≥10% of pts unless otherwise indicated).

	All Pts ≥ 60 y, n (%) (N = 80)		Group A: Pts 60-74 y, n (%) (n = 58)		Group B: Pts ≥ 75 y, n (%) (n = 22)		Group C: Pts < 60 y, n (%) (n = 15)	
	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4
Overall study drug–related AEs								
Mido 50 mg BID	29 (36)	7 (9)	23 (40)	6 (10)	6 (27)	1 (5)	6 (100)	2 (13)
Mido 100 mg BID	27 (34)	11 (14)	17 (29)	4 (7)	10 (46)	7 (32)	9 (100)	4 (27)
Hematologic AEs[a]								
Anemia	4 (5)	4 (5)	3 (5)	3 (5)	1 (5)	1 (5)	1 (7)	0
Thrombocytopenia	4 (5)	4 (5)	2 (3)	2 (3)	2 (9)	2 (9)	0	0
Leukopenia	0	0	0	0	0	0	1 (7)	1 (7)
Nonhematologic AEs								
Nausea	37 (46)	1 (1)	25 (43)	0	12 (55)	1 (5)	11 (73)	0
Vomiting	31 (39)	1 (1)	23 (40)	1 (2)	8 (36)	0	10 (67)	0
Diarrhea	18 (23)	1 (1)	10 (17)	1 (2)	8 (36)	0	4 (27)	2 (13)
Asthenia	6 (8)	0	6 (10)	0	0	0	1 (7)	0
Fatigue	6 (8)	0	5 (9)	0	1 (5)	0	2 (13)	0
Abdominal pain[b]	4 (5)	0	3 (5)	0	1 (5)	0	4 (27)	1 (7)
Headache	3 (4)	1 (1)	3 (5)	1 (2)	0	0	4 (27)	0
Biochemical abnormalities								
Increased AST	9 (11)	3 (4)	7 (12)	1 (2)	2 (9)	2 (9)	1 (7)	0
Increased ALT	8 (10)	3 (4)	6 (10)	2 (3)	2 (9)	1 (5)	2 (13)	0
Increased alkaline phosphatase	3 (4)	0	2 (3)	0	1 (5)	0	3 (20)	0
Increased amylase	0	0	0	0	0	0	2 (13)	0

[a] Occurring in ≥ 5% of pts.
[b] Includes the terms "abdominal pain" and "abdominal pain upper."
ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Results: Of 95 enrolled pts, 58 were aged 60-74 y (Group A; median 67 y) and 22 were ≥75 y (Group B; median 77 y). Fifteen pts were aged <60 y (Group C). More pts in Group A vs Group B had r/r disease at baseline (48% vs 14%, respectively). Pts aged 60-74 y were also more likely to have AML than MDS (91% vs 82% with AML in Group A and B, respectively), a recurrence within 3 mo of study entry (40% vs 9%), and poorer performance status (WHO ≥2: 12% vs 5%). The median duration of exposure to mido was shorter in Group A vs B (44 d [range, 1-332] vs 62 d [range, 12-187]). All pts experienced at least 1 adverse event (AE). The first onset of most AEs occurred by cycle 2. Pyrexia was the only AE with first onset occurring during or after cycle 3 in ≥ 10% of pts (n=9, 11%). Study drug–related AEs were experienced by 70% of pts (69% and 73% of pts in Group A and B, respectively) and were mainly grade 1/2, with gastrointestinal (GI) toxicity occurring most frequently (Table 1). Rates of drug-related AEs of any grade were slightly higher in pts aged 60-74 y than in pts ≥ 75 y, with the exception of thrombocytopenia, nausea, diarrhea, and anorexia. Drug-related grade 3/4 AEs were less frequent in pts aged 60-74 y (17% vs 36% in Group A vs B) and most commonly included hematologic AEs and laboratory abnormalities. No drug–related grade 4 GI AEs were reported. Drug-related AEs (any-grade and grade 3/4) were more frequent with 100 mg BID vs 50 mg BID in pts aged ≥75 y, but this dose effect was not observed in pts 60-74 y. Serious AEs occurred in 58 pts (73%; 42 and 16 pts in Groups A and B, respectively) and most commonly included febrile neutropenia (n=18), pneumonia (n=18), and pyrexia (n=12). Twelve (21%) and 4 (18%) pts in Groups A and B, respectively, discontinued study treatment due to AEs, most commonly due to infections or respiratory, thoracic, and mediastinal disorders. Of the 27 pts who died on study, more deaths occurred in pts aged 60-74 y (36% and 27% of pts in Groups A and B), a higher proportion of whom had r/r disease.
Summary / Conclusion: Overall, AEs occurring on mido monotherapy were less frequent in pts aged 60-74 y than in the small group of pts ≥ 75 y. The most common AEs were low-grade GI events that resolved with dose modification or interruption. There were only slight differences in the rates of GI AEs (nausea, diarrhea) between pts aged 60-74 and ≥ 75 y, and fewer AEs, including GI AEs, were observed in pts aged ≥ 60 than pts < 60 y.

P073

HAEMOSTATIC PARAMETERS AS PROGNOSTIC FACTORS IN ACUTE MYELOID LEUKEMIA

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Background: Haemostatic abnormalities are apparent at presentation in a subset of patients with nonpromyelocytic acute myeloid leukemia (AML). Recent studies indicated that a high level of fibrinogen at presentation is correlated with a poor outcome for patients with AML (Lancet. 2010;376(9757):2000-8; ASH abstract 2011: No 3593).
Aims: The aim of this study was to determine the role of haemostatic parameters as prognostic factors for overall survival (OS), complete remission (CR) rate, disease-free survival (DFS) and early death (ED) in patients with AML.
Methods: This single-center study involved 302 adult patients with nonpromyelocytic *de novo* AML during follow-up of 48 months. At presentation of disease the following haemostatic parameters were evaluated at diagnosis as risk factors for OS, CR, DFS and ED: fibrinogen level > 4 g/L, prothrombin time (PT) <60%, activated partial prothrombin time (aPTT) > 35s, D-dimer level >250 ng/mL and International Society for Thrombosis and Haemostasis Scoring System for disseminated intravascular coagulation (ISTH DIC score) ≥ 5. Patients were treated according to Medical Research Council (MRC) 12 protocol, with 50% reduction of the anthracycline dose for subjects aged > 60 years. Patients aged >75 years received hydroxyurea per os. Risk factors were identified using univariate and multivariate analysis.
Results: The mean patients' age was 57 years (range 19-79 years). Multivariate logistic regression analysis indicated that a high fibrinogen level was the most significant factor for poor OS (P<0.001, relative risk (RR) = 2.168; 95% CI 1.574-2.986). In addition, multivariate analysis identified a high fibrinogen level as the most significant factor for both - lower CR rate (P=0.001, RR = 0.378; 95% CI 0.203-0.706) and shorter DFS (P=0.016, RR = 1.971; 95 % CI 1.135-3.423). In contrast, multivariate analysis identified an ISTH DIC score ≥ 5 to be the most important haemostatic parameter for ED (P=0.006, RR 2.371; 95 %CI 1.282-4.385).
Summary / Conclusion: This study has shown that a high fibrinogen level at presentation of nonpromyelocytic AML is significantly correlated with poor OS, CR rate and DFS, while ISTH DIC score ≥ 5 is associated with a higher ED rate. According to data in the literature high fibrinogen levels in AML have a potential role in resistance to chemotherapy. A high concentration of fibrinogen may also be a marker of increased inflammation in AML patients, which needs to be confirmed in further investigations.

P074

SUCCESSFUL SALVAGE TREATMENT WITH CLOFARABINE AND CYTARABINE (ARA-C) IN RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA

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Background: Relapsed/refractory AML patients have a poor prognosis, with CR rates of 1%>30%, unless allogeneic hematopoietic stem cell transplantation (HSCT) is an available option. Although retrospective modeling studies have demonstrated the prognostic value of selected parameters, responses with salvage therapies remain still poor. It was previously established the activity of clofarabine plus cytarabine in AML relapse (clofarabine dosed once daily for 5 days with 40 mg/m^2 followed 4 hours later by ara-C at 1 g/m^2 per day). However, modifications of this combination in AML therapy of relapsed/refractory patients warrant further evaluation.
Aims: To determine the efficacy and safety of clofarabine and cytarabine (Ara-C) in adult patients with relapsed or refractory acute myeloid leukemia (AML).
Methods: Twenty-five patients aged (30-67) years with refractory/relapsed AML were treated at the dose of clofarabine 30 mg/mq on days 1-5 + cytarabine 1000 mg/mq gg on days 1-5. We evaluated the complete remission rate (CRR), duration of remission (DOR) and overall survival (OS). Minimal residual disease (MRD) by molecular targeting was considered in all patients.
Results: Twenty-five patients received clofarabine 30 mg/mq on days 1-5 + cytarabine 1000 mg/mq gg on days 1-5, (followed by gentuzumab therapy in only three patients). All patients had relapsed/refractory myeloid leukemia and had received multiple priors therapies. Six pts had received a prior hematopoietic stem cell transplant (HSCT). Fourteen patients achieved a complete remission (CR); nine patients went on to receive allogeneic transplants after clofarabine/ARA-C salvage. The complete remission rate (CRR) was (56,00 %) The Median of Overall survival for all patients was (149) days (range 12-1152), while the media of Overall survival (OS) was (221.52) days, and we estimated a duration of remission (DOR) as (195.00) days in median (range 41-1131), and (311.36) days in media (we calculated from the first day of remission). Treatment was complicated by neutropenic fever (n=16), grade III-IV mucositis (n=2), skin rush (n=1) grade II- III, hepatic transaminase elevations (n=2). Two (n=5) patient died before their disease status could be evaluated
Summary / Conclusion: Combination treatment with clofarabine 30 mg/mq and ARA-C 1000 mg/mq in adults pts with refractory or relapsed AML resulted in an ORR of (56,00 %) and of the (14) patients who achieved a CR, nine (64.29%) proceeded to HSCT (Five are still alive). The safety profile is acceptable in this relapsed/refractory population, and our results are very similar to previous regimes using higher clofarabine dosages. More studies with this combination in adults are warranted

Chronic lymphocytic leukemia: Translational Research

P075

ACHIEVING BONE MARROW CLL MINIMAL RESIDUAL DISEASE-FREE STATUS WITH FIRST-LINE FCR IS ASSOCIATED WITH IMPROVED PFS AND CAN BE CONSIDERED A PRIMARY TREATMENT OBJECTIVE

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Background: MRD-Free Status After 3 courses of First-Line FCR Achieves Comparable PFS to 4-6 Courses
Aims: To prospectively investigate clinical and biological factors associated with bone marrow MRD status and outcomes in first-line FCR. To determine the prognostic and therapeutic implications of bone marrow MRD in CLL.
Methods: Two-hundred thirty-seven pts with CLL and standard NCI/IWCLL indication for treatment were evaluated for pretreatmet characteristics, including prognostic factors. They received up to 6 courses of standard first-line fludarabine, cyclophosphamide, and rituximab (FCR) between 09/2008 and 09/2012. MRD was prospectively assessed in bone marrow by 4 or 6-color flow cytometry after course 3 and 2 months after 6th or last course of treatment. Categorical and continuous variables were compared using χ^2 and Mann-Whitney tests. Kaplan-Meier estimates were compared using the log-rank test. Cox regression was used for multivariate analyses (MVA).
Results: All 237 pts completed treatment and were evaluable for response assessment by NCI/IWCLL criteria; 61% were male, 21% were older than 65 years, 40% had Rai stage III-IV CLL, 41% had beta2-microglobulin (B2M) ³ 4 mg/L, 61% had unmutated *IGHV* gene, and 18% had FISH analysis positive for trisomy 12 (+12), 21% for deletion 11q and 7% for deletion 17p (del17p). Seventy-five percent of pts received more than 3 total courses of FCR. The complete remission (CR) and overall response (OR) rate was 65 and 97%, respectively. MRD negativity was achieved in 59% of pts at response assessment. Baseline characteristics independently associated with MRD negative status in MVA were Rai stage 0-II (P=0.04), mutated *IGHV* gene (P=0.002), +12 (P=0.02) and lack of del17p (P=0.02). After a median follow-up of 28 months (range 4-53), median progression-free survival (PFS) and overall survival (OS) were not reached. Baseline characteristic associated with a shorter PFS in MVA were unmutated *IGHV* gene (P=0.03) and presence of del17p (P<0.001). When evaluating response variables in MVA, partial remission (P=0.01), non-response (P=0.03) and MRD positivity (P=0.002) were independently associated with shorter PFS. Five groups were defined according to number of FCR courses and MRD status (Figure 1): pts receiving 3 or fewer total courses, MRD positive (MRD3+) and MRD negative (MRD3-); pts receiving more than 3 total courses, negative both after 3 courses and at response assessment (MRD3-/EOT-), positive at both time points (MRD3+/EOT+), or positive after 3 courses, but negative at response assessment (MRD3+/EOT-). PFS was significantly shorter for MRD3+ (median 30 months, P<0.001) and for MRD3+/EOT+(median not reached, P<0.001). No differences in PFS were observed between MRD3-, MRD3-/EOT- and MRD+/EOT- with current follow up. When comparing MRD3+/EOT- and MRD3+/EOT+ according to baseline and treatment characteristics, only a high CR rate was associated with MRD3+/EOT- (P<0.001).
Summary / Conclusion: CLL pts with early stage disease, mutated *IGHV* gene, +12 positive and lack of del17p are more likely to achieve MRD negativity with frontline FCR therapy, which is independently associated with improved PFS. With this early follow up, it appears that if MRD free status is achieved after 3 courses, FCR can be safely stopped without compromising PFS outcome.

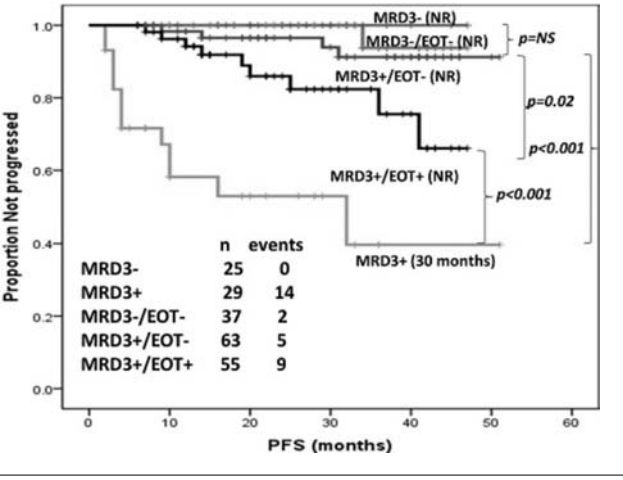

Figure 1.

P076

DEVELOPMENT OF A COMPREHENSIVE PROGNOSTIC INDEX FOR PREVIOUSLY UNTREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Until now, the prognosis and indication for treatment in chronic lymphocytic leukemia (CLL) are guided by the staging systems of Binet and Rai. During the last decade, however, a number of robust predictors of overall survival (OS) have been identified. The multiplicity of new markers, limited information on their independent prognostic value, and a lack of understanding of how to interpret discordant markers have been major barriers to application of these prognostic tools in routine clinical practice.
Aims: We therefore performed a pooled analysis using the database of the German CLL Study Group (GCLLSG) to develop an integrated prognostic index. This index was subsequently validated in an independent cohort of untreated CLL patients (pts) from the Mayo Clinic CLL Database.
Methods: The development of the index was based on data collected between 1997 & 2006 in three GCLLSG phase III trials: 710 in CLL1 ("watch and wait" versus fludarabine (F)), 362 in CLL4 (F versus F and cyclophosphamide (FC)) and 817 pts in the CLL8 trial (FC versus FC and rituximab (FCR)). The external validation was performed on a series of 676 pts with newly diagnosed CLL followed and managed at Mayo Clinic.

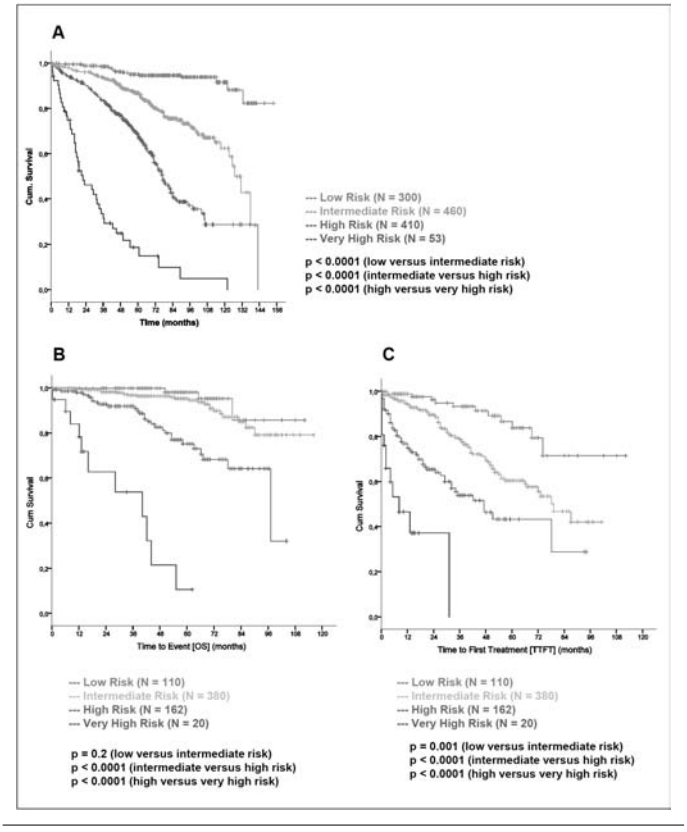

Figure 1.

Results: The GCLLSG data set, a total of 1948 pts (median age of 60 years (range, 30 to 81 years)), was used for the pooled analysis. The study population consisted of physically fit patients at all stages of disease (799 (42.4%) Binet stage A, 717 (38.0%) Binet stage B and 370 (19.6%) Binet stage C patients). After a median observation time of 63.4 months 485 deaths were reported. Multivariate analyses of 23 prognostic factors including baseline characteristics, laboratory results, molecular cytogenetic, mutational status and serum parameters were performed. Eight parameters were identified as independent predictors for OS: sex, age, ECOG status, genetic aberrations del(17p) and del(11q), *IGHV* mutation status, s-TK, and s-β_2m. By using a weighted grading of independent factors, a prognostic index was derived separating four different pts groups: low risk (score 0 - 2), intermediate risk (score 3 - 5), high risk (score 6 - 10) and very high risk (score 11 - 14) (Figure 1A) with significant different OS rates (95.2%, 86.9%, 67.7% and 18.7% survival after 5 years for the low, intermediate, high and very high risk group (P<0.001, respectively)). This prognostic index was then validated in a cohort of 676 pts from the Mayo Clinic. At last follow-up, 163 (24.1%) patients have been treated and 66 (9.8%) have died. At presentation, 396 (57.1%) patients were Rai stage 0, 270 (39.9%) Rai stages I-II and 20 (3.0%) Rai stages III-IV. The four risk groups of the prognostic index for OS were reproduced with 98.3%, 95.4%, 75.4% and 10.8% survival after 5 years (Figure 1B). Slight differences in survival rates by risk category between the two cohorts are likely explained bya shorter observation time (median, 63.4 versus 47.0 months, P<0.001) and high proportion of censored data. The prognostic index predicted survival independent of disease stage for all Rai risk categories (not shown). Furthermore the index score provides accurate estimations regarding the time to first treatment for newly diagnosed CLL patients (Figure 1C).
Summary / Conclusion: Using a multi-step process including an independent external validation cohort, we developed a comprehensive prognostic index for pts with CLL. The prognostic index provides more accurate prediction of both treatment-free and overall survival for CLL patients and appears generally applicable.

P077

THE EXPRESSION OF A SINGLE MICRORNA - MIR-34A – IS A RELIABLE MARKER FOR IMPAIRMENT OF P53-PATHWAY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: We and others have shown that miR-34a is down-regulated in the aggressive CLL subtype harbouring p53 aberration (Mraz, 2009; Zenz, 2009; Asslaber, 2010). However, these studies only specifically studied the expression of miR-34a or few other selected miRNAs that were previously shown to be associated with cancer biology.
Aims: Here we performed an unbiased search for miRNAs that are involved in fludarabine-induced cell death in CLL cells *in vitro* and *in vivo* to investigate the relevance of miR-34a as a marker for impairment of p53 pathway and resistance of CLL to therapy.
Results: To test for miRNAs that are involved in apoptotic pathways in CLL cells we treated *in vitro* ten purified primary CLL B-cell samples with fludarabine (IC50 dose of 3.5 ug/mL, 48hrs), and profiled the expression of 750 miRNAs (TaqMan miRNA Cards, ABI). This identified induction of 15 miRNAs (fold induction [FI] >1.5) with miR-34a being the most prominently up-regulated miRNA (FI 3.7). To verify this observation *in vivo* we obtained peripheral blood from CLL patients treated with a FCR regimen that includes administration of rituximab (R) on day1, followed by fludarabine (F) and cyclophosphamide (C) on day2, 3 and 4. The samples were obtained from patients before administration of therapy, 24hrs after administration of R and 48hrs after FC. The administration of R did not induce miR-34a expression, which was however clearly induced in most samples (96%) after FC administration (FI 2.2, P<0.0001). Additionally, the levels of miR-34a before or after FCR administration were strongly correlated with P53-aberration present in 5 out of 52 FCR-treated patients; with patients harbouring P53-aberration having the lowest miR-34a levels from the whole cohort. Interestingly, CLL cases with deletion of ATM (del11q23, N=19), an up-stream regulator of p53, had similar basal and induced levels of miR-34a compared to cases without del11q23 (N=28). Importantly, when the FCR-treated patients were divided into terciles based on the level of miR-34a after FC, those with the lowest levels of miR-34a experienced significantly shorter time to treatment failure (1.1 years vs. 2.2 years; P=0.037; HR 3.01). These data and the broad screening of fludarabine-induced miRNAs confirmed that miR-34a is the preferential miRNA activated with apoptosis and DNA damage pathway in CLL. This prompted us to develop an assay for absolute quantification of miR-34a that allowed us to determine the copy numbers of miR-34a and to define the precise cut-offs(similarly to assays for bcr-abl quantification in CML). Using this assay, we profiled the expression of miR-34a in 200 CLL patients (miR-34a expression ranged from 1 to 81820 copies/10e6 copies of internal standard). Significantly, miR-34a levels below 2500 copies (N=47) were corre-

lated with shorter overall survival (9.6 years vs. not-reached, HR 2.2 [CI 1.1-4.5], P=0.03). The low miR-34a levels were also apparently associated with the deletion & p53 mutation of 17p13 (N=18) or sole p53 mutation (N=13) in this second CLL cohort (P<0.005).
Summary / Conclusion: Our data provide evidence and novel tool for the use of miR-34a as a marker of p53-aberration, which can be especially useful in cases with sole p53 mutation not accompanied by 17p13 deletion that cannot be discriminated by routine FISH. Patients with sole p53 mutation represents approx. 1/3 of p53 aberrant cases with inferior prognosis similar to cases with del17p13.
IGA MZCR NT11218-6/2010, FR-TI2/254.

P078

CHRONIC LYMPHOCYTIC LEUKEMIA CELLS FROM PAIRED LYMPH NODE AND PERIPHERAL BLOOD SHOW A DIFFERENT PATTERN OF COPY NUMBER ABERRATIONS
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Background: Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of neoplastic B cells in peripheral blood (PB), bone marrow (BM), lymph nodes (LN) and hepatosplenic tissue. BM and LN are most likely the sites of disease maintenance and progression, where the crosstalk with accessory stromal cells prevents CLL cells from apoptosis and enhances their proliferation. Recent data indicate that CLL cells obtained from PB exert a different gene expression profile compared to LN cells.
Aims: Aim of the study was to assess if CLL shows a distinctive qualitative and quantitative pattern of chromosomal alterations in different disease compartments by analyzing copy number aberrations (CNAs) by single nucleotide polymorphism (SNP) arrays in tumor cells extracted concurrently from the PB and LN of each patient.
Methods: Genomic DNA was extracted from paired PB, LN and saliva samples of 10 CLL patients. Cases were selected on the basis of: *i)* availability of CLL cells simultaneously extracted from PB and LN biopsies; *ii)* $CD5^+/CD19^+$ cells in PB and LN >70%; iii) absence of Richter syndrome and *iv)* availability of germline material (saliva). CNA analysis was performed by genomic hybridization using the CytoScan HD array (Affymetrix), according to manufacturer's instruction. The array contains more than $2.6x10^6$ copy number markers, including 750.000 SNPs. Data were analyzed using the Chromosome Analysis Suite (ChAS) Software (Affymetrix). Germline DNA was used to exclude non tumor-related aberrations.
Results: LN samples showed CNAs in all cases, with an average of 4.8 CNAs/case (range1-16). LN CNAs were mainly represented by losses (81%), of which 38% were focal (≤10 genes) and 62% non-focal. The other 19% of CNAs were non-focal gains. PB samples showed CNAs in 9/10 cases, with a mean of 4.4 CNAs/case (range 0-16). A similar pattern to that of LN was found in PB, with 79% of losses and 21% of gains, with a slight increase of focal lesions (45% losses and 17% gains). Overall, a total of 26 CNAs were shared by paired LN and PB samples, while 6 CNAs specific of the LN compartment were not found in the corresponding PB; 2 lesions were specific of the PB compartment. An intra-patient analysis showed that in 3 cases (30%) LN revealed different CNAs compared with the corresponding PB: one exclusive LN lesion (del6q16.3-q26) in case #1, 4 in case #2 (del8p23.3-p11.11, del9p24.3-p13.1, del13q14.1-q31.1 and del13q31.1), while case #3 showed one CNAs exclusive of the LN (del5q14.3-q21.3) and 2 specific of the PB (del6q23.3-q24.1 and del13q14.11). In addition, two shared CNAs were much larger in the LN than PB in case #2 and case #3. The other 7 cases shared the same lesions in both compartments. Among them, it is interesting to note that 8 CNAs of 4 patients were represented at a higher percentage in the LN compared to the PB (mean: 81% in LN and 64% in PB).
Summary / Conclusion: This study shows that a subset of CLL patients with LN involvement reveals a specific pattern of CNAs in LN-derived tumor cells compared to the corresponding circulating counterpart. In fact, LN-derived cells can show either additional or larger CNAs than PB in at least one third of cases, with a greater clonal representation of the common lesions, corroborating the notion that the LN microenvironment contributes to CLL cell proliferation and possible clone selection. Further investigations on the genetic lesions are warranted.

P079

SF3B1 MUTATIONS IN CLL ARE FUNCTIONALLY EQUIVALENT TO ATM GENE ALTERATIONS AND CAUSE DEFECTIVE PUMA AND P21 UPREGULATION IN RESPONSE TO DNA DAMAGE
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Background: Mutations or deletions of the tumor suppressor p53 or its upstream kinase ATM are well-known determinants of poor prognosis in chronic lymphocytic leukemia (CLL). In recent years, genome-wide sequencing has uncovered novel gene mutations that also correspond with poor prognosis. Specifically, recurrent mutations in the splicing factor *SF3B1* and the *Notch1* proto-oncogene have been found. These mutations were (in part) mutually exclusive with *TP53* aberrations, which suggested their overlap in biological function.
Aims: To investigate whether *SF3B1* and *Notch1* mutations affect the p53/ATM axis.
Results: Here, we report results of a comparative analysis of p53 target genes and *in vitro* responses to cytotoxic drugs in CLL samples with *TP53* (n=13), *ATM* (n=18), *SF3B1* (n=20) and *Notch1* (n=10) mutations. Upon irradiation, mRNA induction of p53 targets genes (*p21, Puma, CD95, Bax, PCNA, FXDR)* was decreased in *SF3B1* (overall P<0.01), but not in *Notch1* mutated CLL samples. *SF3B1* mutated samples resembled ATM mutated CLL in displaying a defective but not absent p53 response. At protein level, Puma and p21 induction were defective or absent. This corresponded with decreased apoptosis after *in vitro* treatment with fludarabine. Treatment with nutlin, either alone or in combination with fludarabine, restored cell death induction, again indicating an overlap with ATM dysfunction. Since it is emerging that *SF3B1* mutation correlates with 11q deletion we performed extensive analysis of the coding sequence of the *ATM* gene (exons 1-62) in all *SF3B1* mutated cases. In this cohort there was an overlap of *SF3B1* mutations with *ATM* mutations and/or 11qdeletions in 59% of *SF3B1* mutated cases (10/17; in 3 cases ATM analysis is ongoing), and of 18% (3/17) with *TP53* mutation. Importantly, 4 *SF3B1* mutated cases did not have an *ATM* mutation, 11q deletion or *TP53* mutation, but still these samples displayed an impaired response to irradiation and cytotoxic drugs, indicating that the functional defect can occur independently of *ATM* or *TP53* mutation/deletion.
Summary / Conclusion: In conclusion, the recently described mutations in a splicing factor *SF3B1* in CLL can be linked at the functional level to defective ATM and/or p53 target gene responses, providing an explanation for the poor clinical prognosis of CLL patients with *SF3B1* mutations.

P080

A HIGH NUMBER OF LOSSES IN 11Q CHROMOSOME IS ASSOCIATED WITH SHORT TIME TO FIRST TREATMENT (TFT) AND OVERALL SURVIVAL (OS) IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)
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Background: Genetic abnormalities in CLL define subgroups of patients with different survival. Recently, our group and others have reported that the number of losses in 13q or 17p is associated with the prognosis. CLL patients with 11q- have a bad prognosis, although the exact impact of 11q- in the outcome remains to be elucidated
Aims: To analyze in a multicentric study whether the number of losses in 11q in patients with CLL has an influence in OS and TFT.
Methods: A total of2,493 patients registered in DataBase of CLL of Spanish Group of Cytogenetics (GCECGH) and Spanish Group of CLL (GELLC) were included. Clinical data, FISH information (11q, 12, 13q, 14q and 17p probes) and molecular studies were recorded. Genome-wide expression analysis of clonal B-cell lymphocytes of 11q- patients was also performed using Human Gene 2.0 microarrays (Affymetrix).
Results: A total of 242 patients (10.3%) had 11q-. The final analysis was limited to 197 cases (151 male, median age 65 yr) after excluding cases with monoclonal B-cell lymphocytosis, lack of clinical data or inappropriate follow-up. Most of patients (61%) were in Binet's stage A. In 82 out of 197 patients (42%) 11q- was the sole cytogenetic aberration at diagnosis. Median OS of patients with 11q- was 106 months (CI95%, 97-128) and TFT was 25 months (CI95%, 31-44). Interestingly, in patients with loss of 11q ³40 % of cells (146 cases, 74%), the OS was 90 months (CI95%, 57-123), while in the group with <40 % of losses in 11q, the OS has not been reached (CI95%, 114-157) (P=0.008).In the univariate analysis, clinical stage (P=0.002), B-symptoms (P=0.041), hepatomegaly (P=0.025), splenomegaly (P<0.0001), lymphocyte count >20 x 10^9/L (P= 0.049), a high serum LDH (P<0.0001), β_2M levels (P<0.0001) and high number of cells 11q- (>40) (P< 0.0001) were associated with a short OS. In the Cox analysis for OS, variables included in the final model were serum LDH (P=0.035), β_2M serum (P=0.005) and del11q ≥40 (P=0.004).Regarding TFT, in patients with ≥40% of losses in 11q the median TFT was 18 months (CI95%, 12-24) vs. 44 months (CI95%, 33-55) (P<0.0001).In the univariate analysis, significant variables were clinical stage (P=0.004), serum LDH (P=0.045), serum β2M (P=0.012), high CD38 expression (P=0.022), high ZAP70 expression (P=0.025), unmutated IGVH (P<0.0001) and del11q ≥40 (P<0.0001). In the multivariate analysis, only unmutated IGVH status resulted significant in predicting TFT (P=0.014). No differences in OS or TFT were observed between patients with del11q as a sole cytogenetic aberration vs. del11q plus other cytogenetic aberration, although patients with 11q- and 13q- combination exhibited a trend for a better OS (P=0.06). Regarding gene expression analysis, patients with 11q- showed a distinctive gene expression profile characterized by an activation of NFkB signaling, due to the overexpression of genes such as BTRC and TLR. In addition, an overexpression of URB4, ILK, BSG, SHH, and downregulation of STC1 and CASR, leading to a decreased apoptosis (P=0.006), as well as upregulation of ILK, BTRC, RPL7A and FOXM1, involved in deregulation of the cell cycle (P=0.03) was observed in 11q- patients. Of note, overexpression of JUNB, described as a proto-oncogen was also observed in 11q-patients, which could lead to a higher cell proliferation (P=0.009).
Summary / Conclusion: In patients with CLL, a high number of losses in 11q is associated with a shorter TFT and OS. This group of patients is characterized by an activation of NFkB signaling leading to a decreased apoptosis and high proliferation

P081

NOTCH1 MUTATED IGHV UNMUTATED CHRONIC LYMPHOCYTIC LEUKEMIA CONSTITUTIVELY OVEREXPRESSED NUCLEOPHOSMIN-1 AND RIBOSOME-ASSOCIATED COMPONENTS

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Background: stabilizing mutations of *NOTCH1* have been identified in about 10% of chronic lymphocytic leukemia (CLL) cases at diagnosis, with a higher frequency in unmutated *IGHV* (*IGHV*-UM), immuno-chemorefractory or advanced disease phase CLL. In CLL, all *NOTCH1* mutations disrupt the C-terminal PEST domain (about 80% of which are a 7544-7545delCT frameshift deletion) and cause an accumulation of an active NOTCH1 isoform. Clinically, the presence of *NOTCH1* mutations is an independent predictor of overall survival in CLL and identifies a subset of patients with particularly unfavourable prognosis (Rossi *et al*, Blood, 119, 2012; Del Poeta *et al*, Br J Haematol, 160, 2013).
Aims: to identify molecular/biological features of *NOTCH1* mutated CLL.
Methods: the presence of the *NOTCH1* 7544-7545delCTwas investigated by ARMS-PCR.The percentage of *NOTCH1* DNA in the context of the CLL clone was determined by quantitative real-time PCR (QRT-PCR). Gene expression profile (GEP) was performed by a one-color labeling strategy using the 4x44K platform. Specific gene/protein validations were performed by QRT-PCR, western blotting, immunofluorescence for confocal microscopy and immunohistochemistry. Proliferation of CLL cells by CpG/IL2 stimulation was evaluated by a BrdU uptake assay.
Results: in a cohort of 431 *IGHV*-UM CLL, the *NOTCH1* 7544-7545delCT was found in 97/431 (22.5%) cases. QRT-PCR revealed a percentage of *NOTCH1* mutated DNA ranging from 1 to 37%. CLL carrying the *NOTCH1* 7544-7545delCT (*NOTCH1*-mut, 8 cases; from 11% to 37% of *NOTCH1* mutated DNA) showed higher NOTCH1 protein expression than cases lacking *NOTCH1*-mut (11 cases) employing monoclonal antibodies either recognizing the trans-membrane (mean fold increase=3.0) or the intra-citoplasmic (mean fold increase=2.1) NOTCH1 domain. A GEP comparing purified cells of 5 *IGHV*-UM *NOTCH1*-mut CLL (from 15% to 37% of *NOTCH1* mutated DNA) and 5 *IGHV*-UM CLL lacking *NOTCH1*-mut selected nucleophosmin-1 (*NPM1*) and genes codifying for several ribosomal proteins (*RPS6*, *RPS10*, *RPS17*, *RPS28*, *RPSA*, *RPL7A*, *RPL18*) as significantly up regulated in *NOTCH1*-mut CLL. QRT-PCR validations confirmed GEP results in a wider series of 34 cases (18 *NOTCH1*-mut cases). Western blot in 19 cases (8 *NOTCH1*-mut cases) confirmed a higher NPM1 protein expression in *NOTCH1*-mut cases (1.3-5.2 range of fold increase). Consistently, lymph nodes preparations from *NOTCH1*-mut cases revealed a strong NPM1 staining both in nucleoli and cytoplasms. When intracellular distribution and fluorescent intensity of *NPM1* immunostaining was evaluated, both *NOTCH1*-mut and *NOTCH1*-wt showed a visible nucleoplasmic staining; with an additional cytoplasmic staining visible in *NOTCH1*-mut cases. Finally, when stimulated in-vitro with the CpG/IL2 combination, *NOTCH1*-mut *IGHV*-UM CLL cells proliferated, as detected by a BrdU uptake assay (>10 fold increase over control), and up-regulated NPM1 both at transcript (mean fold increase=2.02 after 18 hours of CpG exposure, P=0.001) and protein (fold increase=1.34 after 6 hours of CpG exposure) levels.
Summary / Conclusion: NPM1 was constitutively overexpressed in *NOTCH1*-mut *IGHV*-UM CLL together with several ribosome-associated components. An increased activity of the ribosomal machinery/DNA-repair mechanisms (Lindstrom MS. Biochem. Res. Int. 2011, 2011) in *NOTCH1*-mut CLL may concur to explain immuno-chemorefractoriness of patients affected by CLL bearing this novel mutation.

P082

CLL: DIFFERENT AGES, DIFFERENT ANTIGEN RECEPTOR PROFILES

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Background: CLL is considered a disease of the elderly, however, 20-30% of patients referred to specialized hematological centers are aged 55 or younger. Limited information is available concerning possible distinctive features of younger patients, especially with respect to the immunoglobulin (IG) repertoire of the clonotypic antigen receptors, which is relevant in view of recent therapeutic developments.
Aims: We conducted a large, multicenter study aiming at obtaining insight into

the clinical and biological characteristics of patients in different age groups, with a special emphasis on immunogenetic profiles.
Methods: The cohort included 4615 patients with the following characteristics: males: 2953/4615 (64%); Binet stage A/B/C: 2154/624/346; mutated/unmutated (M/U) IGHV genes: 2472 (53.5%)/2143 (46.5%); CD38 expression: 905/3176 (28.5%); del(13q): 1383/2284 (61%), del(11q): 362/2248 (16%), del(17p): 258/2272 (11%), trisomy 12: 414/2090 (20%). Based on age distribution, patients were divided in four groups, each representing almost a quartile of the sampled population: A: 71+ years, n= 1048; B: 63-70 years, n=1158; C: 55-62 years, n=1218; and D: <55 years, n=1191.
Results: Advanced age (71+) was associated with Binet stage C (P=0.003), CD38 expression (P=0.015) and trisomy 12 (P<0.001); in contrast, del(11q), del(13q) and del(17p) were equally distributed among the four age groups. The mutational status of the clonotypic IGHV genes was overall similar in all age groups. Notably, however, the four age groups exhibited distinct IGHV gene repertoires: (i) lower frequency of the IGHV4-34 and IGHV1-69 genes in group A (71+) versus groups B-C-D (P=0.008); (ii) higher frequency of the IGHV4-39 and IGHV1-2 genes in group A (71+) versus groups B-C-D (P=0.032); (iii) predominance of the IGHV3-21 gene in groups A-B-C versus D (<55) (P<0.05). Extending the analysis to B cell receptor (BcR) stereotypy, the age distribution of major stereotyped subsets was distinctly different (P<0.001). In particular, patients in subsets #4 (M-CLL, IGHV4-34), #148 (M-CLL, IGHV2-5), #3, #5 and #7 (U-CLL, all IGHV1-69) had significantly younger ages at diagnosis compared to subsets #1 (U-CLL, Clan I genes), #2 (IGHV3-21), #6 (U-CLL, IGHV1-69) and #8 (U-CLL, IGHV4-39). Among 2952 cases with available data, disease progression requiring treatment was seen in 63% of Group A cases, 69% of Group B cases, 72.6% of Group C cases and 75.2% of Group D cases (P<0.001); admittedly, this could also be related to the fact that younger patients are allowed more time to progress before they die of comorbidity. In univariate analysis, advanced age had a significant negative impact on time-to-first-treatment (TTFT) (P=0.04) along with male gender, advanced clinical stage, unmutated IGHV genes, CD38 expression and del(17p). However, in multivariate analysis only advanced clinical stage, unmutated IGHV genes and del(17p) retained significance.
Summary / Conclusion: In conclusion, CLL patients in different age groups exhibit distinct features extending from clinical stage at diagnosis to immunogenetic profiles. The striking IG repertoire differences between younger versus older patients with CLL could reflect different rates of disease progression or different antigen exposure histories, including excessive production of apoptotic and oxidative products due to aging that could stimulate cells with distinctive antigen receptors. Alternatively, repertoire biases in older patients might also be a consequence of the physiological process of immune senescence.

P083

DETECTION OF TP53 DYSFUNCTION IN CHRONIC LYMPHOCYTIC LEUKEMIA BY AN IN VITRO FUNCTIONAL ASSAY BASED ON TP53 ACTIVATION BY THE NON-GENOTOXIC DRUG NUTLIN-3: A PROPOSAL FOR CLINICAL APPLICATION

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Background: Impaired TP53 function through mutations and/or deletions is the most characterized factor associated with chemoresistance in chronic lymphocytic leukemia (CLL). Since direct sequencing, the standard technique for *TP53* mutation detection, does not evaluate TP53 function, a functional assessment of the TP53 pathway may be of interest to identify high risk CLL.
Aims: To develop a short-term functional assay evaluating TP53 and TP53 target gene modulation upon *in vitro* exposure of CLL cells to the non-genotoxic TP53 activator Nutlin-3.
Methods: The functional assay was set-up on cell lines recapitulating all *TP53* genotypes (EHEB, $TP53^{wt/wt}$; RAJI, $TP53^{mut/wt}$; MEC-1 and MAVER1, $TP53^{mut/del}$; HL-60, $TP53^{del/del}$) and evaluated in two multi-institutional cohorts, purposely enriched in CLL bearing *TP53* disruption: a training cohort (TC) of 100 cases and a validation cohort (VC) of 40 cases, characterized by FISH and *TP53* direct sequencing. Cells were exposed to 10μM of Nutlin-3 for 24 hours. TP53 accumulation was evaluated by Western blotting (WB) and TP53 transcriptional activity was determined by quantitative real time PCR (qRT-PCR) of the TP53-target genes *CDKN1A, BAX, PUMA*.
Results: By WB analysis on cell lines, we defined: i) a normal pattern (i.e. absence of basal TP53 and induction after treatment) in EHEB cells; ii) a mutant pattern (i.e. high basal TP53 without increase after treatment) in MEC-1, MAVER-1 and RAJI cells; iii) a null pattern (i.e. absence of TP53 before and after treatment) in HL-60 cells; iv) a detection sensitivity of 5% of TP53-expressing cells by dilution experiments. By WB analysis of the TC, we defined: i) 63 normal patterns (51 $TP53^{wt/wt}$, 12 $TP53^{del/wt}$); ii) 18 mutant patterns (3 $TP53^{mut/wt}$, 15 $TP53^{mut/del}$); iii) 19 intermediate patterns, i.e. an important basal accumulation of TP53 which increased upon Nutlin-3 exposure (11 $TP53^{wt/wt}$, 5 $TP53^{mut/wt}$; 3 $TP53^{mut/del}$). QRT-PCR of TC cases for expression of the TP53-target genes *CDKN1A*, *BAX*, *PUMA* revealed that all the 25 cases with a mutated *TP53* status ($TP53^{mut/wt}$ or $TP53^{mut/del}$) had negligible/no induction of all the three TP53-target genes upon Nutlin-3 exposure, while in the 75 cases with unmutated *TP53* status ($TP53^{wt/wt}$ or $TP53^{del/wt}$) a marked induction of all three genes (P<0.001) was observed. Of note, *CDKN1A* had the greatest amplitude of induction compared to *BAX* or *PUMA* (P<0.001 for both comparisons), suggesting the use of this target for the TP53 functional evaluation. In particular, a value of about 5-fold increase for *CDKN1A* was able to segregate: i) all the 18 mutant patterns from the majority (56/63) of normal pattern cases (including the 12 $TP53^{del/wt}$ cases); ii) in the context of cases with an intermediate pattern by WB, the 8 cases with a *TP53* mutated status from the 11 $TP53^{wt/wt}$ cases. In addition, this approach was able to identify 7 cases with a normal WB pattern which failed to up-regulate *CDKN1A*, i.e. putatively carrying defects on the DNA damage pathway other than TP53 defects. The proposed functional assay was separately validated "in blind" (5 independent data analysers) in a VC which included 13 $TP53^{wt/wt}$ cases, 3 $TP53^{del/wt}$ cases, 12 $TP53^{mut/wt}$ cases and 12 $TP53^{mut/del}$ cases (sensitivity 0.9, 95% CI 0.78-1; specificity 0.875, 95% CI 0.713-1).
Summary / Conclusion: The combined evaluation of TP53 and *CDKN1A* modulation upon Nutlin-3 exposure may represent a useful low-cost functional test to identify TP53 dysfunctional cases that escape FISH and direct sequencing. This approach may contribute to refine the prognostic assessment of high risk CLL.

P084

ATM INACTIVATION DISTURBS ATM-P53 PATHWAY IN RESPONSE TO DNA DAMAGE INDUCED BY DOXORUBICIN BUT NOT FLUDARABINE IN CLL CELLS

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Background: Abnormalities of *ATM* gene are frequent in chronic lymphocytic leukemia (CLL) patients and represent important prognostic factor. *ATM* defects are commonly assessed through 11q deletion (11q-) monitoring, nevertheless complete ATM inactivation stems from biallelic *ATM* defects (mutation/11q- or two mutations) or from sole *ATM* mutation manifesting dominant-negative effect. After DNA damage caused by ionizing radiation (IR), it is well established that ATM plays crucial role in response to dsDNA breaks (DSBs), especially through triggering the p53 pathway. The impact of ATM inactivation in response to conventionally used DNA damaging drugs is much less understood although this inactivation could have important predictive value.
Aims: Our aim was to assess ATM inactivation impact on *in vitro* CLL cells response to fludarabine and doxorubicin, with respect to the ATM-p53 pathway activation.
Methods: To assess p53 pathway activation after drug exposure we used: (a) Real-time PCR to analyze p53-downstream gene (*CDKN1A* (*p21), BBC3* (*PUMA), BAX,* and *GADD45*) induction after 24 h treatment of CLL cells with fludarabine and doxorubicin or after2, 10, 24 h IR exposure (5 Gy in total, 0.3 Gy/min), (b) Western blotting (WB) for total p53 and Ser15-p53 after 24 h fludarabine treatment; these experiments were performed on wt samples with artificially inactivated ATM (inhibitor KU55933) and on CLL samples harboring *ATM* mutation(s), and (c) WB for Ser1981-ATM and Ser15-p53 to evaluate immediate ATM activation in mutated samples after 1 h IR exposure.
Results: The samples with inactivated ATM exhibited clearly impaired induction of all four p53-downstream genes after doxorubicin, but not fludarabine treatment (3/3 artificially inactivated samples and 16/20 *ATM*-mutated samples from our previous study; 14 samples harbored biallelic defect and two harbored single mutation at hot-spot codon 3008). Since doxorubicin is supposedly radiomimetic drug, we verified the ATM-dependent response to DSBs using IR. We performed the same analysis on 8 *ATM*-mutated samples and 5 samples with preserved ATM function (i.e., wt or sole 11q-) and observed similarly impaired response in ATM-defective group, however with the exception of *BAX* gene that had preserved induction. The preserved p53-downstream pathway response after fludarabine prompted us to analyze p53 accumulation and activation. Firstly, we confirmed that fludarabine creates DSBs (gH2AX accumulation after 24 h exposure) and then showed that in relevant proportion of cases the p53 stabilization is present (2/4 artificially inactivated samples and 3/4 *ATM*-mutated samples; the last mutated sample demonstrated partial p53 stabilization). Our observations show subtle ATM impact on studied response to fludarabine suggesting the involvement of other signaling kinase(s) in the p53 pathway activation. To further analyze loss of ATM function within the DNA damage response cascade, we also monitored ATM autophosphorylation on Ser1981 and p53 phosphorylation on Ser-15 in two *ATM*-mutated samples after IR. One *ATM*-mutant showed obvious-

ly diminished autophosphorylation and both mutants completely lost any activity towards p53 activation and stabilization.
Summary / Conclusion: CLL cells lacking ATM activity manifest clearly impaired p53 pathway activation after doxorubicin, while this response appears to be normal after fludarabine. It seems that ATM inactivation is prominently manifested in the end of ATM-p53 pathway while initial processes are less influenced. The work was supported by grants FR-TI2/254, NT13519 and MUNI/A/0723/2012.

P085

SEQUENTIAL MRD ANALYSIS IN CLL DEMONSTRATES EXPONENTIAL EXPANSION WITH A PATTERN THAT CANNOT BE PREDICTED FROM PRE-TREATMENT DOUBLING TIME.

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Background: Minimal residual disease (MRD) is an independent predictor of disease-free and overall survival in B-cell chronic lymphocytic leukaemia. There is a correlation between the levels of residual disease and the time to relapse which suggests that CLL cells probably undergo exponential expansion even at a very low level. However the kinetics of disease progression when CLL cells represent less than 1% of total leucocytes is not clearly defined.
Aims: To obtain pilot information on the pattern of relapse at low level which could be valuable for the design of clinical trails aimed at consolidation, maintenance and MRD eradication.
Methods: Peripheral blood samples from 30 individuals with CLL who achieved MRD-negative status after treatment were prospectively assessed for minimal residual disease using multi-parameter flow cytometry with a detection limit of 0.004% every 3-6 months. Inclusion criteria for this analysis were the availability of at least 4 sequential samples with detectable residual disease with no therapeutic intervention during the follow-up period and 18/30 patients were evaluable.
Results: CLL cells showed an exponential increase from the first point of detection in all evaluable cases. There was a median of 6 (range 4-19) informative time-points in the 18 cases. The Pearson correlation coefficient between log CLL cell level and time since first MRD-positive sample was a median 0.981, range 0.903-0.998. Doubling times after treatment were significantly lower than pre-treatment (pre-treatment median 6.3 months vs. post-treatment median 2.3 months, paired T-test P=0.03). Although the expansion was exponential it was noted that in many individuals there was an apparent pause in progression followed by a change in the expansion rate when the CLL cell level approach normal B-cell levels, i.e. approximately 500/µL. We further analysed 8 patients with sufficient data to evaluate expansion kinetics when the CLL cell levels were below 500/µL and compared to the rate of expansion when CLL cell levels were above 500/µL. The doubling time was on average twice as long when the CLL cell levels were above 500/µL compared to the initial rate (paired T-test P=0.049) although individuals with an initial doubling time less than 4 months typically maintained a rapid doubling time (n=5). Changes in the doubling time from less than 6 months to more than 12 months were seen in 2/8 cases.
Summary / Conclusion: The results from this pilot data support the hypothesis that expansion of CLL cells even at the lowest detectable levels follows an exponential pattern. Cases with a rapid doubling time before treatment also show rapid expansion after treatment but otherwise the post-treatment CLL doubling rate is not predictable from pre-treatment lymphocyte doubling time. In many cases the rate of expansion changes as CLL cell count approaches normal B-cell levels. Analysis of a larger series of cases is required to understand the pattern of expansion and identify optimal time-points to re-introduce treatment. The data provides biological support for the application of MRD as a surrogate end-point in CLL treatment.

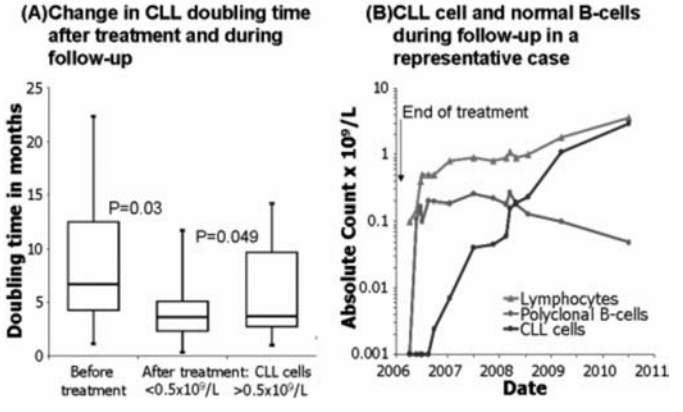

Figure 1.

P086

NOTCH1 MUTATIONS ARE ASSOCIATED WITH THE 14Q DELETION IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND LYMPHOCYTIC LYMPHOMA (LL)

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Background: The deletion of the long arm of chromosome 14 (del14q) is a rare (<5%) but recurrent event in CLL. The size of the deletion is variable, and its molecular consequences are unknown.
Aims: The aim of our study is to characterize this abnormality in a large cohort of patients.
Methods: The Groupe Francophone de Cytogenetique Hematologique (GFCH) collected data from 89 patients with CLL or LL, harboring a del14q. Morphological review was performed for 65 of them and immunological review for 48. All karyotypes were reviewed by members of the GFCH. Fluorescence in situ hybridization (FISH) analysis was performed with 5 probes (CEP12, 13q14, TP53, ATM, 6q21), an IGH probe, and BACs RP11-35D12 and RP11-226F19 covering the ZFP36L1 gene on 14q24.1. SNP-array analysis (illumina omni1, omin2.5) was performed for 37 patients. The *IGHV* status was analyzed locally, or in our laboratory. The mutation hotspots of *NOTCH1* (exon 34), *SF3B1* (exons 14-16), *XPO1* (exons 14-15), *MYD88* (exon 5) and *TP53* (exons 4-10) were analyzed by direct sequencing.
Results: Patients were classified as 49 CLL Matutes score 4-5, 5 atypical CLL score 3 (all CD5+, CD23+), and 27 LL. The sex ratio M/F was 1.02. Among CLL, there were 35 (66%) stages A and 18 (34%) stage B/C. The median time from diagnosis to first treatment was 17 months for CLL, 1 month for LL. Eight patients were excluded with a Matutes score<3 or not evaluable. The karyotype showed chromosomal translocation in 26/79 (33%) (10 balanced, 16 unbalanced) and was complex (> 3 abnormalities) in 26/79 (33%) cases. Using karyotype and FISH, we observed 28/79 (35%) trisomy 12, 12/79 (15%) 13q14 deletions, 11/80 (14%) *TP53* deletions, 5/79 (6%) *ATM* deletions, 3/76 (4%), 6q21 deletions. The whole CLL cohort showed 15/53 (28%) tri 12, 11/53 (21%) del13q, 8/54 (15%) del*TP53*, 4/53 (7%) del*ATM*, 2/50 (4%) del6q. There was no significant difference between CLL score 4-5 and CLL score 3. *IGHV* status was not mutated in 41/53 (77%) patients, and the gene *IGHV1-69* was rearranged in 21/52 (40%) cases. *NOTCH1* gene was mutated in 14/45 (31%) patients, *SF3B1* in 3/45 (7%), *XPO1* in 2/45 (4.5%), *MYD88* in *0/43*, *TP53* in 6/43 (14%) cases. Comparing LL to CLL, there was no significant difference regarding cytogenetic and molecular abnormalities except for trisomy 12, more frequent in LL (13/26 (50%) vs 15/53 (28%), P=0.08) and 13q14 deletion, less frequent in LL (1/26 (4%) vs 11/53 (21%), P=0.09). The 14q deletions appeared distributed along chromosome 14 from bands q11 to q32. The centromeric and telomeric breakpoints of the 14q deletion were investigated by FISH or SNP-array when material was available, and allowed us to categorize the patients in 4 groups: Group 1: 37/77 (48%) patients, with IGH and ZFP36L1 loci rearranged; Group 2: 17/77 (22%), with ZFP36L1 deleted and IGH not deleted; Group 3: 7/77 (9%), with ZFP36L1 and IGH not deleted; Group 4: 16/77 (21%), all the other combinations. Group 1 showed a deletion del14q24.1-14q32.3 of about 38 megabases, which broke in or near *ZFP36L1*, and in the *IGH* gene. It included 26 (70%) LLC score 4/5 and 11 (30%) LL. When compared to the other patients, patients in Group 1 showed significantly more trisomy 12 (18/36 (50%) vs 7/40 (17.5%), P=0.004), and *NOTCH1* mutations (9/18 (50%) vs 4/26 (15.5%), P=0.02). Of note *NOTCH1* mutation was not statistically correlated with trisomy 12. *IGHV* status was unmutated in 22/24 (92%) patients in Group1, 12/24 (50%) harboring the gene *V1-69*.
Summary / Conclusion: When compared to the literature, both CLL and LL with del14q are associated with higher tri12, lower 13q deletion, higher del*TP53*, higher unmutated *IGHV* status, with an over-representation of the *V1-69* repertoire, and higher *NOTCH1* mutated, some of them being poor prognostic factors. The size of the 14q deletion is variable, with in about half of the cases a recurrent interstitial deletion 14q24.1-14q32.3. Among all 14q deletions, patients with both IGH and ZFP36L1 loci rearranged are more specifically associated with trisomy 12, *NOTCH1* mutated, *IGHV* unmutated, and the *V1-69* gene.

P087

UNRAVELING THE HETEROGENEITY OF T-CELL PROLYMPHOCYTIC LEUKEMIA (T-PLL)

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Background: T-cell prolymphocytic leukemia (T-PLL) is a proliferation of small to medium sized prolymphocytes with a mature post-thymic T-cell phenotype. T-PLL accounts for 2% of all mature lymphocytic leukemias in adults over the age of 30. T-PLL is a heterogeneous disease with a wide range of clinical, morphological and molecular features which occasionally impedes the diagnosis. Despite current knowledge of the biology, T-PLL patients remain to have a poor prognosis with a short median survival of 7.5 months due to an aggressive disease progression and limited therapeutic responses.
Aims: To further understand the biology of this disease and to identify possible subgroups we selected 27 T-PLL cases for a comprehensive analysis of phenotypic and genotypic features.
Methods: Multi-color flow cytometry, micro-array gene analysis, TCR rearrangement analysis.
Results: For all 27 T-PLL clonality was confirmed, but no stereotyped T-cell receptor beta (TRB) usage was observed. Cytogenetic analysis showed complex aberrations, including frequent occurrence of the inv(14)/t(14;14) aberration. In accordance with literature, our T-PLL cohort consists of 56% CD4+/CD8-, 33% CD4+/CD8+ and 11% CD4-/CD8+, whilst no other clear subgroups could be observed by extensive eight-color flowcytometry. Interestingly, when comparing the T-PLL phenotype to that of normal T-cell subsets, T-PLL mostly cluster with normal memory T-cells and less with naïve and effector T-cells, suggesting that memory T-cells might be the normal counterpart of T-PLL. Preliminary micro-array-based gene expression data on 23 T-PLL and these normal T-cell subsets confirms this suggestion. Furthermore, unsupervised analysis of the gene expression data is suggestive of at least 2 T-PLL subgroups, but these T-PLL subgroups do not seem to directly correlate with morphological variants of T-PLL (blastic vs. lymphocytic).
Summary / Conclusion: Overall, our findings seem to support the idea of subgroups within the T-PLL entity, and further investigations are required to establish the potential clinical implications of this heterogeneity.
This work was supported by an unrestricted grant from Genzyme and Sanofi.

P088

EPIGENETIC INACTIVATION OF MIR-34B/C IN ADDITION TO MIR-34A AND DAPK1 IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: *TP53* mutation or del(17p) is only found in only 5% to 10% of chronic lymphocytic leukemia (CLL) patients at diagnosis. The inactivation of other members in TP53-centered network, may also block TP53 downstream pathway. *DAPK1* gene, as the upstream gene of TP53, could trigger TP53 activation upon oncogenic cellular transformation and its methylation may contribute to CLL progression by blocking the apoptosis of leukemia cells. Moreover, since *miR-34* family is a transcriptional target of TP53, its methylation might also lead to the perturbation of the TP53 tumor suppression pathway.
Aims: We postulated that components of TP53-centered tumor suppressor network, *miR-34b/c*, in addition to *DAPK1* and *miR-34a* might be inactivated by DNA hypermethylation in CLL. Moreover, we tested if *miR-34b/c* methylation might correlate with *miR-203* or *miR-124-1* methylation.
Methods: *miR-34b/c*, *miR-34a* and *DAPK1* methylation was studied in 8 normal controls, 7 CLL cell lines, and 78 diagnostic CLL samples by methylation-specific polymerase chain reaction. MEC-1 cells were treated with 5-Aza-2'-deoxycytidine for reversal of methylation-associated miRNA silencing. Tumor suppressor function of *miR-34b* was illustrated upon over-expression of precursor *miR-34b* in MEC-1 cells.
Results: *miR-34b/c* promoter was unmethylated in normal controls, but completely methylated in 4 CLL cell lines. *miR-34b/c* expression was inversely correlated with *miR-34b/c* methylation. 5-Aza-2'-deoxycytidine treatment led to promoter demethylation and *miR-34b* re-expression in MEC1 cells. Moreover, over-expression of *miR-34b* resulted in enhanced cellular death and inhibition of cell proliferation. In 78 primary CLL samples, *miR-34a*, *miR-34b/c* and *DAPK1* methylation was detected in 2.6%, 17.9% and 34.6% of patients at diagnosis respectively. Furthermore, 39.7%, 3.8% and 2.6% patients had methylation of one, two or all three genes respectively. Overall, 46.2% patients had methylation of at least one of these three genes. Besides, *miR-34b/c* methylation was associated with methylation of *miR-34a* (P=0.03) and *miR-203* (P=0.012).
Summary / Conclusion: *miR-34b/c* is a tumor suppressor miRNA frequently methylated, and hence silenced in CLL. Together with *DAPK1* methylation, *miR-34b/c* methylation may contribute to the disruption of the TP53-centered tumor suppressor pathway. Moreover, the association of miRNA methylation in CLL warrants further study.

P089

GENOMIC LANDSCAPE OF PRIMARY ULTRA-HIGH RISK AND REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA: RESULTS FROM THE CLL2O TRIAL

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Background: Ultra-high risk chronic lymphocytic leukemia (CLL) can be defined by the presence of *TP53* loss and/or mutation, refractory disease or early relapse (<24-36 months) after treatment with purine analogue-based combinations. The underlying pathogenic mechanisms are yet only partly understood.
Aims: To obtain a comprehensive registry of the genome in primary ultra-high risk and refractory CLL, 83 samples obtained from the CLL2O trial were screened for copy number alterations (CNAs) and copy-neutral loss of heterozygosity (CN-LOH) by Affymetrix 6.0 single nucleotide polymorphism (SNP) arrays in parallel with chromosome banding analysis (CBA).
Methods: 52 cases were treatment-naïve, primary ultra high-risk cases carrying del(17p). The other 31 cases were refractory to fludarabine or bendamustine-based therapy. SNP-array analysis was performed on CD19 sorted CLL cells against intra-individual reference DNA (paired); data was analyzed using dChipSNP, reference alignment and circular binary segmentation. CBA was performed using the immunostimulatory CpG-oligonucleotide DSP30 and IL-2.

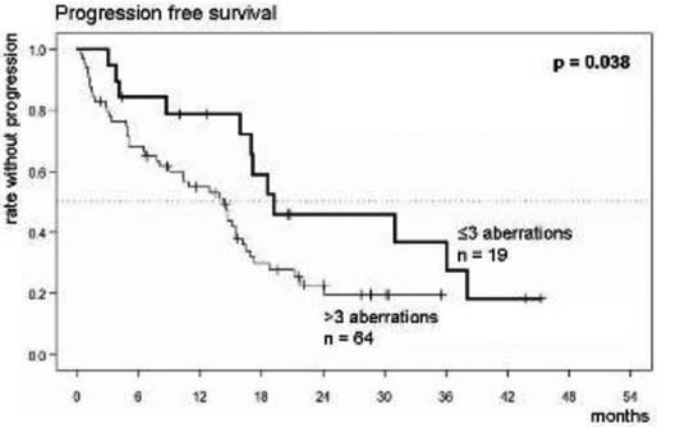

Figure 1. Kaplan-Meier estimates for progression free survival, according to the presence of a complex karyotype detected by combined SNP-array and chromosome banding analysis (defined by >3 aberrations per case).

Results: In total, 499 tumor-specific CNAs were discovered by SNP-array analysis leading to a mean number of 6.01 per case. Compared to data obtained from standard-risk patients at first treatment, this number was strikingly high (CLL8 trial; 1.8 CNAs per case). In contrast, tumor-specific CN-LOH was rare as it was found in only 10 cases. 122 (24%) CNAs detected by SNP-array could not be observed by CBA mostly due to their small size. Of note, 290 (77%) of the remaining 377 CNAs resulted from complex genomic rearrangements. Only 87 (23%) CNAs were described as simple loss or gain of genomic material by CBA. Altogether 280 translocations – mostly unbalanced – could be observed in CBA in 71 cases (range: 1 to 12/case). Translocation breakpoints in at least 5 cases were found in the following cytobands: 17p11 [n=15], 13q14 [n=12], 17p12 [n=9], 14q32 [*IGH*-locus, n=7], 12q24 [n=6], 3q21, 8p11, 13p11 and 19q13 [n=5 each]. Of note, 47 unbalanced translocations in 37 cases had their breakpoint in the centromere resulting in whole arm translocations. Whole arm translocations with concomitant loss of the short arm were the most frequent mechanism leading to del(17p) [n=35; 42% of all 17p deletions] with chromosomes 18 [n=7], 8 [n=5], 15 [n=5] and 17 [isochomosome 17q; n=5] being the most common translocation partners. Other mechanisms leading to del(17p) were complex genomic rearrangements resulting in derivative [n=30; 36%] or dicentric chromosomes [n=5; 6%]. Simple loss of the short arm was

the underlying mechanism in only 13 cases [16%]. Interestingly, up to three different mechanisms of acquiring del(17p) could be observed within one case. Despite the complex and heterogeneous genomic lesions, recurrent concomitant lesions could be identified. The most common ones were gain(8)(q24.21) [n=14; 17%]; del(15)(q15.1) [n=12; 14%], del(10)(q24.32) [n=12; 14%] and del(9)(p21.3) [n=10; 12%]. Cases with loss and/or mutation of *TP53* [n=70] had more genomic aberrations per case than those without [median 6 vs. 4; P=0.02]. Of note, prior therapy had no influence on genomic complexity: treatment-naïve cases had a mean of 6.7 aberrations per case versus 6.0 in cases with prior Fludarabine or Bendamustin-based therapy. In univariate analysis, patients with a complex karyotype defined by more than 3 aberrations had an inferior progression free survival (PFS) [P=0.04] and a trend towards inferior overall survival [P=0.11].

Summary / Conclusion: Primary ultra-high risk (17p- / *TP53* mutated) CLL is characterised by high genomic complexity, similar to refractory CLL. Whereas mutation or loss of *TP53* was associated with increased genomic aberrations, prior therapy did not appear to further increase this. The presence of a complex karyotype was associated with shorter PFS, though its prognostic value has to be further evaluated in multivariate analysis.

P090

CD49D HAS INDEPENDENT NEGATIVE PREDICTIVE POWER FOR OVERALL SURVIVAL IN CHRONIC LYMPHOCYTIC LEUKEMIA: RESULTS FROM MULTI-CENTER POOLED ANALYSIS

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Background: A number of data support the value of CD49d expression as independent prognostic variable in chronic lymphocytic leukemia (CLL). However, the available studies differ for: i) clinical end points employed (overall survival, OS; treatment free survival, TFS); ii) factors included in multivariate analyses; iii) choice of cut-off to define CD49d positivity.

Aims: To perform a worldwide multi-center pooled analysis using individual patient data (IPD) from published as well as unpublished series to evaluate the ability of CD49d to predict OS in CLL.

Methods: Authors provided IPD from published cohorts identified by Medline search by 30 April 2011, as well as from unpublished series. The following variables were collected: date of diagnosis, OS, TFS, CD49d, CD38, ZAP-70, immunoglobulin mutational status (*IGHV*), del17p and del11q chromosomal aberrations, age, stage, absolute lymphocyte count (ALC), and β2 microglobulin concentration. We performed a pooled analysis with a fixed effect model, applied multivariate Cox proportional regression analysis, stratified for study site, and recursive partitioning to rank the relative importance of CD49d with respect to the other flow cytometry prognostic factors in CLL (CD38, ZAP-70). The optimal CD49d cut off to predict OS was chosen with a training/validation strategy.

Results: IPD from3,267 patients was initially available. After excluding 265 patients for missing data, the remaining2,972 CLL patients were included in final analysis (1,556 published; 1,416 unpublished). The optimal CD49d expression cut-off was investigated by applying both data-driven and outcome-driven methods. Although these methods failed to agree on a single cut-off, the value of 30% CD49d positive CLL cells was finally chosen because of its slightly higher outcome discrimination capacity, (C-index 0.61 vs 0.59 for 30% and 45% respectively; P<0.0001). By pooled analysis of the merged database CD49d expression ≥30% associated with a 2.5 increase in the hazard of death (HR=2.5; 95% CI, 2.1-3.0), resulting into an OS decrease of 7% at 5-years (94% in CD49d- CLL versus 87% in CD49d+ CLL) and 23% at 10-years (84% in CD49d- CLL versus 61% in CD49d+ CLL) for CD49d positive patients compared to CD49d negative cases. OS shortening was explained by the higher progression rate displayed by CD49d positive cases. Indeed, CD49d positive patients showed a significantly lower probability of remaining treatment free at both 5-years (68% in CD49d- CLL versus 42% in CD49d+ CLL), and 10-years (50% in CD49d- CLL versus 24% in CD49d+ CLL. In a multivariate Cox model for OS stratified for study site, CD49d, but not CD38 and ZAP-70 expression, remained an independent prognosticator (adjusted HR=2.0, 95% CI, 1.4-3.0) along with age, gender, *IGHV* mutational status, del17p and ALC. Consistently, a recursive partitioning hierarchical full-grown tree model which included the three flow cytometric prognosticators, selected CD49d at the first split indicating that no additional prognostic power was given by CD38 and ZAP-70 after CD49d was considered. CD49d was also at the first split in tree models developed for early stage and young (≤65 years) patients.

Summary / Conclusion: Our large, multi-center study preferentially selected CD49d ahead of CD38 and ZAP-70 as a powerful independent prognostic marker for OS in CLL; a 30% cut-off value provided optimal separation. These findings may have implication for patient stratification in future prospective studies and potential therapeutic efforts targeting CD49d or CD49d signaling.

P091

BIALLELIC LOSSES OF 13Q DO NOT CONFER A POORER OUTCOME IN CHRONIC LYMPHOCYTIC LEUKEMIA: ANALYSIS OF 627 PATIENTS WITH ISOLATED 13Q DELETION

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Background: Losses in 13q as a sole abnormality by FISH confer a good prognosis in chronic lymphocytic leukemia (CLL). Nonetheless, patients with del(13q) are a heterogeneous group. Prognostic differences related to the percentage of altered cells or the size of the deletion have been proved. Regarding the number of deleted alleles, whereas about 70% of the 13q deletions are monoallelic (13qx1), some cases harbour biallelic (13qx2) or mosaic mono/biallelic (13qM) losses (about 15% each). IThe clinical significance of biallelic 13q deletions still remains controversial.

Aims: To describe and compare the characteristics and clinical course of patients harboring isolated monoallelic and biallelic 13q14 deletions by FISH.

Methods: Electronic database containing information from 2452 patients with CLL from 26 Spanish institutions was screened for CLL patients with del(13)(q14) at diagnosis or prior to treatment. Clinical and evolutive data, as well as cytogenetics and FISH for 11q23, CEP12, 13q14 and 17p13 results were analyzed.

Table 1. Baseline patients characteristics at diagnosis.

Patients characteristics	del(13qx1) (N=515)	del(13qx2) (N=54)	del(13M) (N=58)
Median age at diagnosis (range)	66 (28-92)	65 (43-90)	69 (44-88)
Male	314 (61%)	33 (61.1%)	30 (51.7%)
Binet stage (N=616)			
A	454 (89.4%)	43 (84.3%)	53 (93%)
B	38 (7.5%)	5 (9.8%)	3 (5.3%)
C	16 (3.1%)	3 (5.9%)	1 (3.2%)
Absolute white blood cell count ($x10^9$/L)	18.5 (2.9-357)	20.7 (3.8-150)	20 (6.4-63)
Absolut lymphocyte count ($x10^9$/L)	12.9 (1.9-287)	15.3 (1.8-114.9)	13,8 (1.7-60.1)
Hemoglobin (g/dL)	14 (6,4-18)	14 (11-17)	14 (6-18)
Platelets ($x10^9$/L)	196 (40-560)	194 (83-470)	200 (74-450)
Lactate dehydrogenase (IU/L)	314 (81-1420)	318 (103-575)	330 (151-658)
Beta-2 Microglobulin (mg/L)	2.0 (0.6-17.4)	1.9 (1-5.7)	2.3 (1-6.7)
Adenopathies (n=512)	124 (29.5%)	11 (31.4%)	12 (21.1%)
Splenomegaly (n=615)	43 (8.5%)	3 (5.8%)	3 (5.2%)
Hepatomegaly (n=425)	25 (4.9%)	2 (3.8%)	3 (5.2%)
ZAP-70 positive (n=278)	67/223 (30%)	7/19 (36.8%)	3/36 (8.3%)
CD38 positive (n=418)	57/345 (16.5%)	4/33 (12.1%)	6/40 (15%)
Unmutated *IGHV* (n=148)	29/124 (23.4%)	4/18 (22.2%)	1/6 (16.7%)
Abnormal G-banding karyotype	67/251 (26.7%)	5/19 (26.3%)	15/43 (34.9%)
Median follow-up (months)	52 (0-250)	53 (0-196)	39 (0-250)

Results: A total of 627 patients (377M/250F, median age 66) presented isolated del(13)(q14): 515 (82.1%) were monoallelic (13qx1), 54 (8.6%) were biallelic (13qx2), and 58 (9.3%) were considered as mosaics due to the coexistence of monoallelic and biallelic clones (13qM). No significant differences in the clinical characteristics among all three groups were found (Table 1). The median percentage of altered nuclei significantly differed across groups (55% in 13qx1, 72.5% in 13qx2 and 80% in 13qM groups; P<0.001). Focusing in the 13qM group, the median percentage of both monoallelic and biallelic clones found in each patient was not significantly different (35% and 27.5%, respectively; P=0.651). Moreover, no predominance of any of the clones was observed in the 13qM group. After a median follow-up of 50 months (0-250), 195 patients (31.1%) required treatment and 94 (15%) died. No significant differences in the

five-year cumulative incidence of treatment (TtFT) or overall survival (OS) were observed among different groups. The percentage of abnormal cells had a significant impact on the outcome of the studied patients, being 90% the highest predictive power cut-off for a worse TfFT (45% *vs* 28%;P<0.005) and the OS (148m *vs* not reached;P<0.005).
Summary / Conclusion: 1. Patients with biallelic 13q deletions do not show a poorer clinical outcome than those with monoallelic del(13)(q14). 2. Detection of 13q14 deletion in more than 90% of nuclei, is associated to a worse clinical outcome.
Aknowledgements. 2009/SGR541; PI11/1621; RD12/0036/0044 and RD12/0036/0014 (RTICC, FEDER).

P092

A HIGH SENSITIVITY METHOD REVEALED A TWOFOLD-INCREASED INCIDENCE OF NOTCH1 MUTATION WITH ADVERSE PROGNOSTIC IMPACT IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: Our understanding of the genetics of chronic lymphocytic leukaemia (CLL) has advanced significantly in the last few years. We were the first group to show the presence of activating mutations of *NOTCH1* in CLL patients at diagnosis (Di Ianni 2009) and correlate this genetic alteration with an unfavourable clinical outcome (Sportoletti 2010). Whole genome and exome sequencing studies confirmed that CLL genome harbours recurrent mutations of *NOTCH1* that impacted on overall survival (OS). About 80% of *NOTCH1* mutated CLL cases displayed a c.7544_7545delCT frame shift deletion. The currently available approaches for the detection of the *NOTCH1* mutations display a low sensitivity, thus hampering the diagnostic accuracy and increasing the risk of false negatives especially in patients with oligoclonal B cell mutated clones.
Aims: (1) Developing a sensitive, easy, and inexpensive test for the detection of the *NOTCH1* c.7544_7545delCT mutation in blood samples, (2) Assessing the accuracy of this test in a large cohort of CLL patients and correlate *NOTCH1* mutation with other clinical and biological prognostic factors. (3) Evaluating the impact of the *NOTCH1* c.7544_7545delCT mutation on the clinical outcome of CLL patients
Methods: We investigated 303 consecutive unselected CLL patients with median age 63 years (range 30-89). The *NOTCH1* c.7544_7545delCT mutation was screened by using a newly developed allele specific PCR (AS-PCR) and direct Sanger sequencing at CLL diagnosis.

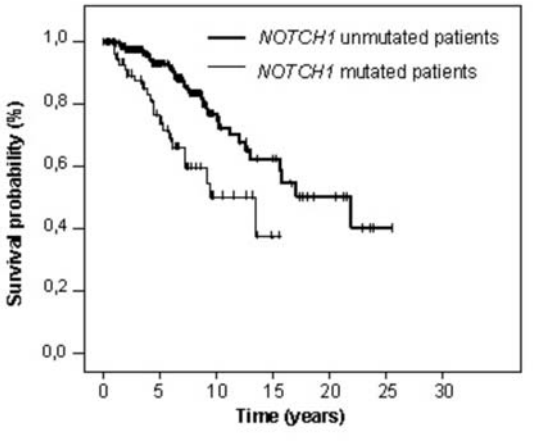

Figure 1.

Results: The analytical sensitivity of our new AS-PCR in serial dilutions of DNA established the lower detection limit to be the 0.1% of mutant alleles. Strikingly, the analysis of 303 CLL samples demonstrated an increased incidence of *NOTCH1* mutation detected by the AS-PCR, compared to Sanger sequencing (20.4% vs 10,5%). In our cohort of patients, *NOTCH1* mutation was associated with adverse prognostic markers such as unmutated IgVH status (280 total available cases, 42/57; P<0.0001) and ZAP-70>20% (189 total available cases 31/49; P=0.0034). In addition, *NOTCH1* mutated patients had more frequently trisomy 12 (132 total available cases 15/39; P=0.0006). To determine whether the AS-PCR detectable *NOTCH1* mutation maintained its prognostic impact in CLL, we estimated its influence on OS, in our cohort of patients.In univariate analysis, patients with *NOTCH1*-mutated CLL showed shorter median OS when compared with unmutated patients (13.46 vs 21.86 years; P<0.001; HR, 2.484; 95% CI, 2.14-6.66) (Figure 1).*NOTCH1* mutation was confirmed to be an independent prognostic factor for OS in a 6 variable model multivariate analysis that included age, sex, Rai Stage, IgVH and ZAP70 (P<0.05, HR 2.053; 95% CI 1.007-4.180).
Summary / Conclusion: The new PCR based approach, exploited in the current study, revealed a significant increased incidence of *NOTCH1* mutation in CLL. Univariate and multivariate statistical analysis proved that *NOTCH1* mutation is an independent prognostic factor retaining its predictive value in CLL. Our diagnostic test allowed us to identify patients with a low allelic burden in the blood demonstrating that the presence of small clonal *NOTCH1* mutated fractions impact on the clinical outcome of CLL patients. Therefore, the AS-PCR determination of *NOTCH1* mutation should be considered in drawing prognostic scores.

P093

PREDICTIVE CLINICAL MODEL FOR UNTREATED DEL(17P13.1) CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS

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Background: The subset 10% of CLL patients (pts) with del(17p13.1) (17p-) karyotype typically exhibit poor response to therapy. There are limited data on clinical outcomes of these pts and no publication of a large group of these patients treated at a single institution.
Aims: We aimed to report the characteristics and time to treatment of *de novo* 17p- CLL patients from initial visit at Ohio State University (OSU).
Methods: We retrospectively reviewed records of 115 CLL pts with 17p- with no prior therapy seen at OSU from 2002-2012. Treatment free survival (TFS) was calculated from date of 1st OSU visit until date of 1st treatment or death from any cause, censoring pts alive and treatment-free. Overall survival (OS) was calculated from date of 1st OSU visit until date of death or last follow-up. TFS/OS estimates were calculated using the Kaplan-Meier method. Proportional hazards models were fit using backwards selection to identify variables significantly associated with TFS & OS.
Results: Median time from CLL diagnosis (dx) to 1st OSU visit was 4.8mos (range: 0 days-19.7 yrs). Median age at 1st OSU visit was 62yrs (40-92), 70% were male, and 92% were Caucasian. At CLL dx, 55%, 35%, and 10% had Rai Stage (RS) 0, 1/2, and 3/4 respectively. Median WBC, hemoglobin, platelets, and LDH were 26.8K/uL (2.4-446.6), 13.5g/dL (5.9-16.8), 186.5K/uL (13-385), and 169U/L (99-1791) respectively. 39% (80 evaluable) had β2microglobulin≥3mg/L. In addition to 17p-, 14%, 55%, and 21% of patients harbored 11q-, 13q- and tri(12) aberrations respectively. Complex karyotype (CK; ≥3 abnormalities including 17p-) was identified in 37% (n=42), and 70% (n=38/54 evaluable patients) expressed un-mutated IgVH status.Of 105 pts followed for TFS, 57 have started treatment (54%) and 8 died prior to treatment, most within 2yrs of visit. Median TFS estimate was 16.2mos (95%CI=6.4-27.0) with a 2-yr TFS estimate of 42% (95%CI:0.31-0.52). At median follow-up of 3yrs, 47 pts have died. Estimated median OS was 4.2yrs (95%CI:3.4-7.8) with an OS estimate at 2yrs of 77% (95%CI:0.68-0.85). In multivariable analysis, the variables significantly associated with shorter TFS were older age (hazard ratio [HR] for 10yr increase:1.53 (95% CI:1.19-1.97 ;p50%. Age and RS remained in a multivariable model for OS (P≤0.0001), yet CK was included instead of 17p -% as an independent significant prognostic factor (P=0.009), and presence of 11q- was not significantly associated with OS.
Summary / Conclusion: Pretreatment characteristics of higher age at referral, higher RS at diagnosis, higher 17p -%, and presence of 11q- can identify pts who will progress quickly and require treatment sooner than those without these adverse risk factors in this population of *de novo* 17p- CLL. The role of 17p-% and CK warrants further study, considering the strong degree of association between the two variables.

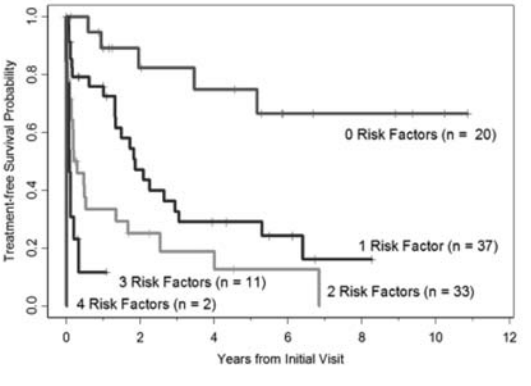

Figure 1.

P094

NOVEL ASSAY FOR THE IDENTIFICATION OF NOTCH1 PEST DOMAIN MUTATIONS USING FRAGMENT ANALYSIS AND ALLELE SPECIFIC PCR

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Background: NOTCH1 is a proto-oncogene with activating mutations described in a variety of malignancies, including acute lymphoblastic leukemia (ALL), mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL). While the prognostic significance of NOTCH1 mutations remains controversial in ALL, recent data suggest that NOTCH1 PEST domain mutations are associated with adverse prognosis in patients with CLL.
Aims: NOTCH1 mutations are found in around 10% of CLL patients at diagnosis, and since this disease has a heterogeneous clinical course and few prognostic markers, we aimed at designing a fast, cost effective and robust assay to detect NOTCH1 PEST domain mutations in patients with CLL.
Methods: While 92% of the mutations in NOTCH1 PEST domain found in CLL are insertions or deletions, only 8% are represented by point mutations. Therefore we decided to use a fragment analysis approach in our assay. Given that a single mutation (c.7544_7545delCT), represents roughly 75% of all PEST domain mutations in CLL we designed a test that can, at the same time, detect the presence of this mutation specifically and also any insertion or deletion in exon 34. We designed a PCR reaction using one FAM-labeled forward primer anchored at codon 2407 and two reverse primers. One specific for the c.7544_7545delCT mutation anchored at codon 2414 yielding a product of 356 base pairs (bp) and one anchored at codon 2425, yielding a product of 391 bp, comprising the hot spot for mutations in the NOTCH1 PEST domain. Primers were designed with Primer3 software (http://frodo.wi.mit.edu/) and the specificity of the reaction evaluated using the tool "PCR in silico" (http://genome.ucsc.edu/cgi-bin/hgPcr?command=start). The test yields three possible outputs: a) A single 391 bp peak: wild type samples; b) Three peaks (391 bp, 389 bp and 356 bp): heterozygous for c.7544_7545delCT; c) Two peaks (391 bp and another bigger or smaller, depending on the size of insertion / deletion): another insertion or deletion, but not c.7544_7545delCT.
Results: We have first studied 46 blood samples from unselected patients with CLL, in several disease stages. NOTCH1 wild type was detected in forty patients. Six patients had a pattern compatible with c.7544_7545delCT NOTCH1 mutation (Figure 1A), and no patient presented with another mutation. DNA sequencing was performed in selected samples, and the specificity of our assay was confirmed (Figure 1B). Recent studies have demonstrated that NOTCH1 mutation occur more frequently in CLL patients with trisomy 12 (2). In order to confirm these findings, we studied a second cohort of 14 CLL patients with trisomy 12. Of these, 7 patients presented with mutated NOTCH1 (50%). Overall the frequency of NOTCH1 mutations in our series was: 12% and 50% in patients without and with trisomy 12 respectively, in agreement with previous reports. All mutated cases (N=13) had c.7544_7545delCT. We were not able to study the correlation between NOTCH1 mutations and clinical features at this time, since clinical information was not available for most patients.
Summary / Conclusion: In conclusion, we have designed a robust, fast and cost effective assay for routine identification of NOTCH1 PEST domain mutations using fragment analysis and allele specific PCR that is suitable for implementation in the clinical setting for CLL patient evaluation.

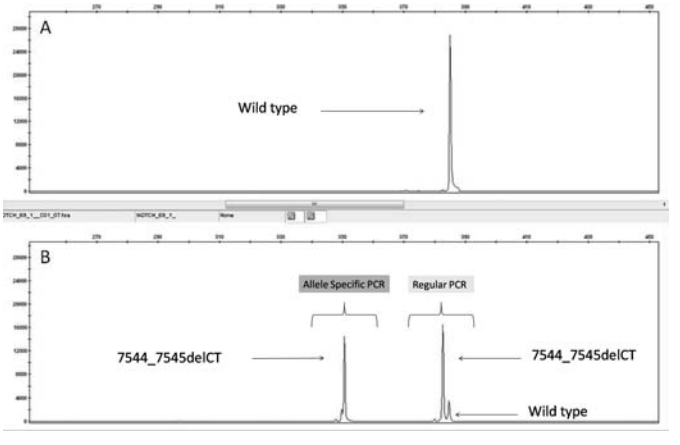

Figure 1. Assay results for NOTCH1 PEST domain mutation.

Chronic lymphocytic leukemia: clinical studies

P095

LONG-LASTING RESPONSES TO LENALIDOMIDE AS INITIAL THERAPY OF ELDERLY PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: We conducted a phase II study evaluating the activity of lenalidomide as initial therapy for elderly pts with CLL. At the time of its initial report, this treatment was associated with an overall response rate of 65% and an overall survival of 88% at 2 years (Badoux, Blood 2011).
Aims: We analyzed outcome, toxicity, clinical and laboratory characteristics of long-term responders in this trial.
Methods: Pts with a response lasting 36 months or longer were defined as "long-term responders" (LTRs). Clinical characteristics, prognostic factors, serum immunoglobulin levels, circulating T cell numbers (up to 36 months) and plasma cytokine levels of LTRs were compared with the rest of the study population using non-parametric and Chi-square tests. Differences were considered to be significant if p was equal to or less than 0.05.
Results: Thirty-four of the 60 pts (57%) are LTRs. Best responses among LTRs consisted of 24 (71%) complete remissions (CR), including 5 pts with MRD-negative CR, and 10 (29%) partial remissions. Median time to failure (TTF) has not been reached for LTRs, after a median follow up of 47 (37-60) months. The median daily dose of lenalidomide at last follow-up in LTRs pts is 5 mg (2.5-10). Twenty-four LTRs are still on therapy and ten have discontinued lenalidomide. Reasons for treatment discontinuation were: progression after 43 months in 1 pt, toxicity in 6 pts (deep venous thrombosis after 41 months in 1 pt, moderate neuropathy after 30 and 39 months in 2 pts, persistent fatigue after 23 months in 1 pt, moderate weight loss after 5 months in 1 pt, immune thrombocytopenia after 11 months in1 pt), infectious complications in 1 pt (sepsis, after 12 months), second malignancy (new onset invasive squamous cell carcinoma of the skin after 26 months) in 1 pt and change of institution in 1 pt. Lenalidomide is often associated with myelosuppression in pts with CLL. Interestingly, LTRs experienced neutropenia during the first 12 months of therapy that later resolved in 83% of pts (Figure 1A); additionally a recovery in hemoglobin and platelets compared to baseline values was also seen in 100 and 77% of LTRs, respectively (Figure 1B-C). We also observed a recovery in the percentage of circulating T cells (CD3+) in 41% ofpts and a recovery in plasma levels of immunoglobulins A, G and M levels in 68, 58 and 52% ofpts, respectively (Figure 1D). As therapy continues until progression, we focused on lenalidomide-related late (defined as toxicities observed after 48 months of therapy) toxicities. Grade 1-2 diarrhea was observed in 2 pts and Grade 1 peripheral neuropathy in 3 pts. One patient developed skin lesions shown to be *in situ* squamous and basal cell carcinomas on biopsy. We compared pretreatment clinical characteristics of LTRs and the other pts on study. We observed that LTRs had lower baseline beta-2-microglobulin (median values: 4 vs 5 mg/L; P=0.005) and were less likely to have a deletion 17q (0 vs 6; P=0.005) but more likely to have trisomy 12 (11 vs 2; P=0.03). Furthermore, baseline plasma levels of IL8, IFNγ, sVEGFR2 and MIP1α were significantly lower in the LTRs (P=0.05, 0.06, 0.05 and 0.02, respectively).
Summary / Conclusion: Lenalidomide as initial therapy of elderly pts with CLL induced responses that are durable, with 57% of pts maintaining their response for more than 3 years. Myelosuppression is observed, but it is transient and resolves past the first year. Compared to the pts with shorter response duration, LTRs were more likely to have lower baseline levels of beta2-microglobulin, IL8, IFNγ, sVEGFR2 and MIP1α and intermediate or favorable cytogenetic abnormalities.

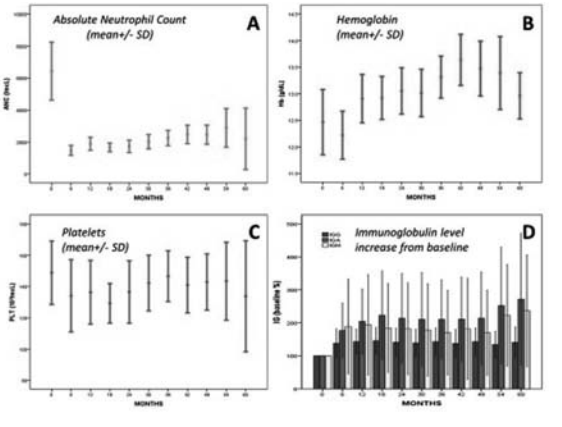

Figure 1.

P096

LOW-DOSE FCR IN ELDERLY/COMORBID PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA (CLL/SLL): UPDATED RESULTS OF PROJECT Q-LITE BY CZECH CLL STUDY GROUP

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Background: Combination of fludarabine, cyclophosphamide and rituximab (FCR) is currently considered the treatment of choice in physically fit patients (pts) with chronic lymphocytic leukemia (CLL). However, many patients cannot tolerate this aggresive regimen because of advanced age and/or serious comorbid conditions which may lead to unacceptable toxicity. Protocols based on low-dose fludarabine have recently demonstrated promising results in small studies.
Aims: Aims: to assess efficacy and safety of low-dose FCR regimen used in elderly/comorbid patients with CLL/SLL; updated results including first data on progression-free survival (PFS) and overall survival (OS) are presented.
Methods: Between March 2009 and July 2012, a total of 207 pts with active disease (CLL, n=196, SLL, n=11) were treated by low-dose FCR at 16 centers cooperating within Czech CLL Study Group. Dose reduction of chemotherapy in comparison to full-dose FCR was following: 50% of fludarabine dose (12 mg/m^2 i.v. or 20 mg/m^2 orally on days 1-3) and 60% of cyclophosphamide dose (150 mg/m^2 i.v./p.o. on days 1-3). Rituximab was administered in standard schedule (375 mg/m^2 i.v. day 1 in 1st cycle, 500 mg/m^2 i.v. day 1 from 2nd cycle). Treatment was repeated every 4 weeks; antimicrobial prophylaxis with sulfamethoxazol/trimethoprim and aciclovir or equivalents was recommended. Data regarding efficacy and safety are currently available in 199 pts; the descriptive characteristics are summarized in Table 1.
Results: Based on intention-to-treat principle, the overall response rate / complete responses (including clinical CR [without bone marrow biopsy] and CRi [with incomplete marrow recovery]) were 79/37% in first line and 64/29% in relapsed/refractory setting. Serious (CTCAE grade III/IV) neutropenia was frequent (57 and 49%) but did not translate into high occurrence of serious infections (14 and 18%, Table 1). The most common causes of death were CLL progression and infections. At the median follow-up of 19 months, median progression-free survival for previously untreated and relapsed/refractory patients was 20 and 15 months; median overall survival has not been reached in previously untreated pts (80% at 2 years) and was 31 months in relapsed/refractory pts. CIRS score and age did not significantly influence PFS or OS.
Summary / Conclusion: Our data show that treatment of elderly/comorbid patients with CLL/SLL using low-dose FCR has promising efficacy in first line as well as relapsed/refractory disease, including reasonable PFS and OS. Toxicity is acceptable and manageable.

Table 1. Basic charcateristics, therapeuic efficacy and severe (CTCAE grade III/IV) toxicity.

	1st line	Relapsed/refractory
Total number of patients	102	97
Age (median, range)	69 (54-83)	71 (58-87)
Males	64%	59%
Advanced Rai stages	56%	66%
Bulky lymphadenopathy	41%	37%
CIRS score (median, range)	5 (0-13)	6 (0-14)
Unmutated IgVH	75%	72%
Del 11q	32%	36%
Del 17p	8%	9%
Number of FCR cycles (median, range)	5 (1-7)	4 (1-6)
Overall response rate	79%	64%
CR + cCR + CRi	37%	29%
Stable disease	9%	21%
Progressive disease	8%	6%
Not evaluable	4%	9%
Neutropenia	57%	49%
Anemia	10%	13%
Thrombocytopenia	7%	19%
Infections	14%	18%

P097

PLATINUM AND HIGH-DOSE CYTARABINE-BASED CHEMOTHERAPY IN ULTRA HIGH-RISK CHRONIC LYMPHOCYTIC LEUKEMIA AND RICHTER'S SYNDROME: RESULTS OF A RETROSPECTIVE MULTICENTER STUDY

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Background: Ultra high-risk chronic lymphocytic leukemia (CLL) and Richter's syndrome (RS) usually display a poor prognosis. Although they are widely used as salvage therapy in many types of lymphomas, platinum and cytarabine (PI + AraC)-based regimens have not been evaluated in large cohorts of patients with CLL or RS.
Aims: The aim of this study was to assess the efficacy of PI + AraC-based regimens in patients with aggressive forms of CLL (high or ultra high-risk CLL or Richter's syndrome).
Methods: This French retrospective analysis included 75 patients with relapsed/refractory CLL or RS, who received at least one course of PI + AraC-based chemotherapy (DHAP±rituximab, ESHAP±rituximab or OFAR) in 4 centers between 2000 and 2012.
Results: Forty-seven patients with relapsed or refractory CLL (including 36 ultra high-risk CLL) and 28 with RS were included. Median age was 62 years (range, 18-79 years). The median number of previous therapies was 3 (range, 1-7), including fludarabine-based regimens (75%) and alemtuzumab (32%), and 61% of the patients were refractory to the last treatment. Tumor mass >5 cm and splenomegaly were present in 32% and 42% of patients respectively. LDH and β2-microglobulin were elevated in 75% of cases. The incidences of 17p and 11q deletions were 40% and 39% respectively. The overall response rate was 60% with 24% complete response (CR) in CLL, and 43% with 25% in RS. The median progression-free survival and overall survival were 11 and 14.6 months respectively. Fludarabine refractoriness and 17p deletion were not associated with a poorer outcome. In multivariate analysis, the only factors associated with a shorter survival were performance status ≥2 (P=0.04) and albumin level <35 g/L (P=0.0004). The main toxicities were myelosuppression (grade III-IV in 80% of cases) and infectious complications (toxic death 15%). Twenty-one patients underwent thereafter autologous or allogeneic stem cell transplantation (SCT).
Summary / Conclusion: Platinum and high-dose cytarabine-based regimens provide high response rate in high-risk CLL and RS. In ultra-high-risk CLL (17p deletion or fludarabine refractoriness), these regimens should be considered as an option for tumor control before allogeneic transplantation.

P098

DOSE-ESCALATION AND PHARMACOKINETICS (PK) OF DIFFERENT LENALIDOMIDE (LEN) STARTING DOSE REGIMENS IN PATIENTS WITH RELAPSED OR REFRACTORY (REL/REF) CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) (CC-5013-CLL-009)

C Wendtner [1,*] M Hallek[2], G Fraser[3], A Michallet[4], P Hillmen[5], J Duerig[6], S Gregory[7], M Kalaycio[8], T Aurran-Schleinitz[9], L Trentin[10], S Stilgenbauer[11], T Kipps[12], B Purse[13], J Zhang[13], N Chen[13], J Mei [13], A Chanan-Khan[14]
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Background: CLL patients (pts) who relapse after purine-analog or bendamustine-based treatments have a poor prognosis. Lenalidomide is an immunomodulatory agent that has shown significant activity in rel/ref CLL. Various doses and schedules have been explored. Here we report the preliminary results on tolerability, efficacy, and PK from the CLL-009 study, in which different doses were investigated to determine the optimum starting dose regimen.
Aims: To investigate primarily the safety and secondarily the efficacy of LEN initiated at 3 starting doses (5, 10, and 15 mg/d) followed by a step-wise dose escalation as tolerated in rel/ref CLL. Exploratory endpoints included PK analyses.
Methods: In this phase2, randomized, double-blind, multicenter trial, rel/ref CLL pts (after ≥ 1 purine-analog or bendamustine-based regimen) were randomized 1:1:1 to receive a starting dose (5, 10, or 15 mg/d) of oral LEN on days 1–28 of each 28-day cycle. All doses were escalated by 5 mg increments every 28 days to reach a maximum dose of 25 mg/d, as tolerated. In case of poor tolerability, dose reductions occurred in 5 mg decrements.
Results: A total of 104 pts were enrolled; median age was 64.5 years (range 32-81). Pts were heavily pretreated-median number of 3 prior treatments-and

had poor prognostic features. The safety population consisted of 103 pts and PK data are available for 26 pts: 11/34 in the 5 mg, 6/34 in the 10 mg, and 9/35 in the 15 mg dose level. For the 103 evaluable pts, 51.4% did not escalate above their starting dose level (50.0%, 38.2%, and 65.7% for the5, 10, and 15 mg groups, respectively). Additionally, 56% of dose reductions occurred in the first 3 treatment cycles. In cycle1, 17.6% (5 mg), 29.4% (10 mg), and 22.9% (15 mg) of pts had dose reductions; in cycle2, dose reductions from the respective starting dose occurred in 20.6%, 11.8%, and 20.0%. Reductions in these 2 cycles were primarily due to hematologic events of neutropenia (41%) and thrombocytopenia (10.3%). 7 pts in cycle 1 and 1 pt in cycle 2 were dose reduced due to tumor flare; the average duration was 13.8 days and each of these patients continued study therapy. However, of the 39 responders (13 in each group) 7.7% (5 mg), 23.1% (10 mg), and 53.8% (15 mg) did not escalate to a higher dose level. 34 responders (87.2%) reached a dose ≥15 mg/d and 38.5% escalated to the maximum allowed dose of 25 mg/d. LEN was rapidly absorbed and eliminated. The maximum plasma concentration was observed 1 hour after dosing and the mean terminal half-life was approximately 3 hours in all starting dose levels (Table 1). The differences in plasma exposure in each group were approximately proportional to the starting doses from 5 to 15 mg/d. The PK pts had a median age of 64 years (range 54–78) and 46% were ≥ 65 years old. It is noted that the mean LEN clearance in pts ≥ 65 years and < 65 years was 184 and 212 mL/min, respectively ($p > 0.05$), consistent with the respective mean creatinine clearance of 78.7 and 89.3 mL/min. Cross-study comparisons suggested that LEN clearance in CLL pts is comparable to patients with multiple myeloma or myelodysplastic syndromes.

Summary / Conclusion: Efficacy data with a longer follow-up will be presented, but at a median treatment duration of 34.0 wks, ORR was 38.2% in this heavily pretreated population. The data suggest that titration of doses up to 15 mg/d or greater correlates with better responses, while starting dose of 15 mg/d appears to be too high. The PK of LEN in CLL pts is consistent with that observed in other disease populations studied.

Table 1. Pharmacokinetic parameters of lenalidomide in CLL patients.

Parameter	5 mg dose (n = 11)	10 mg dose (n = 6)	15 mg dose (n = 9)
T_{max} (h)	1 (0.5–2)	1 (0.5–3)	1 (0.5–4)
C_{max} (ng/mL)	85 (22.6)	220 (45.8)	263 (31.6)
AUC_{24} (ng*h/mL)	414 (21.0)	1,022 (22.7)	1,247 (43.3)
$t_{1/2}$ (h)	3.37 (23.5)	3.35 (43.3)	3.09 (38.2)
CL/F (mL/min)	201 (21.0)	163 (22.7)	201 (43.3)
V/F (L)	58.7 (24.7)	47.3 (31.8)	53.7 (20.5)

Data are expressed as median (range) for T_{max} and geometric mean (coefficient of variation) for other parameters.
Abbreviations: AUC_{24}, area under the curve at 24 hours; CL/F, total clearance; C_{max}, maximum plasma concentration; $t_{1/2}$, mean terminal half life; T_{max}, time to maximum plasma concentration; V/F, volume of distribution.

P099

TOXICITY AND RESPONSE BY COMORBIDITY AND AGE IN A RANDOMIZED STUDY OF ORAL FLUDARABINE AND CYCLOPHOSPHAMIDE WITH RITUXIMAB FIRST-LINE THERAPY OF FIT ELDERLY WITH CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: Combination immunochemotherapy with fludarabine (F), cyclophosphamide (C) and rituximab (R) gave superior progression free and overall survival compared to FC in the CLL8 Study. The median age in CLL8 was 61 years compared to the median age of CLL overall at 72 years. There is debate regarding the tolerability of FCR based therapy in the elderly.
Aims: We aimed to assess the safety and tolerability of F(C)R based therapy in fit elderly patients with CLL requiring treatment in a dose de-escalation study. Fitness was assessed as a Cumulative Illness Rating Scale [CIRS] score ≤6. This analysis was performed on 12 February 2013, seven months after the last recruitment, when all patients should have completed therapy, and focused on toxicity and response by CIRS score and age. The study was supported by Roche Products Australia, and Genzyme (formerly Bayer Schering).
Methods: Previously untreated fit patients with progressive CLL aged ≥65 were randomized to one of three treatment regimens "FR5", "FCR3" and "FCR5" as follows: (i) Fludarabine 24 mg/m^2 orally for five days + Rituximab (375 mg/m^2 C1, 500 mg/m^2 C2-6) iv Day 1 (FR5), (ii) Fludarabine 24 mg/m^2 and Cyclophosphamide 150 mg/m^2 both orally for three days (D1-3) + Rituximab iv D1 (FCR3) or (iii) Fludarabine 24 mg/m^2 + Cyclophosphamide 150 mg/m^2 both orally for five days (D1-5) + Rituximab iv D1 (FCR5), all given at 4 weekly intervals for an intended 6 cycles. Patients were administered their therapy arm with no dose reduction. Therapy was delayed up to 2 weeks if there was grade 3 or 4 toxicity, and if unresolved after 2 weeks, patients were taken off study. If toxicity resolved to grade 2 or less, therapy proceeded.
Results: Recruitment of all 120 randomised patients was completed in July 2012. Median age was 71 (range 65-83) years. Binet stage at registration was progressive A – 20 (16.7%), B – 56 (46.7%) and C – 44 (36.7%). Haematological toxicity and overall response rates (ORR) are as follows for the total patient cohort by CIRS comorbidity score and age bracket but with no analysis by treatment arm. The overall grade 3/4 neutropenia rate was 39.8% and febrile neutropenia / infection was 15.9%. Grade 3/4 toxicity across the CIRS score brackets 0-2, 3-4 and 5-6 respectively was 56%, 39% and 56% for all haematological toxicity, 43%, 31% and 50% for neutropenia, and 15%, 19% and 13% for febrile neutropenia and/or infection. Grade 3/4 toxicity across the age brackets 65-69, 70-74, 75-79, and 80-84 respectively was 52%, 53%, 43% and 20% for all haematological toxicity, 45%, 38%, 43% and 0% for neutropenia, and 18%, 7%, 33% and 0% for febrile neutropenia and/or infection. The overall response rate (ORR) across the CIRS score brackets 0-2, 3-4 and 5-6 respectively was 94%, 87% and 100%, and across age brackets 65-69, 70-74, 75-79, and 80-84 respectively was 93%, 91%, 92% and 100%. Using stringent stopping criteria, 35% stop early due to toxicity, intercurrent illness or patient choice, and 61.9% have a delay during therapy.
Summary / Conclusion: Oral F(C)R therapy appears generally safe and well tolerated in CLL patients aged ≥65 years requiring first-line therapy according to incomplete data 7 months from end of recruitment. For fit elderly patients, neither a CIRS score between 0 and6, nor age bracket appear to be associated with toxicity. ORR is high at 92.4%, and complete remission in 40.5% at Final Staging 2 months after treatment. ORR was similar across all CIRS score and age brackets.

P100

PHASE 1B STUDY OF IDELALISIB (GS-1101) PLUS CHLORAMBUCIL±RITUXIMAB IN PATIENTS WITH RELAPSED AND REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: PI3K-delta (δ) is critical for activation, proliferation and survival of B cells and plays a role in homing and retention in lymphoid tissues. PI3Kδ signaling is hyperactive in many B-cell malignancies. Idelalisib is a first-in-class, selective oral inhibitor of PI3Kδ. It has shown activity as a single agent and in combinations with bendamustine and anti-CD20 mAbs, in patients with relapsed or refractory (R/R) CLL.
Aims: The primary objective of this study is to evaluate the safety of the addition of idelalisib to chlorambucil (Chl) and chlorambucil plus rituximab (R) in pts with R/R CLL. The secondary objective is to evaluate the clinical activity of the combination. Preliminary results are presented.
Methods: Pts required therapy according to IWCLL 2008 guidelines. All pts received idelalisib 150 mg po bid continuously. Those on idelalisib+Chl received Chl 10 mg/m^2 po qd on days 1-7 every 28 days for a minimum of 3 and maximum of 12 cycles, based on achievement of optimal response. Those on idelalisib+Chl+R received, in addition, R 375 mg/m^2 on day 1 of the first 6 cycles. Response was assessed by the investigators based on scheduled CT evaluations and clinical criteria following IWCLL 2008.
Results: 29 subjects were enrolled from March 2011 through August 2012: median age 65 (range: 41-82); M/F 83/17 (%); WHO 0/1/2 79/21/0 (%); current Rai III/IV 3/48 (%). The median number of prior regimens was3, mean3, range 1-11. 48% of subjects were refractory to their last therapy (progressed within 6 months) and 48% had relapsed at >6 mo, 4% unknown. Of 13 subjects treated with idelalisib+Chl+R, 5 (39%) were refractory to rituximab. The ORR was

63% for idelalisib+Chl (1/16 CR, 9/16 PR) and 92% for idelalisib/Chl/R (1/13 CR, 11/13 PR). Median PFS has not been reached. As of 22 Feb 2013, 4 subjects have discontinued therapy (2 from each arm): 1 PD (idelalisib+Chl), 1 death, 1 withdrew consent and 1 other. The median time on idelalisib therapy is currently 8.1 mos (range: 0.9-10.2). The most frequently reported treatment-emergent AEs (≥20% of all subjects) and lab abnormalities are shown in Table 1.

Summary / Conclusion: Idelalisib, when combined with either chlorambucil or chlorambucil plus rituximab, is effective in inducing responses in the majority of relapsed and refractory patients with CLL. The safety profile is not different from what would be expected from the addition of each of the administered drugs. These results support further studies with these combinations in patients with CLL.

Table 1.

Adverse Event	Idelalisib / Chl (N=16)		Idelalisib / Chl/R (N=13)	
	Total (%)	≥Gr 3 (%)	Total (%)	≥Gr 3 (%)
Diarrhea	25	6	46	8
Cough	25	0	30	0
Fatigue	19	0	31	8
Pyrexia	13	0	36	8
Febrile Neutropenia	31	31	8	8
Rash	13	0	31	15
Lab Abnormality				
Neutropenia	88	69	69	46
Thrombocytopenia	69	38	39	15
Anemia	56	19	39	8
ALT/AST elevation	31	0	46	23

P101

PRELIMINARY RESULTS FROM A SURVEY OF 'REAL-LIFE' FIRST LINE THERAPY OF CHRONIC LYMPHOCYTIC LEUKAEMIA IN THE UK

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Background: FCR is widely regarded as standard 1st line therapy for chronic lymphocytic leukaemia. In October 2009 the UK National health Service made FCR freely available to all patients in England for this indication. However, the landmark studies establishing the role of this treatment had patient populations younger than the median age and in generally more robust health, e.g. median performance status in CLL8 is 1.

Aims: To determine the applicability and tolerance of trial derived standard treatment to the unselected CLL patient population To determine the degree to which patients entering trials were representative of the unselected patient population. To determine the range of non-FCR treatments in use. To determine the sequence of 1st and 2nd line treatments in current use and any impact on 2nd line treatment of toxicity from 1st line treatment

Methods: We initiated a prospective survey of 1st line treatment choices and outcomes in CLL from 1st October 2009 at 7 hospitals across England serving a combined population of approximately 3.3 million. The survey consists of short questionnaires at treatment initiation, 6 months post treatment completion and at relapse.

Results: To date 209 patients have received treatment, 6 month follow up is available for 151 of these and 12 month for 43. Median age at treatment initiation is 70, Binet stages are A 22%, B 28% and C 50%. *Treatment choice*:97 were scheduled for full dose FCR (88) or received non-FCR treatment in a trials with an FCR arm (9). The full range of treatments is shown in Table 1. For the 54% (112) of patients not considered suitable for full dose FCR the reasons not to use it were medically unfit 25%, known 17p 0%, patient choice 18%, patient age 33%, other 24%. (including previous non-haematological cancer, omission of Rituximab because of grossly elevated WCC, the perception of FCR as excessive for low disease burden and a desire to avoid marrow suppression). 8% (17) of all patients were treated within a trial. *Treatment completion:*Of patients planned for 6 cycles of full dose FCR only 41% receive this while 26% receive ≤5 cycles, 12% have dose reductions and a further 20% have both cycle number and dose reductions. Data from 6 months after completion of treatment is available for 54 of these and 51% have some cytopenia with 34% having severe cytopenia (neutrophil <1x10^9/L, Hb<100 g/L or platelets <100x10^9/L). Of patients planned to have dose reduced FCR from the outset 61% required further dose reduction and 33% have persisting cytopenia 6 months post treatment. At 12 months 30% of those exposed to FCR have persisting cytopenia and 5 have required second line therapy, in 2 cases choice or dose was altered by post=FCR cytopenia.

Summary / Conclusion: Our survey indicates that: 1. a wide range of 1st line treatment options remain in use. 2. fewer than 50% of patients are felt suitable for full dose FCR. 3. Patients planned for full or reduced dose FCR tolerate it poorly with 60% required unplanned dose reductions. 4. Long term cytopaenia, which could compromise future treatment choices, may be a significant problem following full dose FCR treatment. 5. Patients entering trials of 1st line treatment represent a very small percentage of the patient population

Table 1.

Initial Treatment choice	Number (Percentage)
Full dose FCR	96 (41%)
Reduced dose FCR	29 (14%)
FC	2 (1%)
Chlorambucil + Prednisolone*	15 (7%)
Chlorambucil alone	40 (19%)
Chlorambucil + splenectomy/ splenic irradiation	3 (1.5%)
R-CVP	2 (1%)
R-CHOP	1 (0.5%)
R-HDMP	1 (0.5%)
FC	2 (1%)
FCMR (trial)	6 (3%)
FCM-miniR (trial)	2 (1%)
Rituximab+oral prednisolone	2 (1%)
R-Chlorambucil	2 (1%)
Bendamustine	1 (0.5%)
HDMP	2 (1%)

P102

NOTCH1 MUTATIONS IDENTIFY CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS THAT DO NOT BENEFIT OF AN INDUCTION AND CONSOLIDATION TREATMENT BASED ON RITUXIMAB

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Background: Recent studies showed that stabilizing mutations of *NOTCH1* gene occur in about 10% CLL at diagnosis and are associated with advanced disease and unfavourable prognosis (Rossi *et al*, Blood, 2012; Del Poeta *et al*, Br J Haematol, 2013).

Aims: Here, we investigated whether: i) *NOTCH1* mutations (*NOTCH1*^{mut}) are confirmed to be an independent predictor of poor clinical outcome in a cohort of CLL patients (pts) all treated with the same therapeutic approach, and ii) *NOTCH1*^{mut} are able to identify a subset of CLL pts not benefiting from the use of rituximab (rtx).

Methods: We assessed the incidence and impact of *NOTCH1*^{mut} in 123 CLL symptomatic pts, median age 63 years (37-80), homogeneously treated in first line with six monthly courses of intravenous (25 mg/m^2) or oral fludarabine (30-40 mg/m^2) followed by four weekly doses (375 mg/m^2) of rtx. Out of 123 pts, 43 reached complete remission (CR); the remaining 80 pts either reached CR but remained positive for minimal residual disease detection by flow cytometry (CR/MRD+; n=46), or underwent partial remission/stable disease (PR/SD; n=34). Among them, 21 pts entered follow-up without further therapy (unconsolidated pts), while 59 underwent to a consolidation-maintenance phase (consolidated pts) with rtx (four monthly cycles of rtx at 375 mg/m^2 followed by twelve monthly doses of rtx at 150 mg/m^2; Del Poeta *et al*, Cancer, 2008). *NOTCH1* c.7544_7545delCT mutation was investigated by amplification refractory mutation system (ARMS) PCR using frozen samples collected at presentation.

Results: *NOTCH1*^{mut} were found in 20 of 123 pts (16.3%). Regarding baseline characteristics, there were significant associations of *NOTCH1*^{mut} with trisomy 12 (P=0.03), unmutated *IGHV* (P=0.0001), ZAP-70 >20% (P<0.0001) and CD49d >30% (P=0.0004). Regarding response to therapy, 12 out of 20 (60%) *NOTCH1*^{mut} pts showed only PR/SD vs 22/103 (22%) *NOTCH1* wild-type (*NOTCH1*^{wt}) pts (P=0.002). At extended follow-up (median 68 months), *NOTCH1*^{mut} was associated with significantly decreased response duration (RD), (13% vs 48% at 8 years, P=0.00004, Figure 1) and inferior overall survival (OS), (0% vs 57% at 15 years, P=0.00002). The 59/80 consolidated patients showed a longer RD vs the 21/80 unconsolidated pts [50% vs 0% at 5 years; P=0.001]. Equally, OS was longer in consolidated pts vs unconsolidated pts (81% 0% at 13 years; P=0.0008). Among consolidated pts, *NOTCH1*^{mut} cases (10/59) experienced significantly shorter RD and OS intervals than con-

solidated *NOTCH1*^{wt} pts (RD, 0% vs 61% at 5 years, P=0.004; Figure 2; OS, 38% vs 95%, at 10 years, P=0.004). Of note, RD and OS intervals of *NOTCH1*^{mut} consolidated pts was not dissimilar to that of the 21 unconsolidated CLL pts (p>0.05 in both cases), 8 of them bearing a *NOTCH1*^{mut} genotype. *NOTCH1*^{mut} maintained an independent prognostic impact in Cox regression multivariate analyses, by evaluating: i) RD (*NOTCH1*^{mut} HR 2.96, P=0.02) along with consolidation-maintenance (HR 0.38, P=0.005) and *IGHV* (HR 2.73, P=0.01); ii) OS (*NOTCH1*^{mut} HR 7.31, P=0.002) along with *IGHV* (HR 3.14, P=0.04), *TP53*$^{mut/del}$ (HR 4.18, P=0.02) and age >60 years (HR 4.14, P=0.03).
Summary / Conclusion: Our results confirm that *NOTCH1*^{mut} represent an independent clinical prognostic factor in homogeneously treated CLL pts and identify a subset of CLL pts that does not benefit from the addition of rtx to therapy.

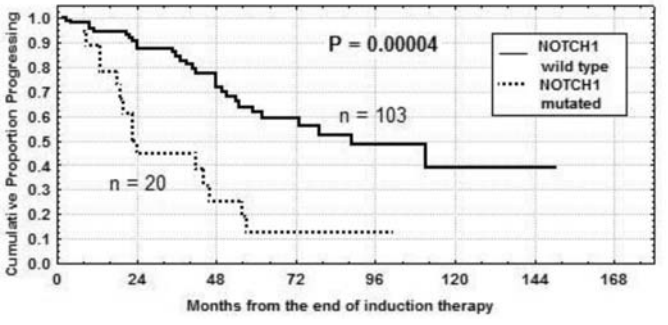

Figure 1. Response duration by NOTCH1 mutations.

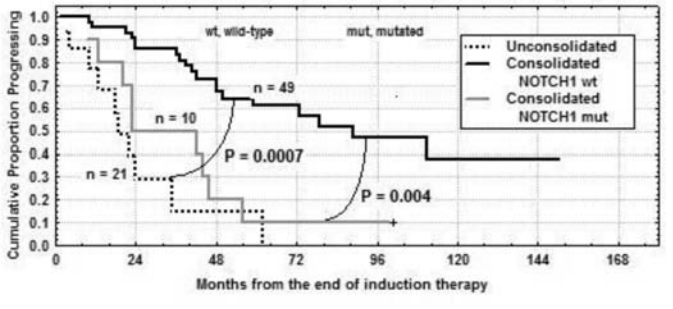

Figure 2. Response duration in unconsolidated vs NOTCH1 wt and NOTCH1 mut consolidated pts.

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INCREASED EXPRESSION OF THE EZH2 METHYLTRANSFERASE AND EVIDENCE OF FUNCTIONALITY IN CLL SUBGOUPS WITH AGGRESSIVE CLINICOBIOLOGICAL PROFILES

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Background: EZH2 is the enzymatic subunit of the polycomb repressive complex 2 (PRC2), which induces gene repression through trimethylation of histone H3 at lysine 27 (H3K27). EZH2 over-expression has been reported in a broad range of both hematopoietic and solid malignancies and associated with poor prognosis. Furthermore, activating mutations of the EZH2 gene are recurrent in B cell lymphomas of germinal center origin. Recently, we demonstrated for the first time that EZH2 is expressed in CLL and, notably, that EZH2 mRNA and protein levels were up-regulated in the aggressive stereotyped subset #1 versus the indolent subset #4.
Aims: In order to obtain deeper insight into the role of EZH2 in CLL pathobiology, here we extended our studies by investigating EZH2 expression and function in a well-annotated cohort of 116 patients with CLL.
Methods: EZH2 mRNA levels were determined in negatively selected CD19+ cells from peripheral blood samples obtained at diagnosis or before any treatment by real-time quantitative PCR (RQ-PCR) using abl as the housekeeping gene. Using Western blotting, we also evaluated (i) EZH2 protein expression; and, (ii) its functional effects, by measuring H3K27me3 levels.
Results: A total of 116 cases were evaluated for EZH2 mRNA expression by RQ-PCR; of these, 55 (47.4%) carried unmutated IGHV genes (98-100% identity to the germline, U-CLL), whereas the remaining 61 cases (52.6%) carried mutated IGHV genes (<98% identity to the germline, M-CLL). Significantly higher EZH2 mRNA levels were identified in U-CLL vs. M-CLL (fold difference, FD, >2, P<0.00001). In accordance with this result, Western analyses of 59 cases (U-CLL, n=26; M-CLL, n=33) revealed significantly higher EZH2 protein expression in U-CLL vs M-CLL (FD=4.5, P<0.0001). Interestingly, we also observed higher levels of H3K27me3 in U-CLL vs M-CLL (FD=4.3, P<0.01), strongly supporting that EZH2 over-expression in U-CLL is functionally relevant. We next evaluated whether the clustering of CLL cases in subsets based on BcR stereotypy might be reflected in subset-biased differences in EZH2 expression, focusing on the largest subsets in the present cohort, namely: (i) subset #1 – IGHV1 clan genes, U-CLL, n=8; (ii) subset #4 – IGHV4-34, M-CLL, n=9; (iii) subset #6 – IGHV1-69, U-CLL, n=8; and, (iv) subset #8 – IGHV4-39, U-CLL, n=7. Significantly (FD>1.7-2.2, P<0.05) higher EZH2 mRNA levels were identified in (i) subset #1 versus subset #4, confirming our previous findings; (ii) subset #1 versus U-CLL subsets #6 and #8 as well as U-CLL in general. Finally, we investigated possible clinical implications and found significantly shorter time-to-first-treatment (P=0.04) in patients with increased EZH2 mRNA levels. However, likely due to relatively small numbers, EZH2 mRNA levels did not retain prognostic significance on multivariate analysis, where only advanced clinical stage and U-CLL status emerged as independent negative prognostic factors.
Summary / Conclusion: EZH2 is over-expressed in aggressive CLL subgroups and seems to have a functional impact as evidenced by distinctive histone marks. These results offer hints about the evolution of CLL through epigenetic activation of oncogenic signaling cascades and inhibition of pro-differentiation pathways. Better understanding of the molecular basis of such regulation in agressive CLL could help in establishing EZH2-mediated epigenetic silencing as a therapeutic target.

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OUTCOMES OF PATIENTS WITH FLUDARABINE-REFRACTORY CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL) – A POPULATION-BASED STUDY FROM A WELL-DEFINED GEOGRAPHIC REGION

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Background: Treatment of fludarabine-refractory (FR) chronic lymphocytic leukemia (CLL) remains unsatisfactory. Several new agents have been or are currently being tested in pivotal, non-controlled phase 2 trials. Knowledge on the natural history of similar groups of advanced-phase CLL and outcome of existing salvage regimens in routine health care is therefore mandatory.
Aims: To study the outcome of consecutive patients with FR CLL from a geographically well-defined area without external referrals. Such patients are likely to represent an important group of non-selected patients that can serve as comparators for data obtained with new orphan drugs in non-randomised phase II trials.
Methods: Patients with CLL (n=1479) were identified from the Cancer Registry in Stockholm (1991-2010). 1301 patient files (94%) were identified and reviewed to identify FR patients. Efficacy and toxicity of salvage therapies were recorded as well as long term follow-up. Patients were also subclassified as follows: FR with bulky lymph nodes (BFR group); double-refractory to both F and alemtuzumab (DR group), or being FR without fulfilling criteria for BFR or DR (group "Other").
Results: Chart review identified totally 92 consecutive, non-referred patients with FR CLL undergoing various types of salvage therapy. Median age was 69 years, most had Rai stage III/IV and had received a median of 3 prior therapies (range 1-9). The overall response rate (ORR) was 20% (all but 3were PR), which was significantly lower in the BFR subgroup (ORR 8%) and the DR group (ORR 20%) than in the group "Other" (ORR 31%) (P=0.01). Median time to treatment failure in all patients was 5 months. The ORR in patients who received antibody therapy was 35% with a median time to start of next therapy of 10 months. Early deaths (within 8 wk) occurred in 5% and ≥ grade 3 infections in 20% of patients which appeared lower than previously reported from other centers/series. Median overall survival (OS) was 18 months; it was significantly longer in BFR patients (median 29 months) than in the DR group (median 13 months) (P<0.05, log-rank test). There was no significant difference in OS between refractory patients treated in the first decade vs the second time period of this study. Among baseline prognostic factors on survival, only gender reached statistical significance (P=0.01, multivariate Cox regression analysis).
Summary / Conclusion: Our study describes the natural history of fludarabine-refractory CLL in consecutive patients in a region with almost complete follow-up and without influence on the results from external referrals. Such results may be used for comparison with data obtained in non-controlled phase 2 trials on new orphan drugs.

P105

PROLONGED CYTOPENIA RELATED TO FLUDARABINE, CYCLOPHOSPHAMIDE AND RITUXIMAB IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Fludarabine, Cyclophosphamide and Rituximab (FCR) is the most effective frontline treatment for Chronic Lymphocytic Leukemia (CLL). This regimen is often associated with transient cytopenia during administration that resolves after treatment. In a fraction of patients (pts), cytopenia persistence can raise concerns for CLL relapse, increased risk of infections, or development of secondary myeloid malignancies (SMM).
Aims: To define laboratory and clinical features associated with the development of cytopenia after frontline FCR in CLL pts. To investigate its association with survival and risk for SMM.
Methods: Cytopenia were graded according to CTCAE4 criterias and considered significant if grade 2-4. Only pts achieving complete remission (CR), CR with incomplete marrow recovery (CRi) and nodular partial remission were included. Categorical and continuous variables were compared using the χ^2 and Mann-Whitney test. Kaplan-Meier estimates were compared using the log-rank test.
Results: Three-hundred pts were treated with frontline FCR at our institution between 1999 and 2002. Two-hundred-seven pts met criteria for analysis. The incidence of cytopenia after treatment at3, 6 and 9 months is described in the Table 1. Baseline characteristics of cytopenic (CY)(72) and non-cytopenic (NCY)(132) pts, including prognostic factors, were compared. CY pts were significantly older (P=0.02), had more advanced Rai stage disease (P=0.01) and more profound baseline neutropenia (P=0.04), anemia (P=0.04) and thrombocytopenia (P=0.001). CY pts had a significantly lower likelihood of completing 6 cycles of FCR (67% vs 87%)(P=0.001). Progression Free Survival (PFS) for CY pts was 135 months and Overall Survival (OS) has not been reached, after a median follow up of 114 months (7-155). No differences in PFS (135 vs 95 months, P=0.31) and OS (both not reached, P=0.84) were observed in comparison to NCY pts, even for CY with persistence of cytopenia at 6 and 9 months. The incidence of SMM was comparable between the 2 groups (2 CY, 5 NCY)(P=NS).
Summary / Conclusion: Cytopenia commonly occurs during FCR. Our analysis indicates that cytopenia is a more frequent finding in older patients, and patients presenting with Rai stage IV disease. Response duration, OS, and the rate of SMM are identical to those seen in patients without cytopenia.

Table 1.

	CYTOPENIA	G2	G3-4
3 months	72/207 (35%)	32/72 (44%)	40/72 (56%)
6 months	45/191 (24%)	20/45 (44%)	25/45 (56%)
New onset*1	13/45 (29%)	8/13 (62%)	5/13 (38%)
Persistence	32/45 (71%)	12/32 (38%)	20/32 (62%)
9 months	24/198 (12%)	8/24 (33%)	16/24 (67%)
New onset*2	2/24 (8%)	0/2 (0%)	2/2 (100%)
Persistence	22/24 (92%)	8/22 (36%)	14/22 (64%)

*1: 11/13 with previous G1 cytopenia
*2: 2/2 with previous G1 cytopenia

P106

FRONTLINE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA WITH DELETION 17P

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Background: Patients (pts) with relapsed Chronic Lymphocytic Leukemia (CLL) bearing deletion 17p (del17p) are very high-risk for poor clinical outcomes.
Aims: We summarize outcomes for first-line treatment of del17p CLL.
Methods: We identified 63 pts with CLL and del17p by FISH who received first-line treatment at MDACC between 1/04 and 12/12. Log-rank test and Cox regression were used for univariable and multivariable analyses.
Results: Baseline characteristics are shown (Table 1). Median time from diagnosis to first treatment (TTFT) was 15 (11-19) months (mos); no association between % del17p positive cells at diagnosis and TTFT was noted (P=.45). First-line therapy consisted of fludarabine, cyclophosphamide, rituximab (FCR) based regimen in 49 pts, rituximab-based in 6 pts, and lenalidomide-based in 8 pts. Ninety pts (30%) achieved complete remission (CR), 2 (3%) nodular partial remission (nPR), 18 (30%) PR, and 24 (37%) were non-responders. Fourteen CR pts (78%) were minimal residual disease free by 4-colour flow cytometry. The median time-to-treatment failure (TTF) was 14 (10-18) mos (43 events); the median follow-up was 33 (1-89) mos. Univariable analyses showed age >65 yrs (P=.04), complex karyotype (P=.02), lack of response to therapy (P<.001) and >50% cells positive for del17p by FISH (P=0.009) associated with shorter TTF. The multivariable model showed karyotype (P=.005) and quality of response (P<.001) independently associated with TTF. Median overall survival (OS) was 63 (43-83) mos (28 deaths). Fifteen pts (23%) developed Richter Syndrome (RS) after a median of 12 (1-27) mos; 8 deaths (29%) were related to RS. Univariable analyses showed that only lack of response was associated with a shorter OS (P=.001).
Summary / Conclusion: Del17p CLL is high-risk for first-line therapy; better response to therapy was observed in young patients with <50 % del17p positive cells by FISH who received FCR. New strategies and agents must aim at both improving response and maintaining remission, particularly in pts with complex karyotype.

Table 1.

Characteristics (N=63)	n	CR/nPR (%)	ORR (%)	PFS (mos)	OS (mos)
Age ≥ 65 y	25	8*	44*	13*	15
< 65 y	38	50	74	18	NR
Rai stage III-IV	27	33	59	17	49
0-II	36	33	64	14	63
B2M ≥ 4 mg/L	43	33	60	14	55
< 4	20	35	65	16	63
IGHV UM	47	34	60	13	52
M	11	36	64	17	NR
Del17p FISH					
≥ 50% cells	45	22*	56	13*	49
<50%	18	61	78	36	NR
Karyotype					
complex w del17p	20	15	55	11*	35
complex w/o del17p	9	56	56	13	48
< 3 abn w del17p	6	50	83	NR	NR
< 3 abn w/o del17p	19	42	68	20	NR
FCR	49	43*	71*	16	55
Rituximab/Lenalidomide	14	0	29	10	50

*p < 0.05 (Chi-square or log-rank)

P107

THE IMPACT OF NOVEL RECURRENT GENETIC MUTATIONS ON THE EFFICACY OF ALEMTUZUMAB CONTAINING TREATMENT IN HIGH RISK CLL PATIENTS: RESULTS FROM THE HOVON68 TRIAL

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Background: Alemtuzumab treatment is effective in CLL patients with 17p deletion and with chemo refractory disease, indicating that alemtuzumab may act independently of the ATM/p53 axis. However, the impact of *ATM* mutations on alemtuzumab efficacy is less well defined. Moreover, it is currently not known whether alemtuzumab is also effective in patients with the recurrent somatic mutations, which were recently discovered in CLL by next generation sequencing. These include *SF3B1* and *NOTCH1* which have been shown to be associated with impaired prognosis.
Aims: To study the incidence and clinical impact of the novel recurrent genetic mutations in high risk CLL patients treated with alemtuzumab in combination with fludarabine and cyclophosphamide (FCA).
Methods: In the HOVON68 trial, treatment naïve CLL patients with a high risk profile defined as either 17p deletion, 11q deletion, trisomy 12, unmutated IGHV and/or VH3-21, were randomized between treatment with 6 cycles of FC (Oral fludarabine 40 mg/m^2 day1-3 and cyclophosphamide 250 mg/m^2 day1-3) and FC + alemtuzumb (30 mg sc, in cycle 1 day-1 to +1, in cycles 2-6 day1 only). We evaluated 119/272 (43.8%) trial patients based on availability of DNA. This cohort was representative for the complete trial population as there were no differences in main prognostic factors, including age, clinical stage, IGHV mutational status, ZAP-70 or cytogenetic aberrations (17p-, 11q-, trisomy 12, 13q-). Mutational analysis of *SF3B1* (ex14+15), *NOTCH1* (ex34), *TP53* (ex4-10), *BRAF* (ex11+15), *KRAS* (ex2+3), *NRAS* (ex2+3), *EZH2* (ex16), *MYD88* (ex3+5)

and *PIK3CA* (ex9+20) was performed by next generation sequencing. Extensive mutational analysis of *ATM* (ex1-62) is currently being performed (analyzed at present: n=19). Complete analysis will be reported at EHA.
Results: 19 (16%) *SF3B1*, 12 (10%) *NOTCH1*, 16 (13%) *TP53*, 5 (4%) *BRAF*, 5 (4%) *KRAS*, 2 (2%) *NRAS* and 1 (1%) *MYD88* mutations were found. Thus far *ATM* mutations were identified in 5 (out of 19) patients. Mutations in *TP53* correlated with 17p deletion (P<0.0001), *SF3B1* mutations with 11q deletion (P=0.008) and *NOTCH1* mutations with trisomy 12 (P=0.001). The overall response rate (ORR) and complete response (CR) were decreased in patients with *TP53* mutation (P=0.001 and P=0.01 respectively) but not in those with the other mutations. FCA improved ORR in patients with *TP53* mutations (14% vs 67%, borderline-significance P=0.06). However in patients with the other mutations there were no differences in ORR/CR between the two treatment arms.
The median follow-up was 42.5 months (range: 4-82 months). Progression free survival (PFS) was impaired in patients with *TP53* mutation (P<0.001) but not in patients with *NOTCH1* mutation (P=0.82). FCA improved PFS in patients with *TP53* mutation (median 3 vs 16 months, P=0.02), as well as in *SF3B1* mutated patients (median 33 vs 44 months, P=0.05). Overall survival (OS) was only decreased in *TP53* mutated (P=0.02) patients and there was a trend for improved OS due to FCA (median 24 vs 67 months, P=0.09).
Summary / Conclusion: Novel recurrent mutations were found in this high risk CLL treatment naïve patient group, in frequencies comparable to those reported in recent literature. As expected, *TP53* mutations had an adverse prognostic impact as to ORR, CR, PFS and OS, which was overcome by alemtuzumab. In addition, we found that patients with an *SF3B1* mutation might benefit from alemtuzumab. This latter finding is in line with our recent observation suggesting that *SF3B1* mutations affect the ATM/p53 axis.

P108

UPDATED RESULTS OF A PHASE II STUDY OF LENALIDOMIDE AND RITUXIMAB IN RELAPSED/REFRACTORY (R/R) CHRONIC LYMPHOCYTIC LEUKEMIA.

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Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with variable clinical course. Patients with R/R disease have a very poor outcome. Response rates to salvage therapy are 20 to 40%; however they are short-lasting. Lenalidomide (L) has shown encouraging and significant response duration in phase II studies with acceptable toxicity. It is postulated that lenalidomide enhances the antibody dependent cytotoxicity (ADCC) of rituximab (R) by potentiating NK cell responses.
Aims: The primary endpoints included objective response rate (ORR) [complete (CR) and partial (PR) remission], clinical benefit [stable disease (SD) + ORR] and safety and tolerability of the combination regimen. Secondary endpoints were time to treatment failure (TTF), overall survival (OS) and assessment of adverse events (AEs).
Methods: This phase II study evaluated a combination of L+R in R/R CLL. The regimen consisted in dose-escalated L=2.5 mg (days 1-7), 5 mg (days 8-14) and 10 mg (days 15-21) followed by 7 days of rest in a 28-day cycle; for cycle 2 and beyond L=20 mg was given on days 1-21 on a 28 day cycle. R was dosed at 375 mg/m^2 IV weekly for 4 weeks starting on day 15 of cycle 1. In high tumor burden disease (lymphocytes count ≥25,000/μL) R was given prior to lenalidomide. Treatment was continued until disease progression or if unacceptable toxicity ensues. Responses were assessed according to IWCLL 2008 criteria.
Results: Twenty four patients were enrolled in the study. Median age was 62.5 years (41-79). Male/female ratio was 1.67. Patients characteristics included: Rai stage III-IV=13 (54%), bulky disease=11 (45.8%), median number of prior chemotherapy regimens=3 (range 1-5), fludarabine refractory=4 (16.7%), unmutated IgVH =13/19 (54.2%), CD38 ≥30%=9/23 (37.5%), ZAP-70=5/15 (20.9%). Cytogenetic abnormalities included trisomy 12 (10/24), del11q (9/24), del13q (9/24) and del17p (3/24). Five patients did not complete more than 3 cycles [hypercalcemia (1), seizures (1), ischemic stroke (1), severe hematological toxicity (1) and withdrawal of consent (1)] and one patient did not initiate L (due to infusion reaction to R). The ORR in 18 evaluable patients was 61.1% and on the intention to treat (ITT) analysis was 45.8%. The clinical benefit (ORR+SD) was 83.3% in evaluable patients. The median time-to-response and median duration of response were 4.1 and 22.5 months, respectively. The ORR was independent of age, gender, Rai Stage, bulky disease, hierarchical genetic abnormalities, IgVH mutational status or fludarabine refractoriness. The regimen was well tolerated. The most common grade 3/4 AEs were fatigue (33.3%), infections (25%) and diarrhea (12.5%). Most common causes of infections were pneumonia (20.8%) and sinusitis (8.3%). Hematological toxicity consisted of grade 3/4 neutropenia (50%) anemia (8%) and thrombocytopenia (4%). Tumor flare reaction was observed in eight cases (33.3%). The TTF in the evaluable patients was 17.7 months and in the ITT analysis was 14.3 months. After a median follow-up time of 31.2 months the OS was not reached. By univariate analysis, achieving a response predicted a better overall survival.
Summary / Conclusion: The combination of lenalidomide and rituximab is a feasible alternative for the treatment of R/R active CLL, including high risk and heavily pretreated patients, with significant and durable responses and an acceptable toxicity profile.

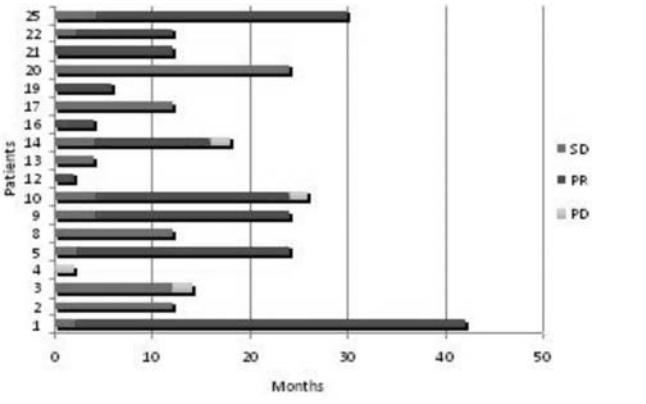

Figure 1.

P109

A MULTICENTER, PHASE IV OBSERVATIONAL SAFETY STUDY OF OFATUMUMAB IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): A EUROPEAN RESEARCH INITIATIVE ON CLL (ERIC) STUDY

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Background: Ofatumumab was given a conditional approval in the EU on April 2010 for the treatment of CLL refractory to fludarabine (F-ref) and alemtuzumab (A-ref), encouraging the retrieval of further data in patients treated in a "daily life" setting and to investigate treatment safety.
Aims: The main objective of the study was to obtain information on the safety profile of ofatumumab in patients with CLL treated outside clinical trials. The secondary endpoints were efficacy, progression free survival (PFS), and overall survival (OS).
Methods: This was an observational, retrospective study. Patients were eligible for the study regardless of prior treatments or disease status and provided they had not been included in phase II or phase III ofatumumab clinical trials. Data on patients' characteristics at diagnosis, prior treatment, toxicity to therapy, response rate, PFS and OS were recorded.
Results: One hundred and twenty patients were screened, of which 103 from 25 centers in 10 European countries were eligible for the study. There were 71 males; median age at initiation of ofatumumab was 64 years (range, 38-84); 66% patients were in advanced clinical stage;54% were F-ref, 70% A-ref and 41% were both. One hundred and sixty one toxic events were reported in 68 patients, with 28 (17%) of them being considered as ofatumumab-related. Infusion reactions occurred in 19 (30%) patients (grade III-IV: 21%). Neutropenia was reported in 26% patients (grade III-IV: 22%), thrombocytopenia in 15% (grade III-IV: 12%) and anemia in 15% (grade III-IV: 7%). The non-hematological adverse events were infection (29%), dyspnea (10%), fatigue (7%), fever (7%), rash (7%), cough (5%), diarrhea (4%) and nausea (1%). A correlation was observed between the number of prior lines of therapy and

hematologic toxicity. Autoimmune hemolytic anemia was recorded in one patient. One patient developed Richter's syndrome while receiving ofatumumab. Two heavily pre-treated patients presented progressive multifocal leukoencephalopathy.The overall response rate (ORR) was 23% and the median PFS and OS were 5 and 12 months, respectively. The main causes of death were disease progression (61%) and, infection (28%).
Summary / Conclusion: These results show that with the only exception of the ORR (lower in this study) all endpoints (PFS, OS) and safety profile in CLL patients treated with ofatumumab in daily practice are consistent with those observed in phase II and III clinical trials.

P110

THE PROGNOSTIC VALUE OF THE NUMBER OF CELLS WITH A DELETION OF 13Q14 IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: The presence of isolated deletion 13q14 (del13q14) in patients with chronic lymphocytic leukemia (CLL) is a favourable prognostic factor of the disease. However, patients with CLL differ in the number of cells with del13q14, as well as in the degree of expression of Zap-70 and the level of thymidine kinase (TK). Zap-70 and TK are considered as the independent predictors of overall survival (OS) and the outcome of CLL.
Aims: Determine the prognostic value of the number of cells with del13q14 in association with Zap-70 and TK.
Methods: We identified 124 patients with CLL. The median age was 61 years. Twenty-five were in Binet stage A, 84 in stage B and 15 in stage C. Chromosomal abnormalities were determined by FISH. Moreover, the expression of Zap-70 and the contents of serum TK were determined by flow cytometry (cut-off value 20%) and a radioenzyme method accordingly (cut-off value 20 U/L). All researches were performed during the time of diagnosis before specific therapy.
Results: Del13q14 was detected as the only chromosomal abnormality or in combination with other chromosomal disorders in 77 (62%) and 47 (38%) patients accordingly. Patients with a single del13q14 were divided into 2 groups according to the number of cells with this aberration. The first group included 41 (53%) patients with del13q14 in ≥ 60% of cells, the second one – 36 (47%) patients with del13q14 in < 60% of cells. Among patients with del13q14 in ≥60% of cells positive level of protein expression Zap-70 and the content of TK >20 U/L in serum were detected in 39 (95%) and 40 (98%) patients accordingly. At the same time, among patients with del13q14 in < 60% of cells positive expression of Zap-70 and high levels of serum TK were not detected in any case. Median follow-up was 68 months. Median OS in patients with del13q14 in <60% of cells was 144 months, median time to first treatment (TFT) was 42 months. In patients with del13q14 in ≥60% of cells median OS was 65 months, but median TFT – 13 months (P=0.034 and P=0.002, respectively). By univariate analysis, OS was significantly shorter in patients older than 60 years (P=0.017), Binet stage B or C (P<0.001), with del13q14 in ≥60% of cells (P=0.002), the content of TK ≥20 U/L (P=0.023) and the positive expression level of Zap-70 (P<0.001). During undertaking of the multivariate analysis by the independent predictors of OS and TFT the level of expression of Zap-70 (P=0.014; p=0.023) the contents of TK (P=0.036; P=0.009), and the number of cells with del13q14 (cut-off value 60%) (P=0.016; P=0.025) were determined in patients with CLL.
Summary / Conclusion: Patients with del13q14 in ≥ 60% of cells have a poorer prognosis than patients with del13q14 in < 60% of cells. For a more precise stratification of patients into risk groups a set of factors should be used simultaneously, including the number of cells with del13q14, Zap-70 and TK which will identify patients with a poor prognosis more accurately.

P111

FINAL RESULTS OF A MULTICENTER PHASE IB SINGLE AGENT STUDY WITH THE NOVEL ANTI-CD20 MONOCLONAL ANTIBODY UBLITUXIMAB (TG-1101) IN PATIENTS WITH RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)
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Background: Ublituximab (TG-1101, previously LFB-R603) is a novel, chimeric monoclonal antibody (mAb) targeting a unique epitope on the CD20 antigen. Ublituximab has been glycoengineered to enhance affinity for all variants of FcγRIIIa receptors and therefore demonstrates greater antibody-dependent cellular cytotoxicity (ADCC) activity than rituximab and ofatumumab, particularly against tumor cells that express low CD20 levels. A weekly x 4 dose regimen of ublituximab was found to induce rapid, profound and sustained blood lymphocyte depletion in patients (pts) with advanced stage CLL in a first-in human dose-escalation phase I study (Part 1). The second part of the study (Phase Ib) was designed to evaluate a fixed, weekly x 8 dose regimen for safety, pharmacokinetics (PK) and efficacy of ublituximab in pts with CLL relapsed after at least one prior course of therapy with fludarabine.
Aims: Herein, we report the final results of the Phase Ib study.
Methods: From April 2010 to August 2011, 12 pts were enrolled at 9 centers in France and followed for 12 months. Eight infusions of ublituximab were administered once weekly consisting of an initial dose of 150 mg followed by 7 doses of 450 mg, with no maintenance therapy. Safety was the primary endpoint (CTCAE v. 3.0), with PK and efficacy (IWCLL, Hallek 2008) secondary endpoints.
Results: Median age was 69.5 years [62–77], median time from diagnosis to inclusion was 10.4 years [4.0–23.6], median prior therapies was 3 [range 1–8]. Seven pts (58%) received at least one prior rituximab-containing regimen. Abnormal cytogenetics: 10/12 (2 with 17p del). Bulky (>5cm) lymph node enlargement was observed in 4 pts (33%), splenomegaly in 9 pts (75%), and hepatomegaly in 4 pts (33%). Median lymphocyte bone marrow infiltration was 85% [40–94]. PK data showed an increase of mean Cmax, AUC_{∞} and $t_{1/2}$ term from the first to the eighth infusion from 23.4 to 220.5 mg/L, 732 to 50, 760 mg.h/L, and 13.4 to 147.8 h, respectively whereas mean CL decreased from 424 to 38.6 mL/h. Median lymphocyte count at baseline was 46.6 ($x10^9$/L; after 1 month (M1) = 1.5 (94%↓); M4=1.4 (91%↓) and M6=2.0 (89%↓). 12/12 patients (100%) achieved a peripheral lymphocyte response. All pts but one received the planned 8 infusions without any dose reduction. One patient was prematurely withdrawn due to a concomitant secondary leukemia. Most frequent drug-related AEs were infusion related reactions (IRR) (75% of the pts, including 33% of pts with Grade 3 IRR). Other Grade 3/4 AE's > 10% included: neutropenia (67%) and increase ALT/AST (17%). All AEs were reversible spontaneously or with supportive care intervention. Response was evaluated at M4 for the 11 evaluable pts, with an initial response rate of 64% (7/11) with a confirmed response at M6 in 5/11 pts (45%) pts (all PRs). 4/11 pts achieved stable disease. At the 1 year follow-up, no responders had progressed demonstrating all confirmed responses were durable, despite no ublituximab maintenance therapy. Median PFS was not reached at the 12 month follow-up.
Summary / Conclusion: Ublituximab induced a durable 45% ORR in pts with advanced stage CLL at a relatively low dose regimen (total 3300 mg over 8 weeks, with no maintenance). PK data indicates that the dose and the schedule of administration could be optimized. Toxicity was manageable and typical for anti-CD20 therapy, enabling combination with novel agents. Phase I/II studies evaluating ublituximab at doses > 450 mg as a single agent, in addition to combination therapy with lenalidomide in pts with CLL and NHL are currently ongoing. Monthly maintenance with ublituximab has been incorporated into ongoing clinical trials.

P112

CLL CELLS ARE HIGHLY SENSITIVE TO THE INHIBITION OF PI3K-P110 ALPHA ISOFORM WHICH IS ASSOCIATED WITH DOWNREGULATION OF CD23
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Background: PI3K/Akt signalling cascade is emerging as a promising therapeutic target in chronic lymphocytic leukemia (CLL). However, CLL cells highly express all four isoforms of PI3K (alpha, beta, gamma and delta). Therefore, the advantage of using pan- or isoform-selective PI3K inhibitors remains to be extensively explored.
Aims: The primary aim of the work is to compare the effect of different pan and isoform selective PI3K inhibitors on the viability of CLL cells in a well characterized cohort of CLL patients in terms of clinical stage, cytogenetics and IgVH mutational status.
Methods: Exposure of CLL cells to the inhibitors is performed in suspension culture and in co-culture with bone marrow stromal cells (BMSC) to minimize the spontaneous apoptosis rate of CLL cells. The inhibitors included pan PI3-K inhibitors (LY294002, wortmannin and PI-103), and the isoform selective inhibitors against p110-a: (N-((1E)-(6-Bromoimidazo[1,2-a]pyridin-3-yl)ethylene)-N′-methyl-N″-(2-methyl-5-nitrobenzene)sulfonohydrazide), p110-b: (TGX-221), p110-c: (5-(2,2-Difluoro-benzo[1,3]dioxol-5-ylmethylene)-thiazolidine-2,4-dione) and p110-d: (IC87114 and CAL-101). Cell viability was assessed by FACS (annexin V/Pi staining) and MTT assay. Induction of apoptosis was confirmed by detection of Caspase and PARP cleavage using western blotting. In addition, effects on the activation status of the central PI3K pathway molecules Akt and PTEN were analysed by western blotting and intracellular FACS.
Results: The results revealed that the p110-a isoform selective inhibitor was the most effective in inducing apoptosis in CLL cells compared to other inhibitors at equal concentrations. Dose and time kinetics showed that the IC50 ranged between 10 and 40 nM within 24 hours of incubation with some variation between patients. Inhibition of p110a subunit led to a rapid induction of apoptosis (within 6 hours) at nanomolar concentrations (10-100nM). The induction of apoptosis was accompanied by Caspase and PARP cleavage and a decrease in CD23 surface expression as assessed by FACS. Decrease in CD23 and Mcl-1 expression could also be detected by western blotting. In parallel, p110a-inhibitor induced a significant de-phosphorylation of Akt particularly at Thr308 and de-phosphorylation of PTEN at several residues in its tail domain. PI3K-alpha inhibitor was highly effective in suspension culture and could overcome the supportive effect of BMSC in co-culture. The response to PI3K-alpha was observed in all patients tested independently of the clinical stage, cytogenetics and IgVH mutation status. In addition, downregulation of surface CD23 expression correlated to the response to PI3-K inhibitors.

Summary / Conclusion: The data confirm the importance of PI3K as a therapeutic target in CLL and reveal the high sensitivity of CLL cells to the inhibition of p110a isoform in particular. The results also demonstrate that CD23 expression may represent a reliable biomarker for monitoring the response to PI3-K inhibitors. Therefore, PI3K alpha may represent an important therapeutic target in CLL and warrants further investigation and clinical validation.

P946

CLL PATIENTS CARRYING 13Q DELETION SHOW CLINICAL HETEROGENEITY ACCORDING TO IGVH MUTATIONAL STATUS AND CD38 EXPRESSION

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Background: Chronic Lymphocytic Leukemia (CLL) is one of the most common and indolent hematological malignancies characterized by a heterogeneous clinical course and outcome. Over the last years several clinical or biological markers have been identified as prognostic factors. In particular, patients carrying chromosome 13q14 deletion (13q-) have been usually considered to have a good prognosis. Despite this, some of these patients require treatment a few years after the diagnosis.
Aims: The aim of this study was to investigate clinical features in 13q- CLL patients by correlating/integrating different prognostic factors, i.e. IgVH mutational status and CD38 expression, in the effort to define a scoring system useful for clinical practice and patients risk stratification.
Methods: Fluorescence *in situ* Hybridization (FISH) was performed in 214 CLL patients, referred to the Hematology Division of Padua University Hospital from 1989 to 2011; 114 out of 214 (53%) showed chromosome 13q14 deletion. Both IgVH mutational status (mutated, MUT or unmutated, UNMUT) and CD38 expression ($CD38^+$ or $CD38^-$; cut-off: 30%, as determined by cytofluorymetric) were available for 105/114 patients. Considering these two parameters in 13q- group, we developed a scoring system assigning score 0 to 13q- patients which were MUT&$CD38^-$, score 1 to 13q- patients which were UNMUT&$CD38^-$ or MUT&$CD38^+$, and score 2 to 13q- patients which were UNMUT&$CD38^+$ (Figure 1A). Statistical analyses for time to first treatment (TTT) and overall survival (OS) were performed using Kaplan-Meier Log-Rank test; p values <0.05 were considered significant.
Results: On the basis of the above mentioned scoring system, 13q- CLL patients were stratified in 3 different groups: 65/105 (59%) patients with score 0; 30/105 (29%) with score 1 and 10/105 (9%) with score 2 (Figure 1B). The number of treated patients was 28% for score 0, 43% for score 1 and 60% for score 2; 2% with score 0, 7% with score 1 and 17% with score 2 patients died. According to this scoring system, the analysis of TTT and OS of our 13q- patients stratified them in 3 statistical different groups (P<0.002 for TTT, Figure 1C and P<0.001 for OS, Figure 1D). In particular, score 0 patients showed an indolent disease and only the 16% of their require therapy after 3 years from diagnosis and @ 5 years OS of 100%; conversely, score 2 patients had an aggressive clinical course with @ 3 years TTT of 70% and @ 5 years OS of about 53%.
Summary and Conclusions: Chromosome 13q deletion has usually been expected to assign a favorable outcome to CLL patients. Despite this, some patients are treated or die few years from the diagnosis, demonstrating that not all 13q- CLL patients have the same clinical behavior, with some of them showing an aggressive clinical course. Herein we have underlined this clinical diversity demonstrating that 13q- patients UNMUT&$CD38^+$ (defined as score 2) have a shorter TTT and OS than score 0 patients (MUT&$CD38^-$). Hence the need to develop a prognostic system, including low risk CLL, that allows risk stratification and helps in timing and choice of therapy as well as to define following scheduling.

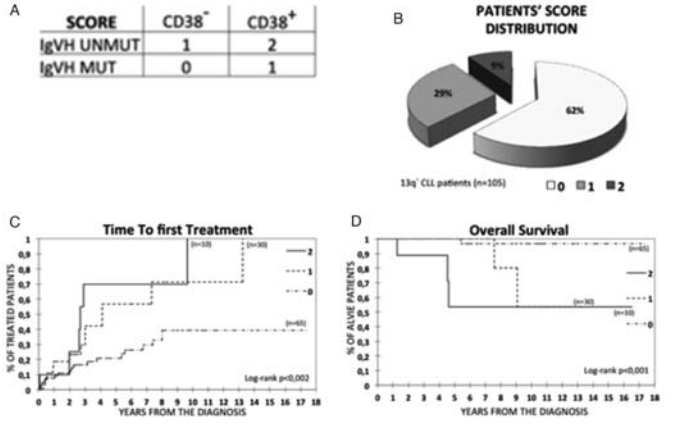

Figure 1.

Chronic myeloid leukemia - Biology 1

P113

IMATINIB OR DASATINIB TREATMENT OF CHRONIC MYELOID LEUKEMIA REDUCES CIRCULATING MYELOID-DERIVED SUPPRESSOR CELLS AND INCREASES THEIR CD40 EXPRESSION

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Background: Tyrosine kinase inhibitors (TKIs) such as imatinib or dasatinib are first line therapy for chronic phase chronic myeloid leukemia. Studies show that TKIs can modulate cells of the immune system, possibly affecting anti-tumor immunity of treated patients as well as giving rise to immunological adverse events. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of myeloid cells that regulate T cell activation during immune responses. MDSCs utilize different mechanisms for suppressing immune responses, including up-regulation of the enzyme arginase 1 (Arg1) which leads to inhibition of T cells and other immune cells. Moreover, MDSCs can affect the immune system by induction of T regulatory cells (Tregs) which regulate the immune system by inhibition of effector cells. MDCSs and Tregs are expanded in patients with different solid tumors, and increased levels have been associated with worse prognosis. The mechanism by which Tregs are induced by MDSCs has not been extensively studied, but involvement of the CD40/CD40 ligand (CD40L) pathway has been suggested.
Aims: To investigate samples from patients treated with imatinib or dasatinib for the presence of immune escape mechanisms such as MDSCs, Arg1 and Tregs and to investigate the CD40 expression. Samples were also analyzed for the presence of natural killer (NK) cells and T memory cells.
Methods: Patient samples were from the clinical trial "Randomized Study Comparing the Effect of Dasatinib and Imatinib on Malignant Stem Cells in Chronic Myeloid Leukemia (NordCML006)". The study was performed according to the declaration of Helsinki and all patients gave their written informed consent. Samples were analyzed at baseline and after one and six months of treatment by flow cytometry and ELISA.
Results: Patient MDSC levels decreased with TKI treatment and at 6 months there was a significant difference compared to baseline (P<0,05). The leukocyte count at baseline, reflecting the tumor burden, correlated with the MDSC level at baseline (Spearman r: 0,6713, P<0,0001). The median Arg1 concentration at baseline (44,8 ng/ml, range 14,5-277 ng/ml) was higher than has been reported for control subjects and treatment with TKIs significantly decreased the level of Arg1 after one and six months (P<0,05 and P<0,05, respectively). The baseline Arg1 concentration was correlated with the level of MDSCs at baseline (Spearman r: 0,5238, P=0,005). CD40 expression on MDSCs was low in patients at baseline (median 12,6%, range 6,5-32,3%) and increased during treatment (6 months: median 33,6%, range 9,2-64,8%) (P<0,05). Treg levels were significantly increased at one and six months of treatment (P<0,05 and P<0,05, respectively). Natural killer cells were increased by TKI treatment (P<0,05 median 10,6%, range3,4-44,4% at baseline and median 15,7%, range2,6-49,0% at 1 month of treatment) and there was a non significant trend towards increasing T memory cells.
Summary / Conclusion: Dasatinib and imatinib decrease MDSC levels while simultaneously promoting expression of CD40 on myeloid cells which may explain the increase of effector lymphocytes such as NK- and memory T cells. Taken together, our results indicate that TKIs, even though shown to be immune inhibitory in many *in vitro* studies, can be immunostimulatory *in vivo* because of a combination of inhibition of regulatory cells and stimulation of effector cells. These results may aid the understanding of which patients could benefit from TKI discontinuation after achieving a complete molecular response.

P114

THE AXIS SHP-1/SHP-2 MODULATES VEGFR2 SIGNALING IN CML CELL LINES: VEGFR2 DEREGULATION MAY BE IMPLICATED IN IMATINIB (IMA) RESISTANCE

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Background: The Imatinib (IMA) discovery revolutionized the treatment and management of CML patients and represented the gold standard therapy for this disease. However, although the vast majority of patients respond to the therapy, the resistance is emerging as a significant problem. Our previously published data showed that SHP-1 plays a key role in the response to IMA treatment through negative regulation of SHP-2[1]. Thus, we evaluated the putative factors able to activate SHP-2 in Ph+ CML cells resistant to TKI activity (named KCL22R). Gene expression profile of KCL22-R cell line revealed an up-regulation of Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) respect to the TKI sensitive cell line KCL22S. In this regard, several groups showed that the signaling from VEGFR-2 is necessary for proliferation, chemotaxis and cell survival of several tumor systems.
Aims: The aim of this study is to evaluate a possible involvement of VEGFR-2 signaling in the regulation of IMA responsiveness in CML cells resistant to TKI treatment.
Methods: We assessed the level of gene and protein expression of VEGFR-2 in KCL22 cell lines by RT-qPCR and western blot (WB) analysis. Moreover, we performed co-immunoprecipitation assays to evaluate a putative interaction between the two phosphatases SHP-1 and SHP-2 with VEGFR-2. Then, we carried out WB to specifically demonstrate that this interaction is functional. *in vitro* cellular experiments were done to assess the role of VEGFR-2 to modulating cell viability in CML cells resistant to IMA treatment.
Results: First, we demonstrated that VEGFR-2 is expressed at higher level in KCL22R respect KCL22S cell line, at both mRNA (2.2±1.2 vs 0.1±0.14 VEGFR-2/ABL mRNA copy numbers; P=0.01) and protein level. In addition, we demonstrated that VEGFR-2 forms a complex with SHP-1 and SHP-2 in KCL22S cell line but not in KCL22R cell line, in which we detected only the interaction with SHP-2, since SHP-1 is down-regulated. Indeed, the ectopic SHP-1 expression in KCL22R cell line allow the interaction VEGFR-2/SHP-1. Recently, Bhattacharya *et al*, demonstrated that SHP-1 regulates negatively VEGFR-2 signaling by dephosphorylation of specific tyrosine residues in HUVEC cells. Thus, we hypothesized that SHP-1 may have an important role in the down-regulation of VEGFR-2 signalling in Ph+ cells sensitive to TKI treatment, and on the contrary, the VEGFR-2 activation in resistant Ph+ cells due to the down-regulation of SHP-1 may account for a novel mechanism of tumour escape. Indeed, we demonstrated that VEGFR-2 *activation sites* (Y996 and Y1059) remain phosphorylated after IMA treatment only in KCL22-R but not in KCL22-S cell line and that the ectopic SHP1 expression in resistant cell line is associated with dephosphorylation of the same sites. Thus, to evaluate if the activation status of VEGFR2 was correlated with expression levels of its ligand VEGF, we performed ELISA assay on KCL22 supernatant cells. This analysis revealed that VEGF is equally produced in both sensitive and resistant cell lines and that IMA treatment reduces its level. To better clarify the functional role of VEGFR2 activation in IMA resistant cell line, we treated KCL22S and R with the VEGFR-2 inhibitor *sorafenib*. Sorafenib treatment induced apoptosis in 60% of KCL22R, whereas it does not produced effects on KCL22S viability.
Summary / Conclusion: Taken together, our dataindicate the involvement of VEGFR-2 in the signaling pathways, Bcr-Abl independent, implicated in IMA resistance and that the axis SHP-1/SHP-2 may modulate VEGFR2 signaling in CML cell lines.
Reference
Esposito N, *et al*. Blood 2011.

P115

SYNERGY BETWEEN JAK INHIBITOR RUXOLITINIB AND TYROSINE KINASE INHIBITORS (TKIS) TO OVERCOME DRUG RESISTANCE RELATED TO BONE MARROW (BM) STROMA MICROENVIRONMENT IN CHRONIC MYELOID LEUKEMIA (CML)

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Background: Patients with CML in chronic-phase (CML-CP) treated with Imatinib has shown an overall survival rate of 85% in a 8 year result update of IRIS trial, but only a minority of them achieve complete molecular response (CMR). Although second-generation TKIs yield higher rates of CMR *versus* Imatinib there is no still evidence to support the eradication of CML stem cells. Recent evidence suggests that upon TKI treatment, CML stem cell survival is *BCR-ABL* kinase independent phenomena. The BM is a dynamic microenvironment with high concentration of soluble factors that regulates haematopoiesis, enhances leukemia blast survival and modulates their resistance to pharmacological treatment. Indeed, enhanced survival in leukemia stem cell (LSC) protective microenvironments, such as the BM niche, also contributes to LSC persistence.
Aims: We assess the potential role of BM mesenchymal stroma cells (BM-MSC) in mediating resistance to TKIs in LSC derived from CML patients. Moreover, we evaluate if the IC50 of three clinical relevant TKIs: Imatinib, Nilotinib and Dasatinib significantly increase when Ph+ cell lines are treated in the presence of stroma conditioned media (SCM) produced by BM-MSCordirect MSC interaction.Last, we evaluate if Ruxolitinib synergizes with TKIs in the induction of apoptosis in CML cells.
Methods: K562 Ph+ cell line was treated with TKIs in the presence of either RPMI medium, defined as regular media (RM) or human stroma cell line HS-5 serum-free supernatant, named HS5/SCM. After 72hrs of treatment we evaluate apoptosis induction by Annexin-V staining. IC50 was calculated based on the level of viable cells residual after treatment with increasing doses of drugs in the presence or absence of HS-5/SCM. CD34+ cells from BM samples of CML-CP patients were treated with TKIs and tested for the clonogenic potential in Colony Forming Cell (CFC) assay.
Results: The apoptosis of Ph+ cell line K562, treated with clinical doses of Imatinib, Nilotinib or Dasatinib on HS-5 monolayer is significantly reduced (18%±13%, 50%±6%, or 10%±10%, respectively), respect to RM (46%±12%, 84%±15%, or 53%±20%, respectively). Moreover, the TKI-resistance is also related to soluble factors produced by HS-5 cells. Indeed, apoptosis is greatly reduced when K562 cell line is treated with Imatinib, Nilotinib or Dasatinib in the presence of HS-5/SCM (20%±9%, 29%±18%, or 17%±6%, respectively), respect to RM. Furthermore, the IC50 of Imatinib, Nilotinib or Dasatinib is significantly increased when K562 cell are cultured on HS-5/SCM (5309.29nM, 381.14nM, 2.31nM, respectively) vs the IC50 observed when Ph+ cells are cultured in RM (564.97nM, 14.26nM and 1.13nM, respectively). Moreover, we prove that HS-5/SCM cell exposition induces BCR-ABL independent STAT3 activation, without significant modification of cell cycle phases. These preliminary evidences were further confirmed in CD34+ primary cells derived from BM CML-CP patient's samples. Indeed, Imatinib, Nilotinib or Dasatinib show a slight ability to impair the formation of CML colonies in the presence of HS-5/SCM (55%, 46% and 41% colonies, respectively, relative to untreated controls). However, combination of Ruxolitinib/Imatinib or Ruxolitinib/Nilotinib demonstrated a substantial improvement of reducing the formation of CML colonies, relative to untreated controls (5% and 4%, respectively). Importantly, Imatinib, Nilotinib, and Ruxolitinib, alone or in combination did not significantly impair the formation of normal erythroid and myeloid colonies.
Summary / Conclusion: This data suggests that stroma related drug resistance has a relevant role in TKIs responsiveness in patients with CML. Combination therapy applying TKI and Jak inhibitor Ruxolitinib, has a potential value to overcomethe "protective effect" of BM stroma microenvironment.

P116

CHRONIC MYELOID LEUKEMIA WITH VARIANT T(9;22) REVEALS A DIFFERENT SIGNATURE FROM CASES WITH CLASSIC TRANSLOCATION.

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Background: The t(9;22)(q34;q11) generating the Philadelphia chromosome and the *BCR/ABL1* fusion gene represents the cytogenetic hallmark of chronic myeloid leukemia (CML). About 5-10% of CML cases show variant translocations with the involvement of other chromosomes in addition to chromosomes 9 and 22. The prognostic significance of variant t(9;22) was unclear and debated in the pre-imatinib era, whereas recent studies of large CML series showed that the presence of variant translocations has no impact on the cytogenetic and molecular response or on prognosis. However, the molecular bases of differences between CML patients with classic and variant t(9;22) have never been elucidated.
Aims: The aim of this study is to perform a gene expression profiling (GEP) by microarrays to identify a signature discriminating CML patients bearing variant rearrangements from those with classic t(9;22)(q34;q11) and to unveil the molecular bases of CML heterogeneity in chronic phase.
Methods: Conventional and molecular cytogenetic analyses allowed the identifications of 12 CML cases with classic t(9;22) and 8 cases with variant translocations. Total bone marrow RNA was labeled and hybridized on the Agilent SurePrint G3 Human GE 8x60K Microarray slide. To validate microarray data, quantitative real-time polymerase chain reaction experiments were performed. Microarray data were analyzed using two different gene annotation databases (Database for Annotation, Visualization and Integrated Discovery, DAVID; Ingenuity Pathways Analysis, IPA).
Results: A set of 59 genes was identified as differently expressed in CML cas-

es with variant t(9;22) rearrangements. Querying the DAVID database showed that the enhanced biological process in our gene set involved the intracellular protein kinases cascade. The kinases list included 5 genes: *TRIB1* (tribbles homolog 1), *STK17B* (serine/threonine kinase 17b), *PTK2B* (PTK2B protein tyrosine kinase 2 beta), *C5AR1* (complement component 5a receptor 1) and *ZFP36* (zinc finger protein 36, C3H type, homolog). Further IPA analysis yielded strong indications that 19 out of 59 dysregulated genes from our dataset are involved inthe "Haematological System Development and Function, Tissue Morphology, Cellular Development" network. A central role in this network is played by several proteins that are known to be activated in *BCR/ABL1* cells, namely ERK1/2 (extracellular signal-regulated kinases), p38MAPK (p38 mitogen-activated protein kinase), JNK (c-Jun N-terminal kinase), and cell cycle regulator AKT *(*RAC-alpha serine/threonine-protein kinase). Noteworthy, the upregulated kinase genes, previously revealed by DAVID analysis, are also enclosed in the network identified by IPA. Moreover, TRIB1, PTK2B and C5AR1 kinases are involved in the regulation of the RAS/MAPK pathway.
Summary / Conclusion: In conclusion, our GEP analysis performed on CML cases with variant t(9;22) improved the understanding of the biological mechanisms at the basis of the CML heterogeneity. Overall, our results reveal that in CML cases with variant t(9;22) there is an enhancement of the MAPK pathway deregulation already known to underlie the CML pathogenesis and point out the role of interesting candidate genes, such as *TRIB1, PTK2B,* and *C5AR1*. These findings show that kinases are a common target of molecular alterations in hematological disorders and reinforce the idea that a perturbed action of signal transduction pathways is one of the hallmarks of cancer.

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ALIGNMENT STRATEGIES ON INTERNATIONAL SCALE FOR BCR-ABL1 MRNA QUANTIFICATION METHODS: ARE THEY EQUALLY VALID?
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Background: Serial quantitative measurement of BCR-ABL1 mRNA levels in peripheral blood of patients undergoing tyrosine kinase inhibitor (TKI) therapy have proven relevant to aid clinical decisions. Harmonization efforts for improving comparability of the results between laboratories are ongoing worldwide and it is now accepted that results should be expressed on the international scale (IS). The process by which laboratories can access to IS has been extensively described (S. Branford, blood 2008) and applied to European laboratories under the aegis of the European Treatment and Outcome Study (EUTOS) for CML program. It is based on establishment of a laboratory-specific conversion factor that allows alignment of local results on IS. More recently, a genetic reference panel for quantification of BCR-ABL1 mRNA has been approved by the World Health Organization (WHO) and made available for manufacturers, in order to calibrate secondary reference reagents.
Aims: We report here results from a comparative study performed between two commercial kits designed for quantification of BCR-ABL1 mRNA and calibrated on the WHO reference panel and two "in-house" methods routinely used in two EUTOS French laboratories. Main objectives of this study was (i) to evaluate the comparability of the two IS alignment strategies; (ii) to evaluate the added value of kits on interlaboratory reproducibility and (iii) to measure the impact of a preanalytical delay on the results.
Methods: Overall, 4 methods were compared. M1: "in-house" method developed in Saint-Louis hospital (SLS), Paris; M2: "in-house" method developed in Lille hospital (Lille); M3: Xpert BCR-ABL Monitor (Cepheid) run in SLS and Lille; M4: BCR-ABL MBCR IS-MMR DX (Ipsogen) run in SLS and Lille. Sixty CML patients under TKI therapy, with BCR-ABL1 levels expected between 10 and 0.001%, were included in the study. Upon reception, samples were systematically splitted into two. One part was processed within a working day (D0, <8 hours) and another part was stored at room temperature and processed on the next day (D1, 24-36 hours). Processing included leukocytes retrieval and RNA extraction using Trizol for M1, M2 and M4, or lysis of 0.2ml of blood into the Xpert BCR-ABL Monitor lysis buffer (LB) for M3. Trizol or LB samples harvested at D0 an D1 were dispatched in the 2 laboratories and analyzed using M1, M3 and M4 in SLS or using M2, M3 and M4 in Lille. It is to note (i) that both laboratories are involved in the EUTOS project and have validated their "in-house" method (i.e.: M1 or M2) specific CF and (ii) that M3 and M4 are IS aligned through calibration procedures based on the WHO genetic reference panel. Acceptable concordance between the results of two methods was defined as achievement of 2 of the 3 following landmarks: (i) ≥ 50% between a 2-fold range; (ii) ≥ 75% between a 3-fold range; (iii) ≥ 90% between a 5-fold range. (Müller MC, Leukemia 2009)
Results: Methods M2, M3 SLS, M3 Lille, M4 SLS and M4 Lille fulfilled the acceptability criteria as compared to method M1, suggesting that both alignment strategies on the IS are equally valid. Interlaboratory comparisons showed that "in-house" as well as kit based methods fulfilled the acceptability criteria and led to comparable reproducibility levels. Finally, comparison between D0 and D1 samples showed that acceptability criteria were met for all 4 methods, suggesting that samples may be processed up to one day after their collection.
Summary / Conclusion: All BCR-ABL1 mRNA quantification methods tested in this study, aligned on the IS either by the EUTOS procedure or by the WHO genetic reference panel, give concordant results as defined by our acceptability criteria. The use of CE-IVD labeled kits led to similar interlaboratory reproducibility than "in-house" methods developed in two highly standardized laboratories. A storage period at room temperature of 24 to 36 hours between samples collection and processing did not lead to discordant results. To our knowledge, this is the first study evaluating the two alternative strategies for IS alignment: Eutos versus WHO reference panel calibration.

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REDUCED EXPRESSION OF CHIBBY IS A COMPONENT OF BETA-CATENIN ACTIVATION IN CHRONIC MYELOID LEUKEMIA STEM CELLS
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Background: Chibby (Cby) is a β-catenin antagonist encoded by C22*orf*2 on chromosome 22q13.1 in close proximity to the Bcr breakpoint involved in the Bcr-Abl rearrangement. It hinders β-catenin binding with Tcf/Lef transcription factors, thereby repressing target gene expression, and drives β-catenin nuclear export and cytoplasmatic relocation in a stable tripartite complex encompassing 14-3-3 scaffolding proteins, thereby attenuating β-catenin signaling. Notably, although the Bcr-Abl fusion gene is the causative genetic lesion of chronic myeloid leukemia (CML) and Ph+ acute lymphoblastic leukemia (ALL) the critical signal for proliferation and survival of leukemic stem cells (LSC) is β-catenin.
Aims: The aim of our study was to investigate whether Cby has a role in β-catenin activation in Bcr-Abl+ hematopoiesis and, in particular, in the LSC compartment.
Methods: In first instance we analyzed C22*orf*2 location in Bcr-Abl+ cells. We performed FISH analysis on mononuclear cell fractions (MCF) of 15 CML patients at diagnosis. We used 2 different probes mapping the entire region on chromosome 22q13.1. We then evaluated by means of quantitative PCR and Western blot (WB) analyses the Cby expression levels in mononuclear cell fractions (MNCs) from bone marrow samples of 30 CML patients at clinical diagnosis and 8 ALL patients compared to a pool of MCF from healthy donors. Moreover, we compared Cby expression levels in the putative stem cell compartment (CD34+) and MCF. Finally, we studied the epigenetic modifications (methylation) of the C22*orf*2 promoter in MCF and CD34+ cells of 5 CML patients at diagnosis.
Results: FISH analyses were carried in MCF of CML patients. They showed that one C22*orf*2 allele was invariably translocated to the derivative 9 chromosome and fused to upstream Bcr sequences. WB analyses revealed a significant Cby reduction (>than 70% compared to healthy controls) in the great majority (19/30) of CML patients and all 8 ALL patients. Notably, Cby reduction was associated with a significant increase of nuclear β-catenin. The analysis of gene expression in 4 CML patients at diagnosis and at the moment of major molecular response (MMR) under Nilotinib therapy confirmed that Cby reduction is restricted to the Bcr-Abl+ leukemic clone. However, it was not contingent upon transcriptional events, as proved by the marginal reduction of C22*orf*2 transcripts associated with Bcr-Abl expression. Further analysis carried in 5 CML patients at diagnosis showed that C22*orf*2 transcript levels were significantly reduced in the putative stem cell compartment (CD34+) compared to MCF. These findings support that Chibby transcriptional down-regulation occurs at the level of an early progenitor compartment still retaining stemness traits. Further work provided evidence of the epigenetic control on C22*orf*2 transcription. In particular, we found a significant increment of C22*orf*2 promoter methylation in CD34+ cells from CML patients compared to MCF and to CD34+ cells from healthy donors.
Summary / Conclusion: Our results indicate that transcriptional Cby downregulation associated with Bcr-Abl is restricted to a stem cell compartment and mediated by epigenetic events encompassing the gene promoter methylation. Further investigation is presently underway to evaluate the Bcr-Abl-associated mechanisms affecting the Cby protein stability in more differentiated leukemic progenitors.

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DIFFERENTIAL ACTIVATION OF MTOR KINASE IN CHRONIC AND BLASTIC PHASE OF CHRONIC MYELOGENOUS LEUKEMIA

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Background: Introduction of tyrosine kinase inhibitors (TKI) into clinical practice transformed chronic myeloid leukemia (CML) into chronic disorder. This holds true for majority of patients, but there is still a significant group of patients who develop resistance to the treatment and progress to blast crisis. Residual leukemia stem cells (LSCs) that are resistant to TKI may play a role of a "ticking bomb" contributing to the relapse of the disease. It has been shown that mTOR (mammalian target of rapamycin) kinase is engaged in BCR-ABL1-positive leukemias, moreover this pathway may be responsible for development of drug resistance in course of CML treatment. Therefore inhibitors of this pathway may provide interesting therapeutic approach to overcome drug resistance and to target LSCs.
Aims: Analysis of mTOR pathway activity in chronic phase of the CML in comparison to acute phase of the disease and potential involvement of ERK (extracellular signal-regulated kinase) also known as MAPK (mitogen-activated protein kinase) pathway in this activation. Evaluation whether rapamycin can overcome resistance to imatinib of non-dividing CD34+ cells pool (containing LSCs).
Methods: In this study primary CD34+ cells were used as well as CML cell lines (human K-562 and murine myeloid 32Dcl3 cells BCR/ABL-transformed). Primary cells were obtained after informed consent from the patients with CML in different stages of disease. CD34+ cells were isolated from peripheral blood leukocytes using magnetic beads systems and were cultured with addition of growth factors or starved. Activation status was determined by Western blotting using phosphospecific antibodies. Response of non-dividing CD34+ cells pool (containing LSCs) to rapamycin and/or imatinib was assessed by flow cytometry using CellTrace® CFSE dye.
Results: We focused our study on CML CD34+ progenitor cell pool from different phases of the disease, which contains primitive LSCs as well as early progenitors. First, we tested the contribution of ERK to activation of mTOR pathway in CD34+ cells. ERK inhibition (by MEK inhibitor -U0126) was effective in cells from all phases of the disease, however the resulting suppression of TORC1 activity varied. In CML-CP CD34+ cells mTOR was inactivated by ERK inhibition, while this effect was not observed in the CML-BC CD34+ cells. This indicates, that mTOR activation mode may change with the progression of CML (becoming ERK independent in more advanced stage of the disease) and that ERK contributes differently to mTOR signaling in chronic and blastic phase of CML. We also showed, that rapamycin in low, selective dose of 20nM effectively inhibited TORC1 in CD34+ CML cells regardless of the phase of the disease. In order to verify whether rapamycin can overcome resistance to imatinib, CML-BC CD34+ were analyzed by flow cytometry for the number of CD34+ CFSE-containing cells upon treatment with imatinib, rapamycin or both inhibitors after several days of culture. We used selective dose of rapamycin, that was shown to inhibit TORC1 in CML-BC CD34+ primary cells and corresponded to the serum level of this inhibitor in clinical conditions, however the number of non-proliferating, CML-BC CD34+ $CFSE^{high}$ cells was unaffected by imatinib, rapamycin or the combination of both of them.
Summary / Conclusion: In summary, we demonstrated, that mTOR activation in CML CD34+ progenitor cells is ERK-dependent in chronic phase of the disease, and ERK-independent in blast crisis. Our results suggest diverse regulation of mTOR pathway during progression of CML. We also showed, that inhibition of BCR-ABL1 and/or TORC1 did not influence the number of non-dividing CD34+ CML cells (which contain LSCs).

P120

MYELOID-DERIVED SUPPRESSOR CELLS ARE INCREASED IN CHRONIC MYELOID LEUKEMIA AND EXERT IMMUNE SUPPRESSIVE ACTIVITY

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Background: In some solid tumors it has been demonstrated that a subpopulation of myeloid cells, defined as "myeloid-derived suppressor cells" (MDSCs), plays an important role in inducing T cell tolerance by production of arginase 1 (arg1) that depletes microenvironment of arginine, an essential aminoacid for T cell function. Since chronic myeloid leukemia (CML) patients have high levels of immature myeloid cells it is of interest to investigate if these cells have MDSCs phenotype and activity.
Aims: The aim of this study was to analyze MDSCs and investigate their immunosuppressive activity in CML patients.
Methods: MDSCs were analyzed in peripheral blood (PB) of healthy donors (HD; n=20) and 30 CML patients at diagnosis. Twenty-one of them were also analyzed during treatment with tyrosine kinase inhibitors (TKIs). Granulocytic MDSCs (G-MDSCs) cells were identified as cells CD11b+CD33+CD14-HLADR-, while the monocytic MDSCs (Mo-MDSCs) as CD14+HLADR by cytofluorimetric analysis. Using real time PCR, arg1 expression was also assessed in PB HD and CML patients at diagnosis and during TKIs therapy Purification of granulocytes, monocytes and lymphocytes from PB was performed by a positive magnetic separation kit (EasySep, STEMCELL Technologies). Arg activity was measured in granulocyte lysates using a colorimetric test after enzymatic activation and arginine hydrolysis. To evaluate the activation of CD3+ T lymphocytes after mitogen stimulation, we analyzed at 24, 48, 72 h the following markers: CD69+, CD71+, DR+. T cell proliferation was analyzed by CFSE labeling after 72 h. Microvesicles (MV) were isolated from CML serum at diagnosis (n=5) by sequential ultracentrifugation.
Results: CML patients showed high levels of Mo- and G-MDSCs at diagnosis in comparison to HD (63±8 and 83±12,2% respectively in CML vs4,9±2,1 and 55,8±5,3% respectively in HD; P<0.001) while after TKIs therapy MDSC levels returned to normal values. Either in PB and in the purified granulocytes subpopulation, arg1 expression was 30 fold higher in CML at diagnosis (P<0.001) than HD and decreased after therapy. Arg enzymatic activity resulted increased in granulocytes of CML patients (n=10) compared to HD (n=10) (P<0.001). The suppressive function of CML granulocytes was measured by their ability to inhibit the proliferation and activation of HD T cells. CML myeloid cells from 4 patients exerted a significantly suppression both on activation and proliferation of HD T cells (P<0.001). CML as well as HD T lymphocytes showed a normal activation *in vitro* which was significantly lost when they was incubated with CML serum (n=4). In addition, an increase of Mo-MDSCs *in vitro* was observed after incubation of HD Mo (n=4) with CML serum (n=6) (29±13%;P<0.0001) or microvescicles (8±2,8%;P<0.05) vs control serum.
Summary / Conclusion: G- and Mo-MDSCs are increased in CML patients at diagnosis and decrease during TKIs treatment. CML granulocytes have high arg1 activity and immunosuppressive activity. Moreover, CML serum as well as CML microvesicles increase the percentage of Mo-MDSCs.

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CLINICAL SIGNIFICANCE OF EARLY MOLECULAR RESPONSES IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH FIRST-LINE SECOND-GENERATION TYROSINE KINASE INHIBITORS

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Background: Recent studies have been established the efficacy of second-generation tyrosine kinase inhibitors (2G-TKI) as first-line therapy in chronic myeloid leukemia (CML). 2G-TKI treated patients achieved faster responses compared with imatinib treated patients. Therefore, detailed analyses for clinical significance of early molecular responses in patients treated with 2G-TKI are needed.
Aims: The aim of this study was to evaluate clinical significance of early molecular responses in CML patients treated with first-line 2G-TKI.
Methods: 87 patients were newly diagnosed as CP CML, and started therapy dasatinib (n=13), nilotinib (n=24), bosutinib (n=13), and radotinib (n=37) without prior treatment except hydroxyurea or anagrelide. Among them, 79 patients with available quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) data at 3 months were analyzed for this study. Failure free survival (FFS) includes death resulting from any reason, progression to AP or BP, or treatment failure by ELN.
Results: A total of 79 first-line 2G-TKI treated CP CML patients (including 54 men and 25 women) were included. Their median age was 40 years (range, 18-73). The percentages of patients with low, intermediate and high Sokal risk scores were 42%, 37% and 20%, respectively with unknown Sokal risk scores in 1%. At 3 months, patients showed BCR-ABL1 ≤0.01% (n=2, 2.5%), >0.01 to ≤0.1% (n=11, 13.9%), >0.1 to ≤1% (n=37, 46.8%), or >1 to ≤10% (n=20, 25.3%) or >10% (n=9, 11.4%). For 68 patients with qRT-PCR data at 6 months, they showed BCR-ABL1 ≤0.01% (n=6, 7.6%), >0.01 to ≤0.1% (n=26, 32.9%), >0.1 to ≤1% (n=29, 36.7%), or >1 to ≤10% (n=6, 7.6%) or >10% (n=1, 1.3%).
After a median follow-up of 16.9 months (3.9-65.1 months) after first-line treatment, no progression to AP/BP occurred. At the time of analysis, 61 (77%) patients remained first-line therapy. Primary reasons for first-line therapy discontinuation included loss of CCyR (n=3), primary resistance (n=1), intolerance (n=13), and others (n=1). In patients with BCR-ABL1 ≤10% at 3 months, significantly higher rates of cumulative incidence (CI) of CCyR by 1 year (98% vs 60%, P=0.002) were observed, compared with that of patients with BCR-ABL1 >10%. They also had significantly better 1-y FFS (95.7% vs 60.0%, P=0.003). Patients with BCR-ABL1 ≤1% at 3 months had a trend for better CI of CCyR by 1 year (97.7% vs 85.9%, P=0.061). However, there were no significant differences in 1-y FFS. Molecular response with BCR-ABL1 ≤10% at 6 months was associated with better 1-y FFS (100% vs 94.1%, P<0.001), compared with those who achieved >10% response at 6 months, and BCR-ABL1 ≤1% at 6 months had significantly better 1-y FFS (95.7% vs 60.0%, P=0.003).
Summary / Conclusion: Our data showed that BCR-ABL1 ≤10% at 3 months was a predictor for CI of CCyR at 1year and FFS. BCR-ABL1 ≤10% and ≤1%

cut-offs at 6 months had a significance for FFS. It suggested that the 3 and 6-month qRT-PCR assessment may apply prognostic information in CML patients treated with first-line 2G-TKI. However, further clinical investigations in a larger patient population with longer follow-up are needed.

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DEEP SEQUENCING OF THE BCR-ABL KINASE DOMAIN REVEALS A FREQUENCY OF 35INS INSERTION/TRUNCATION HIGHER THAN EXPECTED

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Background: The spectrum of Bcr-Abl kinase domain mechanisms that confer resistance to tyrosine kinase inhibitors (TKIs) in Philadelphia-positive (Ph+) Leukemia is quite heterogeneous. Not always molecular events underlying drug-resistance can be explained by presence of mutations; Bcr-Abl KD insertions/deletions can be an alternative mutational mechanisms. The recent development of "deep-amplicon sequencing" (DS) technologies has opened the way to a more accurate characterization of molecular aberrations in Ph+ Leukemia with higher sensitivity of screening for know and unknown mutations.
Aims: We took advantage of a DS approach in order to fully characterize the spectrum of insertions and deletions in CML and Ph+ ALL patients who had developed resistance to one or multiple lines of TKI therapy.
Methods: We set up a Bcr-Abl KD mutation screening assay on the Roche GS Junior instrument that allows to reliably detect sequence variants and deletions or insertions with a lower detection limit of 1%. A total of 67 samples from 26 CML and 13 Ph+ ALL patients who had developed resistance to one or multiple TKIs (Imatinib, Dasatinib, Nilotinib) were selected for this analysis. In order to reconstruct the dynamics of growth of mutations we evaluated their presence in a serial follow-up samples collected during TKI therapy in 6 patients.
Results: DS revealed a 35-base insertion (35INS) in 18/26 (69%) CML and 11/13 (84%) ALL Ph+ patients with an abundance from 1% up to 96% of all Bcr-Abl transcripts**.** Interestingly DS highlighted an increased expression of 35INS over time in 6 patients (growth ranged from 2% to 96% within a few months). This insertion is known to retain a stop codon which causes the loss of 653 C-terminal amino acids of Bcr-Abl resulting in early termination and a truncated Bcr-Abl1 protein missing a significant portion of the C-terminal regulatory regions. In addition DS detected 2 in-frame deletions in 3 samples, with an abundance from 2% to 19% . This not previously described variants include a 72-nt deletion (1233-1304) between the junction of Abl exon 6 and 7 that causes the loss of 24 amino acids (aa 359-383) and a 42-nt deletion in exon 7 (1258-1299) which leads to loss of 14 amino acids (aa 371-384).
Summary / Conclusion: Our results show that DS technologies on the GS Junior instrument allow a more accurate characterization of mutational status of patients in comparison to conventional sequencing methods. The higher sensitivity of DS approach allowed to highlight, both in CML and in Ph ALL+ patients, a frequency of 35INS higher than previously reported (60%). The 35INS thus seems to be very frequent in CML and Ph+ ALL patients who develop resistance to one or multiple lines of TKI therapies but its abundance is dynamic in individual patients and seems not to be related to TKI therapy. In line with our results, recent 35INS *in vitro* studies have demonstrated that this insertion is kinase-inactive and should not contribute to TKI-resistance. Although this insertion does not predict for a specific TKIs-resistance its role in Ph+ Leukemia merit additional studies and further analysis of a larger number of samples will be needed to better understand its biological and clinical relevance.
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ABERRANT MICRORNA EXPRESSION IN CHRONIC MYELOID LEUKEMIA

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Background: miRNAs are non-coding regulatory RNAs that control gene expression at the post-transcriptional level. Deregulated miRNA expression has been discovered in numerous tumors and it is now clear that they contribute to cancer development and progression. Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder characterized by the fusion oncogene BCR-ABL. This oncogene encodes the tyrosine kinase BCR-ABL whose expression is essential for sustained proliferation of leukemic cells and has become a target for therapy. Imatinib, a selective BCR-ABL tyrosine kinase inhibitor (TKI), induces apoptosis of BCR-ABL+ cells and is currently the first line therapy for newly diagnosed CML patients. Non-negligible incidences of imatinib resistance emphasize the need to identify novel biomarkers significant for CML pathogenesis, progression and resistance.
Aims: Current miRNAs expression data in CML is inadequate and their role in CML is still poorly understood. We aim to find miRNAs acting as biomarkers in CML pathogenesis. We will also determine potential target genes and signaling pathways affected by these signature miRNAs.
Methods: miRNA expression was profiled using Agilent human miRNA microarrays and verified with Applied Biosystems TaqMan. Results were analyzed with Partek genomics suite software. Bioinformatics analysis was performed using: Ingenuity systems, KEGG database, Venn diagrams
Results: We used 2 BCR-ABL+ cell lines, K562 and Meg-01 to identify a CML specific, BCR-ABL–dependent miRNA expression pattern. MiRNA expression profiling was performed in reference to a pool of healthy blood. In addition, we studied the expression profile of K562 cells treated with imatinib and dasatinib. According to unsupervised hierarchical clustering, healthy blood samples were clustered separately from K562 and Meg-01 cells. Untreated K562 cells were clustered separately from treated ones and imatinib treated K562 cells were clustered closely to dasatinib treated cells. Validation was done by real-time PCR. We continued by focusing on miRNAs whose expression level in K562 and Meg-01 cells was opposite to their expression in healthy blood. Venn diagram revealed 73 miRNAs meeting this criterion. Within these miRNAs, we focused on those whose expression in K562 cells was routed back to normal levels following TKI treatment. Fourteen miRNAs; miRNA-9, 23b, 30e, 132, 154, 193b, 320a, 320b, 454, 500, 564, 671-5p, 765, and 892 satisfied this criterion and were further analyzed. Of these miRNAs, miRNA-9 whose expression was high in the CML cell lines was also high in 30% of the tested CML patients. Similarly, miRNA-454 whose expression was low in the CML cell lines was also low in 30% of the tested CML patients. The expression of both of these miRNAs was routed back to normal levels in these patients following imatinib treatment. TargetScan revealed that miR-454 targets genes such as TGFβR1, p21, E2F2, MAPK1, and SMAD4 known to be associated with CML. MiR-9 was found to target ACVR1B, CCNDBP1, LZTS2, SOCS4 and BCL2L11. Reassuringly, the analysis identified CML as the main diseases associated with these miRNAs. MAPK, ErbB, mTOR and focal adhesion were the central molecular pathways related with these expression patterns.
Summary / Conclusion: Further patient studies are needed however, we hope that with these preliminary data, we will identify novel deregulated miRNAs and highlight new candidate gene targets allowing for a better understanding of the molecular mechanism underlying CML development and propose possible new avenues for therapeutic treatment.

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A NEW NON CANONICAL ROLE OF IKB ALPHA IN CHRONIC MYELOID LEUKEMIA PATHOGENESIS

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Background: Background: IkappaBalpha (IκBα) is commonly known as inhibitor of the transcriptional factor (TF) NFκB. Under specific stimuli IκBαis phosphorylated, subsequently ubiquitinated and eliminated by proteasome. As consequence free NFκB enters the nucleus and regulate the transcription of many genes involved in immune and inflammatory responses, as well as genes regulating cell proliferation and survival, including IκBα.
Aims: Aims: In recent years many studies have focused their attention on IκBα; it has been found involved and deleted in some disease such as Hodgkin

lymphoma and glioblastoma, suggesting its important rule as tumor suppressor. Moreover IκBαis described to be able to physically interact with the tumor suppressor protein p53 and as consequence to decrease the p53 transcription activity. For these reasons our group decided to study IkBαin Chronic myeloid leukaemiaIκBα, where its rule is poorly investigate.
Methods: Methods: mRNA derived from 25 CML Philadelphiapositive (Ph+) chronic phase and 8 normal donor bone marrow were analyzed for IκBαexpression with real-time quantitative PCR. Subsequently fresh cells derived from patients were used for immunoprecipitation, western blot analysis and immunofluorescence assay, in order to understand protein level and localization. Ph+ cells were also incubated with 10 μM Imatinib mesilate, the common Bcr-Abl inhibitor, for 6 hours and subsequently processed for WB and IF.
For biochemical studies, 293T cells were transfected with IκBαexpression vector alone or in combination with Bcr-Abl expression vector and analysed after treatment with 10 μM Imatinib mesilate and MG-132, a known inhibitor of proteasome.
Results: Results: Despite real time quantitative analysis shown no significant difference between CML patients at diagnosis and control samples, IκBαprotein level was increased in patients greater than in donors. This results was confirmed both by western blot of patients cells lysate than immunofluorescence, where we observed a strong cytoplasmatic IκBαlocalization. After Imatinib incubation, CML patients cells show reduced levels of IkBα, suggesting a strong control kinase-dependent. To investigate this hypothesis we performed the same experiments in 293T cells transiently transfected with IκBαand Bcr/Abl. *in vitro* studies confirmed all our observations; Bcr/Abl plasmid transfection didn't modify the IκBαmRNA, but we observed a strong increased of IκBαprotein; by immunofluorescence we confirmed that also IκBαlocalization, that is mainly diffuse in nucleus and cytoplasm when it is alone, became predominantly cytoplasmatic when it is co-transfected with Bcr/Abl. Treatment of 293T cells with MG-132, a proteasome inhibitor, shown a strong increase of IκBαonly when it is transfected alone, suggesting the ability of Bcr/Abl to stabilize IκBαproteinat a post-translational level. The non canonical IκBαrole as interactor of p53 led us to investigate this mechanism in our CML Ph+ model; in 293T transfected we shown that in presence of Bcr/Abl these two protein physically interact, in a stronger way than in basal condition and as a consequence p53 activity is drastically reduced. The same results was also confirmed in CML patients.
Summary / Conclusion: Summary/conclusions: this work shows how Bcr-Abl induces an higher amount of IκBαprotein and as consequence promotes IκBα-p53 interaction. It will be important to analyse if Imatinib is able to dissociate the interaction between IκBαand p53, in order to identify a new role of p53 on cellular response after tyrosine kinase treatment.

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L-AMINO ACID OXIDASE ISOLATED FROM BOTHROPS PIRAJAI INDUCES APOPTOSIS IN BCR-ABL POSITIVE CELLS AND POTENTIATES IMATINIB MESYLATE EFFECT

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the presence of Philadelphia chromosome and by *BCR-ABL* which encodes the BCR-ABL oncoprotein. Although imatinib mesylate (IM) is effective for CML treatment, patients in accelerated and blastic phases of the disease are often refractory to this therapy. Therefore potential new drugs are being investigated to improve the efficiency of the CML therapy. The L-amino acid oxidase from *Bothrops pirajai* (BpirLAAO-I), a toxin isolated from snake venoms is capable of inducing apoptosis in various tumor cell lines.
Aims: 1) To evaluate the BpirLAAO-I effect on BCR-ABL positive cells, 2) To determine the BpirLAAO-I combined with IM effect on HL-60.BCR-ABL. 3) To evaluate the BpirLAAO-I effect on normal peripheral blood mononuclear cells (PBMC).
Methods: To determine the BpirLAAO-I potential as apoptosis inducer, a hundred thousand cells of HL-60 (sensitive to chemotherapeutic drugs) and HL-60.BCR-ABL (HL-60 cells transfected with *BCR-ABL* gene) were cultured with different concentrations of BpirLAAO-I (0.5; 1.0; 1.5; 2.0; 2.5 and 5.0μg/mL) for 18h. One million cells of HL-60.BCR-ABL were also incubated with 10μM IM during 2 and 18h or with 10 μM IM combined with BpirLAAO-I at the concentrations of 0.5; 1.0; 1.5; 2.0 and 2.5 μg/mL for 18h. Also 1mM of Actinomicin-D (ACT-D), an apoptosis inducer agent was used as control. Apoptosis was quantified by Hypotonic Fluorescent Solution (HFS) method by flow cytometry and the results were expressed as the mean of the percentage of hypodiploid nuclei. The HL-60 and HL-60.BCR-ABL apoptosis activation was confirmed detecting caspases3, 8 and 9 expression by Western blot. The phosphotyrosine and c-abl expression was detected to evaluate the BCR-ABL kinase inhibition also by Western blot. To evaluate the effect on normal PBMC, five hundred thousand cells were treated with different concentrations of BpirLAAO-I (3.125; 6.25; 12.5; 25.0 and 50.0μg/mL) and the cytotoxicity assay was performed by MTT. The results were expressed as percentage of cell viability.
Results: The BpirLAAO-I was able of inducing apoptosis on HL-60 and HL-60.BCR-ABL cell lines in a dose-dependent manner and was capable of inducing apoptosis in HL-60.BCR-ABL mainly in concentration of 2.5 ug/mL (52.37% ±3.2). As expected the ACT-D induced apoptosis only in HL-60 (76.9%±6.1). The HL-60 and HL-60.BCR-ABL apoptosis was confirmed by caspase3, 8 and 9 activation. We also observed a significant increase of apoptosis when BpirLAAO-I is combined with IM on HL-60.BCR-ABL compared to these cells treated only with IM or with BpirLAAO-I. The data obtained from the association of BpirLAAO-I with IM by Western blot suggested that BpirLAAO-I potentiates the inhibition of BCR-ABL tyrosine kinase activity. BpirLAAO-I did notinduced cytotoxicity against normal PBMC.
Summary / Conclusion: The results indicate that BpirLAAO-I presents a antileukemic potential against HL-60.BCR-ABL cells and potentiates IM effect but not affects PBMC normal cells. Since some patients are resistant to IM, the description of news drugs or adjuvant for CML treatment is relevant.
Supported by: CNPq, Fapesp and NAP-TOXAN-USP.

P126

IL-2, IL-12 AND IL-15 PREVENT REACTIVE OXYGEN SPECIES ACCUMULATION ON NATURAL KILLER CELLS FROM CHRONIC MYELOID LEUKEMIA PATIENTS FOLLOWING INTERFERON-ALPHA, IMATINIB AND DASATINIB THERAPY

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Background: Tyrosine kinase inhibitors (TKIs) change dramatically the oncologic treatment in chronic myeloid leukemia (CML) as also in other tumors. However the complete remission of the disease is normally not achieved but instead the patient enters in a chronic free symptomatic state during TKI administration. Cytotoxic lymphocytes such NK cells could have an important contribution in immunotherapy against tumors. However, in many cancers (e.g. CML), extracellular reactive oxygen species (ROS) produced by phagocytes are the main responsible for the immunosuppressive state of NK cells due to the uncontrolled expansion of myeloid cells. NK cells can perform lytic action over CML cells. However, they normally have decrease expression of membrane receptors *in vivo,* which are necessary for the cytotoxic action over BCR-ABL positive cells.
Aims: We aimed to investigate alterations of ROS levels in NK cells of CML patients due to oxidative burst from phagocytes, taking into account the type of treatment and disease progression.
Methods: In this work, we analysed 109 peripheral blood samples from 50 CML patients. CML patients were under IFN-α, Imatinib or Dasatinib therapy. Production of ROS was evaluated using flow cytometry by the conversion of dihydrorhodamine 123. In addition, peripheral blood NK cells and monocytes from 6 healthy individuals were sorted and co-cultured under different conditions, to evaluate the NK cell capacity to resist to ROS production.
Results: As expected, myeloid cells presented a substantial increase of ROS production in peripheral blood of CML patients. However, we also found chronic increased ROS levels in blood NK cells of CML patients comparing to healthy controls after *in vitro* PMA stimulation. We found that $CD56^{dim}$ $CD16^{+}$ NK cells had increased levels of ROS than $CD56^{bright}$ $CD16^{-}$ subset, confirming an increased susceptibility of the cytotoxic subset. The expression of NKp46 and CD16, two important receptors in antitumor response, was significantly decreased in NK cells from CML patients. TKIs were found to influence ROS levels in NK cells, mainly in patients treated with Imatinib 600 mg. IFN-α revealed a minor impact on ROS in NK cells. Interestingly, stimulation of NK cells with IL-2, IL-12 and IL-15 before contact with stimulated monocytes revealed a protective role, decreasing ROS accumulation in sorted NK cells from healthy controls.
Summary / Conclusion: NK cells from CML patients presented a significant increase of ROS levels. A combination of costimulatory cytokines (IL-2, IL-12 and IL-15) prevents NK cells from immunosuppressive state induced by myeloid cells. Refinement of current therapeutic protocols increasing NK cell functional properties seems to be a promising field to explore in CML therapy.

P127

EFFECT OF NILOTINIB ON THE FUNCTION OF CAROTID ARTERY ENDOTHELIAL CELLS

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Background: Chronic myeloid leukemia is a clonal myeloproliferative disorder caused by abnormally proliferating hematopoietic stem cells. CML is treated

with tyrosine kinase inhibitors that inhibit BCR-ABL1 chimeric protein. Nilotinib is a more active second generation tyrosine kinase inhibitor, synthetically derived from imatinib. There have been concerns about the possible pro-thrombotic effects of nilotinib especially on patients having cardiovascular risk factors. The potential mechanism behind the increased risk of thromboembolic events is still not clear.

Aims: In this study, we aimed to evaluate possible harmful effects of nilotinib on endothelial cells. To this aim, we examined proliferative capacity and secretory functions of healthy human carotid endothelial (HCtAE) cells in response to Nilotinib.

Methods: MTT cell proliferation method was used to determine antiproliferative effects of Nilotinib on HCtAE cells. HCtAE cells were incubated 5-, 10- and 100 nM doses of Nilotinib for 72 h. Then, in order to assess the endothelial function, Nitric Oxide (NO), von Willebrand Factor (vWF), tissue plasminogen activator (tPA), plasminogen activator inhibitor 1 (PAI 1) and endothelin 1 (secreted from endothelial cells) levels were evaluated with ELISA from tissue culture supernatants.

Results: There were slight decreases in cell proliferation in response to Nilotinib. Nilotinib increased the secretion of t-PA, PAI 1 and vWF in a dose dependent manner when compared with untreated control group. ET-1 secretion was lower in 5 nM and higher in 10 and 100 nM Nilotinib treated cells as compared to untreated cells. Regarding NO secretion, lower levels were observed in 5 nM and 10 nM and higher levels were detected in 100 nM Nilotinib treated cells as compared to untreated control group cells.

Summary / Conclusion: It cannot be explicitly concluded that nilotinib effects the endothelial cell functions in a pro-thrombotic or anti-thrombotic fashion. In addition, the results obtained from this *in vitro* designed study may not correctly reflect the *in vivo* affect of the drug. We may finally conclude that our study is a preliminary pilot study trying to establish the effect of nilotinib on carotid artery endothelial cells. Some further studies may clarify the possible pathogenetic mechanisms involved in the pro-thrombotic process caused by nilotinib and may induce therapeutic approaches to decrease the incidence of this harmful side effect.

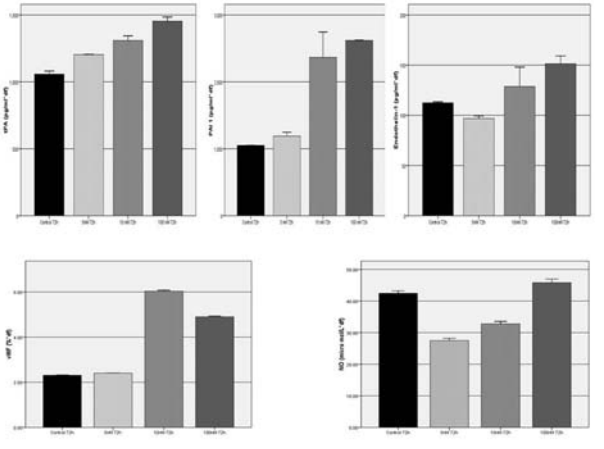

Figure 1.

P128

STANDARDIZATION OF MRD DETECTION IN CML BY USE OF A PEER-GROUP CONVERSION FACTOR

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Background: The goal of treatment with tyrosine kinase inhibitors in patients with CML is achieving a major molecular response (MMR). Due to the complexity of the reverse-transcription quantitative polymerase chain reaction (RQ PCR) for measurement of the BCR-ABL fusion transcripts and the use of different control genes, a universal and reproducible unit of reporting is mandatory for standardization. In 2006 an international scale (IS) was proposed where 100% IS defined the standardized baseline and 0.1% IS defined MMR. In order for laboratories to report results in IS, the European treatment outcome study (EUTOS) for CML has proposed the concept of reference laboratories. By a hierarchical and serial strategy of sample exchange a conversion factor (CF) is calculated for each participating laboratory. Since July 2010 an increasing number of participants of the external quality assessment scheme of UKNEQAS (Sheffield, UK) report results in the IS. In 2010 the World Health Organization established primary reference material for manufacturers. Subsequently, secondary reference material recently became available for clinical laboratories. However, to date variability in test results between laboratories remains high and as CF's might need to be recalculated over time, the use of reference material is expensive and time-consuming.

Aims: The aim of this study is to establish a laboratory-specific CF by making use of the available results of an external quality assessment scheme (UKNEQAS) for BCR-ABL fusion transcript analysis and to confirm this method by use of reference material.

Methods: RQ PCR was performed on two ViiA 7 Real –Time PCR Systems (Applied Biosystems, Foster City, CA, USA) according to the guidelines of the Europe Against Cancer (EAC) program. ABL was used as a control gene and results were reported as ratios (BCR-ABL/ABL %). Analytical sensitivity was determined by calculating the chance of detecting 5 plasmid BCR-ABL molecules when each sample was analyzed in duplicate. A lab-specific CF was calculated by comparing our pre-conversion results for 10 past UKNEQAS samples (period July 2010 to July 2012) with the mean post-conversion values reported by the UKNEQAS. The newly defined CF was confirmed by the use of reference material (Philadelphia p210 Q-P210 standard).

Results: Based on the UKNEQAS results, a CF of 0.37 and 0.46 was calculated for both analyzers, respectively. An analytical sensitivity of 5 molecules BCR-ABL was accepted with the chance of having a false negative result of 1.63%, corresponding to 0.011% IS (95% CI: 0.007-0.015) for both systems after conversion with our newly defined CF. Highly similar CF's were obtained for both systems when using the commercial reference material (0.41 and 0.46 respectively), confirming the calculated CF's based on peer-group comparison.

Summary / Conclusion: In conclusion, we propose a novel approach for standardization of molecular monitoring of BCR-ABL in patients with CML. We believe this is an achievable and acceptable, less time-consuming and less expensive approach for routine laboratories as compared to the EUTOS concept of reference laboratories and commercial reference material. Whether the use of a conversion factor based on peer group comparison will improve between-laboratory variability remains to be elucidated.

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BIOLOGICAL CHARACTERISTICS AND DYNAMICS OF BCR-ABL1 MULTIPLE MUTATIONS IN TYROSINE KINASE INHIBITOR RESISTANT CML

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Background: BCR-ABL1 kinase domain (KD) point mutation causes resistance to tyrosine kinase inhibitors (TKI) in chronic myeloid leukemia (CML) patients through impaired binding of TKI to the target site. Recent studies have reported that multiple mutations detected in 2-9% of patients with imatinib (IM)-resistant CML were associated with poor response rate and survival outcomes. However, biological characteristics and dynamics of multiple mutations are still not assessed with a quantitative serial follow-up data in the same populations.

Aims: The aim of this study was to investigate biological characteristics and dynamics of multiple mutations in the serial samples from the patients carrying multiple mutations using subcloning and sequencing.

Methods: Since 2002, 414 CML patients were screened for mutation analysis due to sign of resistance to TKIs including imatinib (IM), nilotinib (NIL), dasatinib (DAS), bosutinib (BOS), radotinib (RAD) or ponatinib (PON) at Seoul St Mary's Hospital using direct sequencing and allele specific oligonucleotide-polymerase chain reaction (ASO-PCR). Among them, 31 patients carried ≥ 2 BCR-ABL1 kinase domain mutations. We analyzed 137 samples from these 31 patients using subcloning and sequencing (in total, 2737 colonies were sequenced). By cloning and sequencing, two or more missense mutations present in the same clone were defined as compound mutation and co-existence of single missense mutations in the separated clones was defined as polyclonal mutation. Co-existence of single missense mutation and compound mutation harboring two or more missense mutations in the same clone was defined as mixed mutation.

Results: In a total of 2737 colonies from 137 samples, 1596 (58%) colonies harbored compound mutations with a median 2 (range, 2 – 7) mutations, and 905 (33%) colonies with a single mutation and 236 (9%) colonies with wild type were observed. We identified 700 different mutations encompassing 278 residues in the kinase domain. The residues most frequently involved in missense mutations were T315 (11.5%), E255(5.4%), M244 (4.2%), V299 (4.1%), E459 (3.5%), F359 (2.9%), and Y253 (2.9%). By cloning and sequencing of each sample, most samples (117/137, 85%) was in the type of mixed mutation, that is co-existence of single missense mutation and compound mutation harboring two or more missense mutations in the same clone. In the next sample from the time of first detection by cloning and sequencing, multiple mutations were changed though different ways; among 30 patients with available next sample, 9 (30%) patients had an acquisition of different point mutation in same clone. In 33% (8/30) patients, new mutation occurred in separate clones. Each sample was observed in AP or BP (23%; 31/137) and CP (77%; 106/137), and on therapy with IM (12%, 16/137), NIL (15%, 21/137), DAS (35%, 48/137), BOS(4%, 6/137), RAD (3%, 4/137), PON (10%, 14/137), and others (18%, 25/137).

Summary / Conclusion: Our data by subcloning and sequencing showed basic characteristics and dynamics of multiple mutations. Samples harboring

multiple mutations showed mixed patterns with co-existence of single missense mutation and compound mutation. Variable dynamic changes of each clone were observed, including an acquisition of different point mutation in same clone and new mutation development in separate clones. In the meeting, we will show further analyzed data about the effect of prior drug exposure and BCR-ABL1 tyrosine kinase activity on dynamical change of multiple mutations.

P130

ESTIMATION OF THE CURRENT SURVIVAL MEASURES IN CHRONIC MYELOID LEUKEMIA: METHODOLOGY AND NEW SOFTWARE TOOLS

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Background: Common ways of survival assessment, the leukemia-free survival (LFS) and the cumulative incidence (CI), are not fully comprehensive for the outcome assessment in chronic myeloid leukemia (CML) because these measures do not account for multiple relapses and leukemia-free periods during the treatment course. Therefore, the concept of the so-called current survival measures, which accounts for the proportion of patients who have lost the first disease remission as well as the proportion of leukemia-free patients who achieved the subsequent remissions, has recently become discussed in the literature. Proper estimation methods and publicly available software tools are needed to make the current survival measures widely usable.
Aims: To demonstrate the validity of the current survival measures. To introduce the methodology, software, and web-based calculator for the CCI and CLFS estimation.
Methods: The current survival measures, the CCI and CLFS, were estimated using nonparametric statistical methods, which are commonly used in survival analysis (Pavlík *et al*, BMC Med Res Methodol. 2011; 11:140). R software package currentSurvival (http://cran.r-project.org/web/packages/currentSurvival/) was also made publicly available for the CCI and CLFS calculation. Moreover, a web-based calculator at http://www.iba.muni.cz/data-analysis-tools/currentSurvival/ will be launched on May 1st 2013. In total, 233 Czech CML patients in chronic phase received the first-line imatinib between July 2003 and December 2011; records were registered in the Czech database INFINITY (http://www.leukemia-cell.org/en/databaze/).
Results: Regarding all 233 patients, the estimated CCI at 3 and 5 years after starting imatinib therapy was 75.4% and 73.4%, respectively. On the other hand, the common CI at 3 and 5 years after starting imatinib was estimated as 85.2% and 87.1%, respectively. Thus, the estimated difference between the CCI and CI curves reached 9.8% and 13.7% at 3 and 5 years after starting imatinib, respectively. Only 185 patients (79.4%) who achieved at least one CCgR were available for the CLFS calculation. The estimated CLFS at 3 and 5 years after achieving the first CCgR was 90.9% and 92.8%, respectively. The LFS was estimated as 74.2% and 64.0% at 3 and 5 years after achieving the first CCgR, respectively. Therefore, at 3 and 5 years after the achievement of the first CCgR, the difference between the CLFS and LFS estimates reached 16.7% and 28.8%, respectively.
Summary / Conclusion: The common CI overestimates the probability of being alive and in CCgR after initiating imatinib therapy, whereas the common LFS underestimates the probability of being alive and in CCgR after the achievement of first CCgR on the imatinib therapy. Thus, both current survival measures, the CCI and CLFS, more reliably illustrate a CML patient's disease status in time because they account for multiple leukemia-free periods during the treatment course. Moreover, the methodology for CCI and CLFS estimation is now available for public use in the form of either R software package currentSurvival or web-based calculator.

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10058-F4 A SMALL MOLECULE C-MYC INHIBITOR DECREASES CIP2A AND REDUCES BCR-ABL1 TYROSINE KINASE ACTIVITY IN CHRONIC MYELOID LEUKAEMIA (CML)

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Background: Disease progression in CML is associated with inhibition of the phosphatase PP2A. PP2A is functionally impaired by cancerous inhibitor of PP2A (CIP2A), leading to the stabilisation of c-Myc. CIP2A protein is a prospective biomarker of disease progression in imatinib treated CML patients (Lucas *et al* Blood 2011; 117: 6660-8). We have previously shown that CIP2A is not suppressed by imatinib treatment and that CIP2A levels rise as patients progress into blast crisis. Moreover, high CIP2A levels are associated with high c-Myc and high BCR-ABL1 tyrosine kinase activity.
Aims: The aim of this work was to inhibit c-Myc using the small molecule inhibitor 10058-F4 in order to disrupt the CIP2A/c-Myc interaction and thus attempt to suppress CIP2A indirectly.
Methods: Cell lines and newly diagnosed chronic phase patients cells were cultured with 60μM 10058-F4 for 48 hours. CIP2A, PP2A, pY^{307}-PP2A and c-Myc and pS^{62}-c-Myc proteins were assessed by flow cytometry and western blotting. mRNA expression was assessed using the Taqman expression assays. c-Myc siRNA was transfected into K562 cells for 72 hours.
Results: 10058-F4 inhibited both c-Myc and pS^{62}-c-Myc (P=0.003) in K562 cells. Reduction in c-Myc protein resulted in a concomitant decrease in both CIP2A protein levels (P=0.003) and BCR-ABL1 tyrosine kinase activity (P=0.003). 10058-F4 treatment decreased both c-Myc and BCR-ABL1 mRNA expression (P=0.002 and P=0.004 respectively). No effect on CIP2A mRNA expression was observed. To investigate whether the decrease in CIP2A protein was a direct result of c-Myc reduction or an indirect effect via BCR-ABL1, AGS cells (a gastric carcinoma line which is CIP2A positive but BCR-ABL1 negative) were treated with 10058-F4 for 48 hours. Again, c-Myc inhibition resulted in a decrease in CIP2A (P=0.001); thus, the decrease in CIP2A is a direct result of c-Myc inhibition and not due to BCR-ABL1. Importantly, the effect of 10058-F4 on decreasing BCR-ABL1 in CML cells is a result of the reduction in both c-Myc and CIP2A, implying that both are upstream of BCR-ABL1. Knockdown of c-Myc resulted in increased PP2A activity, decrease in CIP2A levels (P=0.004) and decreased BCR-ABL1 tyrosine kinase activity (P=0.001). These data suggest that c-Myc and CIP2A act to stabilise each other at the protein level.
Newly diagnosed chronic phase patients cells were cultured with 60μM 10058-F4 for 48 hours (n=6). In samples with high CIP2A protein expression, c-Myc inhibition led to a significant reduction in both CIP2A and BCR-ABL1 tyrosine kinase activity. BCR-ABL1 tyrosine kinase activity was also reduced in samples with low CIP2A expression, suggesting that c-Myc inhibition would be advantageous to all CML patients.
Summary / Conclusion: CIP2A can be targeted by using c-Myc as a surrogate target. Inhibition of c-Myc in K562 and CML primary cells either with 10058-F4 or c-Myc siRNA resulted in the reactivation of PP2A, a decrease in CIP2A and a decrease in BCR-ABL1 tyrosine kinase activity. c-Myc may contribute to disease progression by promoting aneuploidy as a result of deregulated cell division and increased mismatch repair, as well as stabilising CIP2A. Both c-Myc and CIP2A are therefore attractive therapeutic targets for preventing disease progression.

Chronic myeloid leukemia - Clinical 1

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IMPACT OF DRUG TRANSPORTER ABCG2 HAPLOTYPES IN MOLECULAR RESPONSE OF CML PATIENTS IS MODULATED BY IMATINIB DAILY DOSE

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Background: Imatinib and other tyrosine kinase inhibitors (TKIs) have revolutionized the therapy of chronic myelogenous leukemia (CML). Along with parameters such as the initial Sokal score, comorbidities, age, co-medications clinicians need patient-related parameters such as specific genetic profile related to response or tolerance in order to optimize treatment strategies. Preliminary studies have reported polymorphisms in candidate genes like OCT1, ABCG2 or MDR1 implicated in response to imatinib. However, no systematic association study was performed testing constitutive variations in drug transporter genes and allocated dose of imatinib. *This study was approved by the local Human Ethics Committee. Written informed consent was obtained from all patients prior to study participation.*
Aims: To evaluate the role of constitutive variations in drug transporter genes on imatinib response using longitudinal data relative to molecular response in CP-CML patients allocated to either imatinib 400 mg/day or 600mg/day.
To validate results according to early molecular responses (BCR-ABL^{IS} at 3 months <10%) and response of interest (BCR-ABL^{IS} at 12 months ≤ 1%, BCR-ABL^{IS} at 18 months ≤0.1%).
Methods: We use a custom DNA chip covering 857 SNPs covering 94 drug transporter genes to analyses two independent cohorts of CP-CML patients. The first (St-Louis hospital cohort) composed of patients treated with imatinib 400 mg/day (*n*=105) and the second cohort (SPIRIT cohort) composed of patients from the SPIRIT randomized clinical trial allocated to 400 mg/day (*n*=132) or 600mg/day (*n*=107). Association study was performed on cumulative incidence of major molecular response (CI-MMR) using the Fine & Gray model. (Switch in treatment due to treatment toxicity was the competing event).

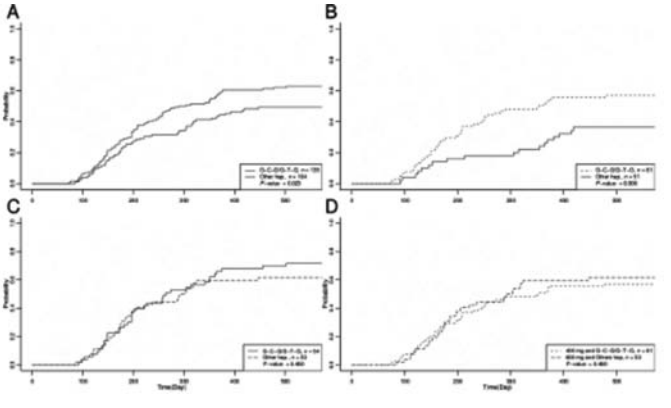

Figure 1.

Results: Two "favorable" haplotypes of ABCG2 gene were associated to higher CI-MMR in patients allocated to imatinib 400 mg/day but not in patients allocated to imatinib 600 mg/day. Patients allocated to imatinib 400 mg/day with at least one copy of the favorable haplotypes had similar outcome as compared to patients with common haplotype receiving 600 mg/day. Interestingly, these favorable haplotypes were common: about half the population present at least one copy of these haplotypes. Quadrant A, B and C of the figure illustrate discrimination of patients with respect to ABCG2 haplotypes globally, in the 400 mg/day arm and in the 600 mg/day arm respectively. Quadrant C shows the similar outcome of patients allocated to imatinib 400 mg/day with favorable haplotype *versus* patients allocated to imatinib 600 mg/day with common haplotype. As expected results were validated in term of early molecular responses (BCR-ABL^{IS} at 3 <10%) and response of interest (BCR-ABL^{IS} at 12 months ≤1%, BCR-ABL^{IS} at 18 months ≤0.1%) only in patients allocated to imatinib 400 mg/day.
Summary / Conclusion: Here we show that response to imatinib is determined by constitutive haplotypes in drug-transporter genes such as ABCG2 in a dose dependent fashion. Moreover, higher response associated with high doses of imatinib could also be reached by patients having favorable haplotypes while treated at low intensity. As about half of the population is targeted by the favorable haplotype, the ABCG2 status in newly diagnosed CP-CML patients could help clinicians to optimize treatment strategies.

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CONTINUED DEEPER MOLECULAR RESPONSE WITH NILOTINIB IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) WITH DETECTABLE DISEASE ON LONG-TERM IMATINIB: ENESTCMR 24-MONTH RESULTS

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Background: Nilotinib (NIL) induced significantly faster and deeper molecular responses than imatinib (IM) in the ENESTnd trial. The 12-month results of the ENESTcmr study demonstrated that patients (pts) on IM with ongoing BCR-ABL positivity who switched to NIL achieved faster, deeper molecular responses than pts remaining on IM.
Aims: The ENESTcmr study evaluates the potential benefit of switching pts with persistent residual disease on long-term IM therapy to NIL. Here, we report the 24-month follow-up of this study.
Methods: ENESTcmr includes pts with Philadelphia chromosome–positive (Ph+) CML-CP who achieved a complete cytogenetic response (CCyR) but had detectable BCR-ABL transcripts after ≥2 years of IM therapy. Pts were randomized to continue their IM dose (400 or 600 mg once daily [QD; n=103]) or switch to NIL 400 mg twice daily (BID; n=104). Molecular response (including major molecular response [MMR, BCR-ABL^{IS} ≤0.1%] and MR$^{4.5}$ [BCR-ABL^{IS} ≤0.0032%]) was determined by RQ-PCR. Confirmed undetectable BCR-ABL was achieved if 2 consecutive samples (with sensitivity of ≥ 4.5 logs) had negative RQ-PCR results.
Results: With 24 months of follow-up, 77% and 91% of pts remained on study in the NIL and IM arms, respectively. The higher rates of discontinuation with NIL were expected, as these pts switched to a new therapy from a well-tolerated long-term therapy. Most discontinuations occurred within the first 12 months on study, with comparable rates of discontinuation across arms between 12 and 24 months. By 24 months, confirmed undetectable BCR-ABL was achieved by significantly more pts who switched to NIL vs pts continuing IM (22.1% vs 8.7%; P=.0087). Twice as many pts achieved and maintained undetectable BCR-ABL on 3 consecutive assessments on NIL vs IM (n=10 vs 5). The increase in the proportion of pts with undetectable BCR-ABL from month 12 to 24 was higher in the NIL arm (9.6 percentage points) vs the IM arm (2.9 percentage points). In pts without MR$^{4.5}$ at study start, significantly more pts in the NIL arm achieved MR$^{4.5}$ by 24 months than in the IM arm (42.9% vs 20.8%; P=.0006), and more pts maintained MR$^{4.5}$ (or better) on 3 consecutive assessments with NIL vs IM (n=18 vs 6). Rates of MR$^{4.5}$ were superior on NIL regardless of response at study start (Table 1), but the difference between the treatment arms was particularly pronounced in pts without MMR at study start (MR$^{4.5}$ achieved in 29.2% vs 3.6% of pts on NIL vs IM; P=.016). No pt without MMR at study start who continued on imatinib achieved confirmed MR$^{4.5}$ or undetectable BCR-ABL (on 2 consecutive assessments). No pt progressed to accelerated phase/blast crisis since the 12-month follow-up. Events were experienced by 3 pts on NIL (confirmed loss of MMR, n=2; death, n=1) and 7 on IM (confirmed loss of MMR, n=4; confirmed loss of CCyR, n=3). Prior interferon therapy (with vs without) and length of prior IM therapy (≤ 36 vs > 36 months) did not significantly affect the rate of MR$^{4.5}$ in either treatment arm. The safety profiles of NIL and IM were consistent with previous studies.
Summary / Conclusion: In pts with detectable residual disease on long-

term IM therapy, NIL induced deeper molecular responses than continued IM, and these responses were more frequently maintained in consecutive assessments. The difference between arms in rates of $MR^{4.5}$ and undetectable BCR-ABL increased between 12 and 24 months. These deeper molecular responses achieved after switch to NIL may increase eligibility for tyrosine kinase inhibitor–free remission studies.

Table 1.

	NIL 400 mg BID (n = 104)	IM 400 or 600 mg QD (n = 103)	*P* Value
Confirmed undetectable BCR-ABL[a] (ITT), %			
By 12 months	12.5	5.8	.108
By 24 months	22.1	8.7	.0087
Response by 24 months (in pts without the response of interest at study start), %			
MMR	(n = 24) 83.3	(n = 28) 53.6	.0342
Undetectable BCR-ABL[a]	(n = 101) 31.7	(n = 100) 17.0	.0106
$MR^{4.5}$ by 24 months by response at study start, %			
Without MMR at study start	(n = 24) 29.2	(n = 28) 3.6	.016
Without MR^{4} at study start	(n = 74) 31.1	(n = 78) 11.5	.003
Without $MR^{4.5}$ at study start	(n = 94) 42.9	(n = 91) 20.8	.0006
Estimated rate of event-free survival, %			
At 24 months	96.6	92.8	.4387
Response by 24 months according to prior interferon use, %			
$MR^{4.5}$, no prior interferon	(n = 59) 54.2	(n = 57) 38.6	With vs no interferon: .1499 (NIL) .5289 (IM)
$MR^{4.5}$, with prior interferon	(n = 45) 40.0	(n = 46) 32.6	
Response by 24 months according to prior duration of IM treatment, %			
$MR^{4.5}$, ≤ 36 months	(n = 18) 55.6	(n = 21) 38.1	≤ 36 vs > 36 months: .4850 (NIL) .8161 (IM)
$MR^{4.5}$, > 36 months	(n = 86) 46.5	(n = 82) 35.4	

[a] ≥ 4.5-log sample sensitivity.

IM, imatinib; ITT, intent-to-treat; NIL, nilotinib.

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IMPACT OF BASELINE MUTATIONS ON RESPONSE TO PONATINIB AND END OF TREATMENT MUTATION ANALYSIS IN PATIENTS WITH PHILADELPHIA CHROMOSOME-POSITIVE (PH+) LEUKEMIAS

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Background: BCR-ABL kinase domain mutations frequently cause tyrosine kinase inhibitor (TKI) failure in chronic myeloid leukemia (CML). Ponatinib, a potent oral pan-BCR-ABL TKI, has shown preclinical activity against all single mutants tested, including T315I.

Aims: The impact of baseline (BL) mutations on response to ponatinib (45 mg once daily) and the end of treatment (EOT) mutation status in patients discontinuing treatment were evaluated in the phase 2 PACE trial.

Methods: Data for chronic phase (CP) CML patients are described herein; data for patients with advanced disease will be presented. Heavily pretreated CP-CML patients (94% received ≥2 prior TKIs, 60% received ≥3 prior TKIs) resistant or intolerant to dasatinib or nilotinib (N=203) or with T315I confirmed at BL (N=64) were enrolled. Among CP-CML patients receiving prior dasatinib or nilotinib (n=256), 84% were resistant to dasatinib and/or nilotinib, 16% were intolerant only. The primary endpoint was major cytogenetic response (MCyR). Minimum follow up at analysis (9 Nov 2012) was 12 mos (median 15 [0.1-25] mos). Sanger sequencing was performed at a single central laboratory.

Results: At BL, no mutations were detected in 136 (51%) patients, 1 mutation was detected in 105 (39%) patients, ≥2 mutations were detected in 26 (10%) patients; 27 unique mutations were observed. Responses were observed regardless of BL mutation status. MCyR rates were: 56% overall, 49% in patients with no mutations, 64% in patients with 1 mutation, 62% in patients with ≥2 mutations; 57% in patients with mutation(s) other than T315I (n=67), 74% in patients with T315I only (n=50), 57% in patients with T315I + other mutation(s) (n=14). Although higher response rates were observed in patients with T315I, multivariate analyses have shown that T315I is not an independent predictor of response. Responses (MCyR) were seen against each of the 15 mutations present in >1 patient at BL: T315I 45/64 (70%), F317L 11/22 (50%), F359V 6/13 (46%), G250E 7/8 (88%), E255K 6/8 (75%), M244V 3/5 (60%), V299L 3/5 (60%), H396R 1/5 (20%), F359I 3/4 (75%), F359C 1/4 (25%), E459K 3/3 (100%), E355A 1/2 (50%), L248V 1/2 (50%), Y253H 1/2 (50%), E255V 1/2 (50%). Ninety-nine patients discontinued (35 adverse event, 20 progressive disease, 14 withdrawal by subject, 11 lack of efficacy, 8 physician decision, 11 other). Of the 99 patients discontinuing, 56 patients had EOT mutations assessed. 5 patients lost a mutation, 46 had no change, and 5 gained mutations. In 2 of the 5 patients, single mutations were newly detected at EOT (E255V [10% of transcripts]; T315I [100% of transcripts]); both patients had a prior history of the same mutation, and responses were achieved in other patients with these mutations at BL. In the other 3 patients, multiple mutations were newly detected at EOT (T315I/M351T [100%/40% of transcripts]; T315I/F359V [100%/90% of transcripts]; Y253H/F359V [100%/100% of transcripts]). In all 3 patients, 1 of the mutations was present at BL and/or the patient had a prior history of 1 or both mutations. In 1 patient, the multiple mutation Y253H/F359V may have been associated with progressive disease; the avg dose intensity of ponatinib in this patient was 26 mg/day. Overall, 11 patients lost MCyR (none with T315I at BL); 6 of the 11 discontinued, and 4 had EOT mutations assessed and no changes from BL were observed (Table 1).

Summary / Conclusion: Responses to ponatinib were observed regardless of BL mutation status. No single mutation conferring resistance to ponatinib in CP-CML has been observed to date. Data with a minimum follow up of 18 mos will be presented. NCT01207440.

Table 1.

CP-CML Patients With Loss of MCyR					
Prior Therapy	BL Mutation	EOT Mutation	Avg Dose Intensity (mg/day)	Reason for D/C	Duration of MCyR (months)
I,D,N	None	None	37	PD	2.8
I,D,N,B	G250E	G250E	15	AE	5.5
I,D,N	None	None	45	PD	5.3
I,D,N	F311L	F311L	29	AE	2.8
I,D	None	ND	23	AE	2.7
I,D,N,B	E255V	ND	28	Lack of efficacy	3.1
I,D,N,B	F317L, E450G	n/a	5	n/a	5.6
I,D,N	F359C	n/a	29	n/a	3.0
I,D	None	n/a	44	n/a	2.8
I,D,B	M244V, G250E	n/a	18	n/a	2.7
I,D,N	F359I	n/a	42	n/a	3.1

BL=baseline; D/C=discontinuation; PD=progressive disease; AE=adverse event; I=imatinib; D=dasatinib; N=nilotinib; B=bosutinib; ND=not done; n/a=not applicable, patient remains on study and has not had post-baseline mutations assessed

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FIFTEEN-MONTH UPDATE OF A PHASE II TRIAL OF THE COMBINATION OF PEGYLATED INTERFERON ALPHA2A+NILOTINIB AS FIRST LINE THERAPY FOR NEWLY DIAGNOSED CHRONIC PHASE CML PATIENTS

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Background: Combining Imatinib (IM) with pegylated interferon alfa2a (PegIFN) seems to improve molecular responses for *de novo* chronic phase CML (CP-CML) patients (pts) compared to IM. Second generation TKI (TKI2) such as nilotinib induce higher levels of cytogenetic and molecular responses than IM in the first line for CP-CML.
Aims: This is the M15 update of the use of nilotinib+PegIFN for front line therapy in CP-CML pts.
Methods: This is a 2-step study, where pts were assigned first to PegIFN (±HU) for 4 wks (90 µg/wk) prior to a combination of nilotinib 300 mg BID+PegIFN 45 µg/wk for ≥ 1 year. The primary endpoint was the rate of $MR^{4.5}$ by M12 confirmed on 2 datapoints. Molecular assessments were centralised and expressed as $BCR\text{-}ABL^{IS}$ in %. All pts have signed up an informed consent to enter the study.
Results: Forty one pts (+1 screen failure) were enrolled in a first step and 20 additional pts were planned if the primary endpoint would not been reached in the first cohort. The current median FU is 20.5 (17–23.2) months (M). Sokal and Euro scores were high for 15% and 5%, interm. for 41% and 54% and low for 44% and 41% respectively. Eutos score was high for 2 pts. Median age was 53 (23-85) years. Two pts had a masked Ph, 3 a variant form, and 1 had an ACA, all pts had a "M" BCR transcript. CHR was obtained in 5%of pts at M1 and 100% at M3. Analysed in ITT, the rates of CCyR at M3, 6, and 12 (= at2,5, 8 and 11 M of TKI2) were 39%, 56%, 71% respectively. Overall 75.6% of pts were in MMR at M12 and 80.6% at M15. The $MR^{4.5}$ rates increase with time: 9.8% at M6 to 29.3% at M15, and the MR^5 rates followed the same pattern (Figure 1). Of note, 87% of pts had a $BCR\text{-}ABL^{IS}$ ≤10% at M3 and, at M12, had significantly higher rates of MMR (77% vs 20%, P=0.028) and $MR^{4.5}$ (20% vs 0%, P=0.034) than pts >10%. One pt progressed to myeloid blast crisis at M6 with no detectable BCR-ABL mutation, and is alive after allogeneic SCT. At last FU, 7 pts went out of study:M2 for non-observance, M6 for seizures related to an extra-dural hematoma in a non-thrombocytopenic pt, M6 for recurrent grade 3 hepatic toxicity, M9 for recurrent grade 3 pruritus, M15, 18 & 18 for coronary stenoses. The mean doses of PegIFN/Wk during the first year were 32.8 µg/wk, significantly higher in pts achieving $MR^{4.5}$ at M12 (45.3 vs 30.2 µg/wk, P=0.02) and the mean doses of nilotinib during the first year (493 mg/day) were not different in pts achieving $MR^{4.5}$ at M12 (505 vs 490 mg/day, P=0.68). At M15, the rate of grade 3-4 hematologic toxicities overall were anemia 2%, thrombocytopenia 24%, neutropenia 24% and pancytopenia 2% of pts. These occurred mainly during M1-M3 (22% neutropenia, 24% thrombocytopenia, 2% pancytopenia, 2% anemia), rarely during M3-M6 (7% neutropenia, 2% thrombocytopenia) and never thereafter. Grade 3-4 non-hema toxicities followed a similar pattern, and overall we observed 20% liver toxicities, 15% of neuro-psy episodes, 10% gut/pancreas disturbances, 2% arthro-myalgias. Three late coronary stenoses occurred at M15, 18 & 18, although no case of PAOD has been reported to date.
Summary / Conclusion: The combination of nilotinib+PegIFN is relatively well tolerated despite frequent initial hematologic toxicities, and provides high rates of $MR^{4.5}$ at M12 and beyond. The cohort n°2 will not be enrolled as the M12 $MR^{4.5}$ rates are beyond planned initial expectations. The M15 and M18 updates will be presented during the meeting

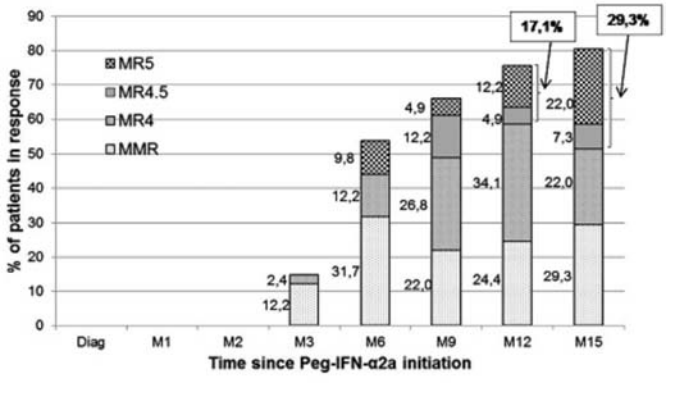

Figure 1.

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EARLY CYTOGENETIC AND MOLECULAR RESPONSES AND LONG-TERM OUTCOME IN EARLY CHRONIC PHASE CHRONIC MYELOID LEUKEMIA (CML) PATIENTS TREATED WITH NILOTINIB

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Background: Early cytogenetic and molecular responses have been associated with a better outcome in patients treated with imatinib, dasatinib, or nilotinib frontline. In particular, BCR-ABL transcript level < 10% at 3 months correlated with: increased 2-year cumulative incidence of CCyR, MMR, and $MR^{4.5}$ with dasatinib (Marin D *et al*, Blood 2012); better progression-free survival (PFS) and overall-survival (OS) at 3 years, with dasatinib (DASISION; Saglio G *et al*, ASH 2012, abs. 1675); higher probability of future MMR and $MR^{4.5}$, and better PFS and OS, with nilotinib (ENESTnd; Hochhaus A *et al*, ASH 2012, abs. 167). There is increasing interest in identifying as early as possible patients who will not respond optimally to tyrosine kinase inhibitors in order to consider treatment modifications.
Aims: To evaluate the impact of BCR-ABL transcript level and cytogenetic response at 3 months on subsequent response and outcome in an independent cohort of patients treated with frontline nilotinib-based regimens in Italy (CML Italian Registry of Nilotinib).
Methods: The CML Italian Registry of Nilotinib includes 215 patients, enrolled in 2 multicenter phase II studies conducted by the GIMEMA CML WP (ClinicalTrials.gov. NCT00481052 and NCT00769327) or treated at the Bologna University Hospital, with nilotinib 300 mg or 400 mg BID as initial treatment. The median age was 53 years (range 18–86). Ten out of 215 patients (5%) had a high EUTOS score. The median follow-up was 33 months (range 21–51 months). At 3 months 196/215 (91%) and 189/215 (88%) patients were evaluable for the molecular and cytogenetic response, respectively. BCR-ABL transcript levels were: ≤1% in 88%; >1% to ≤ 10% in 11%; > 10% in 1%; given the very low proportion of patients with a BCR-ABL > 10% (n. 2), patients have been divided into 2 groups, with a transcript level ≤1% (n=173, 88%), or >1% (n=23, 12%). Cytogenetic response was: MCyR 93% (CCyR 84%; PCyR 9%); less than MCyR 7%. We analysed the rate of MMR at 1 year, and the failure-free survival (FFS, according to ELN 2009 definitions), PFS, and OS (any death included) according to the BCR-ABL transcript levels and to the cytogenetic response at 3 months.
Results: Patients with BCR-ABL <1% at 3 months had a higher rate of MMR at 12 months with respect to those with a transcript level ≤1% (79% vs. 35%, P<0.001). A lower transcript level at 3 months correlated with a better 3-years FFS (92% vs. 74%; P<0.001) and PFS (95% vs. 83%; P=0.009) and a similar OS (96 vs. 86%; P=0.059). Patients with a MCyR at 3 months vs. patients with less than a MCyR had higher rates of MMR at 12 months (77% vs. 46%; P=0.02). Moreover, patients in MCyR at 3 months had a better 3-years FFS (92% vs. 69%; P=0.001) and PFS (96% vs. 77%; p 0.001), but similar OS (96% vs. 92%; P=0.48). Patients with a CCyR at 3 months vs. patients with less than a CCyR had higher rates of MMR at 12 months but similar FFS, PFS, and OS (data not shown).
Summary / Conclusion: In our national experience a very small proportion of patients treated frontline with nilotinib failed to achieve a reduction to < 10% level of BCR-ABL transcript (2/196 or 1%). The cut-off of 1% of BCR-ABL at 3 months in our experience is a reliable surrogate marker of response at 1 year (MMR) and outcome (PFS and FFS), along with the level (MCyR) of cytogenetic response achieved. The absence of these early responses may represent an adverse factor to be considered in the management of patients treated with nilotinib frontline.
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CLINICAL SIGNIFICANCE OF EARLY MOLECULAR RESPONSE IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED FRONTLINE WITH IMATINIB MESYLATE

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Background: Second generation tyrosine kinase inhibitors (TKIs) induce higher cytogenetic and molecular response rates with respect to imatinib (IM) in chronic myeloid leukemia (CML) patients in early chronic phase, but outcome differences have not been clearly demonstrated and IM still represents an important frontline therapeutic option. Early response-related prognostic factors are extremely relevant to optimize the treatment strategy and to consider a switch to 2nd line therapy in patients who are not able to achieve an optimal response. In IM-treated patients, BCR-ABL^{IS} transcript levels >10% at 3 months and >1% at 6 months were able to identify high-risk groups (Marin *et al*, JClinOncol 2011; Hanfstein *et al*, Leukemia 2012). Similar analysis were performed within the IM arms of the ENESTnd trial (Hochhaus *et al*, EHA 2012) and the DASISION trial (Jabbour *et al*, EHA 2012).

Aims: To investigate the prognostic impact of a large BCR-ABL^{IS} transcript amount at 3 and 6 months (in particular, ≤1% vs >1%) on the subsequent response and the long-term outcome of CML patients treated frontline with IM, describing the characteristics of patients with higher transcript levels.

Methods: 559 patients enrolled within 3 trials of the GIMEMA CML WP (ClinTrialsGov NCT00514488/NCT00510926, observational trial CML023) were analyzed. Evaluable QPCR sample at 3 and 6 months: 487/559 (87%) and 492/559 (88%), respectively.Definitions: major molecular response (MMR): BCR-ABL^{IS} ratio <0.1%; molecular response with 4.0-log reduction (MR$^{4.0}$): BCR-ABL^{IS} ratio <0.01% (at least 10.000 ABL copies); failure: according to 2009 ELN recommendations; progression: transformation to advanced phases; all deaths. at any time and for any reason, were included. Patients with events or censored within 3 or 6 months were excluded from the respective analysis.

Results: Median age: 52 years (range 18-84). IM dose: 76% 400 mg, 24% 800 mg. Overall risk distribution: high Sokal score: 22%; high EUTOS score: 7%. Median follow-up: 76 months (range: 7-99; 95% of patients with at least 5-year observation). BCR-ABL^{IS} ratio at 3 months: ≤1% in 336/487 (69%), >1% to ≤10% in 120/487 (25%) and >10% in 31/487 (6%). BCR-ABL^{IS} ratio at 6 months: ≤1% in 425/492 (86%), >1% to ≤10% in 54/492 (11%) and >10% in 13/492 (3%). As expected, baseline characteristics of patients with higher BCR-ABL^{IS} transcript levels were different with respect to patients with lower levels: patients with BCR-ABL^{IS} ratio >1% at 3 months had lower hemoglobin concentration, higher blast % in peripheral blood (PB), larger spleen, higher Sokal and EUTOS scores (patients with BCR-ABL^{IS} ratio >1% at 6 months, in addition, showed also a higher frequency of CCA in Ph+ cells). A detail of response and outcome according to transcript levels at 3 and 6 months is presented in Table 1.

Summary / Conclusion: In a multicentric nationwide experience, the proportion of patients with BCR-ABL^{IS} transcript levels >10% at 3 and 6 months was low. The risk distribution and the presence of a proportion of patients treated with high-dose IM may explain, at least in part, the differences with other published reports. At 3 and 6 months, a BCR-ABL^{IS} cutoff of 1% was a reliable surrogate marker of response and outcome. A cutoff of 10% identified, at 3 and 6 months respectively, two smaller cohorts with inferior response rates, lower failure-free survival, lower progression-free survival and lower overall survival, but the outcome differences did not result statistically significant, probably due to the small number of patients with a BCR-ABL^{IS} ratio >10%. A BCR-ABL^{IS} level >1% at 3 and 6 months represents a warning, requiring a close monitoring. A switch to 2nd generation TKIs should be considered.

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Table 1.

BCR-ABL at 3 months (N = 487)	≤ 1% (N = 336)	> 1% (N = 151)	p	≤ 10% (N = 456)	> 10% (N = 31)	p
CCgR at 1 year, %	87.5	60.3	<0.001	80.9	51.6	<0.001
MMR at 1 year, %	70.5	35.1	<0.001	62.1	22.6	<0.001
MR4 at 2 years, %	23.5	8.6	<0.001	20.0	3.2	0.017
FFS, %	83.7	66.8	<0.001	79.2	68.3	0.104
PFS, %	87.5	81.9	0.004	85.2	90.0	0.771
OS, %	88.4	83.6	0.010	86.5	87.1	0.622
BCR-ABL at 6 months (N = 492)	**≤ 1% (N = 425)**	**> 1% (N = 67)**	**p**	**≤ 10% (N = 479)**	**> 10% (N = 13)**	**p**
CCgR at 1 year, %	88.7	46.3	<0.001	84.3	30.8	<0.001
MMR at 1 year, %	71.1	13.4	<0.001	64.7	7.7	<0.001
MR4 at 2 years, %	21.4	4.5	<0.001	19.6	0	0.143
FFS, %	83.6	57.4	<0.001	81.0	57.1	0.045
PFS, %	87.6	78.4	0.002	86.4	74.1	0.115
OS, %	88.3	80.8	0.010	87.3	76.9	0.078

Responses at each timepoint were compared using χ2 test or Fisher exact test, as appropriate. Failure-Free Survival (FFS), Progression-free Survival (PFS) and Overall Survival (OS) were estimated using the Kaplan-Meier method and compared by log-rank test.

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TARGETED NEXT-GENERATION SEQUENCING FOR THE IDENTIFICATION OF GENOMIC BCR-ABL1 FUSION JUNCTIONS TO QUANTIFY RESIDUAL DISEASE IN CML PATIENTS IN CMR

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Background: Recent studies indicate that 40% of CML patients who achieve complete molecular remission (CMR) on imatinib remain disease-free after drug discontinuation, raising the possibility of an "operational cure". However, the safe introduction of a TKI withdrawal policy would require a reliable and cost effective method of identifying patients with the lowest likelihood of relapse, which is likely to be related to presence residual disease. Preliminary data suggest that PCR of genomic DNA might be more sensitive for the detection of residual disease than one that relies on cDNA and may therefore help to predict outcome post-withdrawal. However, the former method is arduous since it requires a customised patient-specific assay.

Aims: Here we describe a method based on targeted-next-generation sequencing allowing identification of BCR-ABL1 breakpoints from enriched genomic BCR and ABL1 DNA followed by rapid generation of DNA-based qPCR assays.

Methods: The location of the BCR-ABL1 fusion junction was mapped in disease samples from 30 CML patients using Illumina's MiSeq platform. A custom TruSeq DNA target enrichment kit (Illumina) was used to enrich for the BCR and ABL1 genes. The enriching probes were designed via the online tool Design Studio covering both genes plus 50kb upstream and downstream of *BCR* and *ABL1*, respectively. The workflow involved sample quantification, library prep, multiplexed sample pooling (10 sample/run), enrichment-probe hybridisation, template preparation, and sequencing. Subsequent mapping of t(9;22) translocation junctions was performed via a custom designed bioinformatics algorithm.

Results: All breakpoints were successfully mapped. DNA qPCR assays were designed and validated for 9 patients. In clinical samples from patients in complete molecular remission, the RT-qPCR assays detected residual disease in three out of nine patients, demonstrating that DNA-qPCR can detect residual disease in patient samples in which CML cells persist below the detection threshold of RT-qPCR. Furthermore, since assay performance criteria were optimal, we presume that disease levels in the remaining 6 were exceedingly low or completely absent, consistent with the notion that residual disease levels may differ in "CMR" patients.

Summary / Conclusion: NGS-facilitated DNA-qPCR may therefore prove valuable for the stratification of patients with low levels of residual disease and, therefore, in the identification of patients for whom TKI therapy could be safely reduced or stopped.

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LONG TERM MOLECULAR RESPONSE OF CHRONIC PHASE CML AFTER INTERFERON-ALPHA DISCONTINUATION: CURE OR NOT TO CURE CML

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Background: Imatinib (IM) is the standard front line therapy for CP-CML resulting in complete cytogenetic response (CCyR) and major molecular response (MMR) for almost 2/3 of patients (pts). CCyR is associated with improved survival and MMR with progression free survival and stability of the response. Around 10% of these pts achieve a durable undetectable molecular response allowing them candidates for cessation of TKI's according to the STIM study: Sokal risk score, duration of IM, and sex were predictive for the maintenance of molecular response.Before the IM era and besides allogeneic hematopoietic stem cell transplantation, interferon alpha (IFNα) based regimens were used for CML treatment. Maintenance of CCyR and MMR after discontinuation of IFNα has also been reported for rare pts.
Aims: The aim of this study was to analyse the characteristics and molecular response pattern of CML pts after long term discontinuation of IFNα.
Methods: Between 2011 and 2012, 77 CP-CML pts treated with IFNα based regimens and in remission without therapy after IFN discontinuation were reported from 10 European centers. This analysis has been performed on the 60 consecutive cases with an assessable molecular follow-up.
Results: All pts had their CML diagnosed between 1980 and 2001. Median age was 45 years, 57% were female. Sokal risk score was low, intermediate and high for 43 (74%), 13 (22%) and 2 pts respectively. At IFNα discontinuation, all pts were in CCyR. Molecular response was assessed either by non quantitative polymerase chain reaction (PCR n=28) or quantitative PCR (RTQPCR n=28) depending on the technique locally available at that time. Among them, 18 pts had a negative PCR and 20 had an undetectable BCR-ABL transcript by RTQPCR. During follow-up, all pts maintained CCyR: 28 had a sustained undetectable residual disease (UND-MRD) confirmed by RTQPCR, while MRD was detectable in 28. In that latter group, MRD was between 0.1 and 0.001% for the majority of pts (n=25), fluctuation between 1 and 0.001% was observed in 2 cases, and transcript was stable between 1 and 0.1% for 1 pt. Therefore, we distinguished 2 groups: sustained UND-MRD (n=28) or detectable MRD (n=27). As the median follow-up after IFN discontinuation was 125 (41-227) and 119 (43-215) months respectively, comparison between the 2 groups was performed. No difference in term of age, gender, CML presentation, response to IFN was detected. MCyR was more rapidly achieved in the UND- than in the positive MRD group (6.67 versus 12.09 months, P=0.010) but not significantly CCyR (P=0.053) and not for MMR (P=0.913). Median IFN exposure was longer in the detectable MRD group (99 (7-180) vs 75 months (23-191) P=0.030) that suggests a slower response. At the last molecular assessment, 4 pts had lost MMR after a median time off-IFN of 129 months (85-205). All of them had a detectable MRD after IFN discontinuation. Two pts died from other reason than CML.
Summary / Conclusion: This study describes a cohort of CML pts who still maintained their CCyR without treatment after long term IFN discontinuation, whatever their distribution within two groups according to the molecular response pattern: sustained UND or detectable MRD.
Based on a long follow-up, this study illustrates also that "operational cure" of CML does not require the complete eradication of the residual disease.

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EFFICACY AND SAFETY OF RADOTINIB IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS WITH FAILURE TO IMATINIB OR IMATINIB PLUS DASATINIB: 12-MONTH MINIMUM FOLLOW-UP UPDATE

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Background: Radotinib is an orally active, selective Bcr-Abl1 tyrosine kinase inhibitor (TKI), approved for patients with chronic phase chronic myeloid leukemia (CP-CML) resistant to or intolerant of imatinib±dasatinib in Korea. The preliminary phase 2 result of radotinib has been reported to be one of treatment options in patients with TKI-failed CP-CML (Kim SH *et al*., Blood (ASH Annual Meeting Abstracts), Nov 2012; 120:695).
Aims: Here, we update the clinical efficacy and safety results in imatinib or imatinib+dasatinib failed patients with a minimum follow-up of 12 months.
Methods: Philadelphia chromosome - positive (Ph^+) CP-CML patients who failed or were intolerable to TKIs (imatinib or imatinib + dasatinib) were enrolled between July 2009 and November 2011. All patients were treated with radotinib 400 mg twice daily. The primary end point was an achievement of major cytogenetic response (MCyR, Ph^+£35%) by 12 months. Safety parameters were also analyzed.
Results: A total of 77 CP CML patients (18 years of age or over) were enrolled from 12 sites in Korea, India, and Thailand. This analysis includes data from last enrolled patient who received at least 12 months of radotinib therapy. The median age of patients was 43 (range; 22-75) years, and 65 (84.4%) were imatinib-resistant including 3 patients who have dasatinib intolerance with imatinib resistance and intolerance and 12 (15.6%) were imatinib-intolerant including 1 patient who have dasatinib intolerance with imatinib intolerance. With a median follow-up of 23.4 months, treatment with radotinib is ongoing in 36 patients (46.7%) and 33 patients (42.9%) discontinued the treatment including two deaths (2.6%). Median duration of radotinib exposure was 378 (8-1050) days. Overall MCyR rate was 64.9%, including 36 patients (46.8%) complete cytogenetic response and 14 patients (18.2%) partial cytogenetic response. Among the 14 patients with known BCR-ABL1 KD abnormality at baseline, 43% achieved MCyR and 21% achieved CCyR. And MCyR and CCyR rates were higher in patients without mutation. The median time to MCyR was 2.8 months (85 days) and the median duration of MCyR was 20.2 months. Of patients achieving complete cytogenetic response, 30.5% (11/36) achieved major molecular response. Within follow-up durations, 55 patients (71.4%) required dose interruption and 53 patients (68.8%) had dose reduction. Most common grade 3/4 hematologic and laboratory adverse events (AEs) were thrombocytopenia (29.9%), neutropenia (10.4%), anemia (6.5%), and hyperbilirubinemia (33.7%). Common non-hematologic AEs (≥10%) were rash (23.4%), fatigue (16.9%), pruritus (14.3%), headache (13.0%), decreased appetite (10.4%) myalgia (10.4%) and nausea (10.4%). The majority of AEs were easily manageable with temporal dose interruption and/or reductions. In all patients with CP-CML treated with second-line radotinib, estimated progression-free survival and overall survival rate by 12months was 86.3% (95% CI, 75.1-92.7%) and 96.1% (95% CI, 88.4-98.7%), respectively. The PFS rate was higher among patients without a baseline mutation compared with those who had baseline mutations at both 12 months (90.3% vs 69.6%; P=0.0518) and 24 months (83.3% vs 60.9%; P=0.0364).
Summary / Conclusion: With a minimum follow-up of 12 months, radotinib continues to demonstrate efficacy and maintains tolerability in patients with both imatinib or imatinib+dasatinib failed CP-CML. Most of the AEs occurred in the early treatment period, were tolerable, and were easily controlled by dose interruption or reduction.

Table 1. Summary of patients' characteristics, efficacy and safety profile.

Total patients		N=77
Age		47 (24-76) years
Gender	male	54 (70.1%)
	female	23 (29.9%)
Disease status at screening	**Imatinib resistance**	**65 (84.4%)**
	+ dasatinib intolerance	3 (3.9%)
	Imatinib intolerance	**12 (15.6%)**
	+ dasatinib intolerance	1 (1.3%)
Cytogenetic response	Complete cytogenetic response	36 (46.7%)
	Partial cytogenetic response	14 (18.2%)
Dose modification	Dose interruption	55 (71.4%)
	Dose reduction	53 (68.8%)
Grade 3/4 hematologic adverse events	Thrombocytopenia	23 (29.9%)
	Neutropenia	8 (10.4%)
	Anemia	5 (6.5%)
The most common grade 3/4 laboratory adverse event	Hyperbilirubinemia	26 (33.7%)
Common non-hematologic adverse events (> 10%)	Rash	18 (23.4%)
	Fatigue	13 (16.9%)
	Pruritus	11 (14.3%)
	Headache	10 (13%)
	Decreased appetite	8 (10.4%)
	Myalgia	8 (10.4%)
	Nausea	8 (10.4%)

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HIGH RATE OF DEEP MOLECULAR RESPONSE AFTER 5 YEARS OF NILOTINIB 400 MG BID IN EARLY CHRONIC PHASE CHRONIC MYELOID LEUKEMIA - UPDATE OF THE GIMEMA CML WP TRIAL CML0307

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Background: Nilotinib is a potent and selective BCR-ABL inhibitor approved for the frontline treatment of CML. The latest update (4-year follow-up) of the ENESTnd study demonstrated sustained superiority of nilotinib vs. imatinib, with a confirmed significantly decreased risk of progression to accelerated-blast phase (AP/BP) and a higher rate of molecular responses, which were faster and deeper than those observed with imatinib (Kantarjian H *et al*, ASH 2012, abstract 1676). This extended the patient population achieving deeper responses that may potentially enter into Treatment Free Remissions (TFR) trials in the near future. It is important to understand, in patients (pts) treated for a long period with nilotinib, which characteristics are associated with a deep molecular response.

Aims: To evaluate the long-term (5-year minimum follow-up) results, and, in particular the stability of the deep molecular response ($MR^{4.0}$), of pts treated frontline with nilotinib in an investigator-initiated phase II study (GIMEMA CTs CML/0307).

Methods: The GIMEMA CML WP conducted a multicentre phase 2 trial with nilotinib 400mg BID as frontline therapy (ClinicalTrials.gov.NCT00481052). Median follow-up for the present analysis was 55 months (range: 50-62 months). Definitions: $MR^{3.0}$: detectable disease <0.1% BCR-ABL^{IS}; $MR^{4.0}$: either detectable disease ≤0.01% BCR-ABL^{IS} or undetectable disease in cDNA with >10,000 ABL transcripts; stable deep molecular response: $MR^{4.0}$ in at least 3 samples over ≥1 year period without any result with less than $MR^{4.0}$; failures: according to the 2009 ELN recommendations; events: failures and treatment discontinuation for any reason. All analyses have been conducted according to the intention-to-treat principle.

Results: 73 pts enrolled: median age 51 years; 45% low, 41% intermediate and 14% high Sokal risk. Two pts never achieved a $MR^{3.0}$, 1 of these pts progressed to AP/BP (see below), the other is in stable and confirmed CCyR at 48 months. Only 3 pts had a confirmed loss of $MR^{3.0}$ due to poor adherence (all 3 are still on nilotinib). The overall estimated probability of $MR^{4.0}$ was 82%, with a median time to $MR^{4.0}$ of 18 months. During the 3rd year of therapy, 56/73 (76%) pts obtained a $MR^{4.0}$ at least once, stable in 16/73 (22%) pts; fluctuations around $MR^{4.0}$ (always in $MR^{3.0}$) in 37/73 (51%) pts; fluctuations with unconfirmed loss of $MR^{3.0}$ in 3/73 (4%) pts. During the 4th year of therapy (months 37-48), 57/73 (78%) pts had a $MR^{4.0}$ at least once, stable in 21/73 (29%) pts; fluctuations around $MR^{4.0}$ (always in $MR^{3.0}$) in 33/73 (45%) pts; fluctuations with unconfirmed loss of $MR^{3.0}$ in 3/73 (4%) pts. Overall, 19/73 pts (26%) showed a stable $MR^{4.0}$ during the 3rd and 4th year of therapy. Final analysis of the 5th year of therapy is still ongoing and will be presented. Only one patient progressed at 6 months to AP/BP and subsequently died (high Sokal risk, T315I mutation). Overall, 11 pts (15%) permanently discontinued nilotinib: 1 progression to AP/BP; 1 unrelated-death; 7 adverse events; 2 refusal. The estimated probability of overall survival, progression-free survival and failure-free survival was 97% at 5 years; the estimated probability of event-free survival was 83% at 5 years.

Summary / Conclusion: After 5 years of follow-up, the great majority of pts are still on nilotinib, and a significant proportion have a stable deep molecular response ($MR^{4.0}$) and may potentially enter into Treatment Free Remissions (TFR) trials.

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HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH CHRONIC MYELOID LEUKAEMIA: RESULTS OF A GERMAN CROSS-SECTIONAL STUDY IN PATIENTS PREVIOUSLY REGISTERED IN PROSPECTIVE, CONTROLLED CLINICAL TRIALS

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Background: With many treatment options for chronic myeloid leukaemia (CML) providing similarly fortunate survival probabilities, endpoints like health-related quality of life (HRQoL) move into focus. HRQoL might be essential for deciding on treatment strategies. Data on HRQoL in CML are rare (Efficace *et al*., 2011), especially for patients after HSCT.

Aims: We sought to evaluate HRQoL in German patients who had been registered in the prospective, randomized CML studies II to IV (Hehlmann *et al*., 2011, 2007 and 2003). Main interest was the comparison of HRQoL between different treatment strategies.

Methods: In December 2010, the EORTC QLQ 30 questionnaire (including global health status and five functioning scales) was sent out to 241 German CML study participants. With all scales ranging from 0 to 100, 8 points is regarded as a minimally important difference suggesting benefit or harm (Efficace *et al*., 2013). To investigate whether participation could be assumed to be "random", baseline data of responders (R) and non-responders (NR) were compared. Associations between two variables were assessed by the Fisher or Mann-Whitney tests, as appropriate. Level of significance was 0.05.

Results: *A* total of 1634 patients of CML studies I-IV could have received the questionnaire. During January to April 2011, 858 questionnaires (53%) were sent back. Fifteen study centres denied participation. Compared to NR, R were older (median age: 55 (NR) vs. 58 (R), P=0.0426); years since diagnosis (median 6.5 (R) vs. 7.4 (NR)) and the percentage that had been transplanted were lower (18% (R) vs. 24% (NR)). No statistically significant differences were observed regarding sex, Euro score (Hasford *et al*., 1998), or time since transplantation. With 517 patients, 60% (of 858) received 400mg imatinib mono-therapy when answering the questionnaire and 102 (12%) were off therapy after HSCT. Less than 10% received either higher-dose imatinib mono-therapy, imatinib 400 mg + AraC or interferon, nilotinib, or dasatinib. Time since diagnosis was ≤3 years in 156 (18%), > 3 and ≤ 7 years in 309 (36%), and >7 years in 393 (46%) of the patients. Women (352 of 858, 41%) perceived a statistically significant reduction in global health status (mean: 63, P<0.001), role (65, P=0.0016), emotional (60, P=0.0002), and physical functioning (75, P<0.0001) when compared to males (means: 69, 72, 68, and 83 respectively). Cognitive (mean: 77, all) or social functioning (69, all), age, time since diagnosis, and the percentage of transplantations were not significantly different between the sexes. Time since diagnosis was >7 years for 100 patients off therapy after HSCT and for 203 patients receiving imatinib 400 mg mono-therapy. In comparison, global health status (71 vs. 65, P=0.252) and physical functioning (84 vs. 75, P=0.0003) were higher in the off-therapy group. Additional adjusted analysis including age groups will be presented.

Summary / Conclusion: In this cross-sectional study, women showed an impaired global health status, role, emotional, and physical functioning compared to males. In the latter two cases, this perception met the definition of a clinically relevant difference. Long-term survivors of HSCT had an at least comparable overall global health status and a better physical functioning when compared to patients on imatinib treatment more than 7 years after diagnosis. However, the younger age of transplanted patients has also to be taken into account.

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WHICH METHOD BETTER EVALUATES MOLECULAR RESPONSE IN IMATINIB TREATED NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS, BCR-ABLIS OR LOG REDUCTION FROM THE BASELINE LEVEL?

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Background: The molecular response of chronic myeloid leukemia (CML) patients by tyrosine kinase inhibitor treatment could be evaluated either by the BCR-ABL mRNA levels on the international scale (IS) or by the log reduction from the laboratory's individual baseline level. Conversion to the IS is achieved by the application of laboratory specific conversion factors which are acquired through sample exchange with reference laboratories. Because a three log reduction from the laboratory's individual baseline level usually does not equal to 0.1%^{IS} for non-reference laboratories, the patients' molecular response evaluated by these two methods might be different. To date, there is no comparison on these two evaluation methods.
Aims: We tried to investigate which method better evaluates molecular response in imatinib treated CML patients.
Methods: We compared the two evaluation methods in 248 consecutive imatinib treated newly diagnosed chronic phase CML patients. Bone marrow (BM) aspirations for morphology and cytogenetics were performed every 3 to 6 months until complete cytogenetic response (CCyR) was achieved, then every 6 to 12 months, and BCR-ABL mRNA levels were tested in the peripheral blood (PB) or BM samples every 3 to 6 months by real-time quantitative PCR. Only the BCR-ABL mRNA levels in PB were analyzed in the current study. All samples were collected with informed consent. Our laboratory acquired CF by sample exchange with international reference laboratory in Adelaide, Australia. Progression was defined as death for any reason and the development of accelerated-phase or blast-crisis CML as defined by the ELN.
Results: Achieving major molecular response (MMR) during treatment evaluated by them similarly predict progression free survival (PFS, all P<0.0001), whereas significantly more patients were defined as MMR by IS than by log reduction method (173/220 vs 137/220, 78.6% vs 62.2%, P<0.0001) and all MMR patients were alive without progression. Furthermore, the patients' molecular response at 3 and 6 months evaluated by them had similar predictive value on their cytogenetic response at 12 months and molecular response at 18 months: ≤10%^{IS}/ ≥1 log reduction of BCR-ABL at both 3 months (n=65) and 6 months (n=77) associated with CCyR at 12 months (all P<0.001); ≤1% ^{IS} /≥ 2 log reduction of BCR-ABL at both 3 months (n=66) and 6 months (n=77) associated with achieving MMR at 18 months (all P <= 0.003). Both ≤ 10% ^{IS} and ≥1 log reduction of BCR-ABL at 3 months (n=79) and ≤1% ^{IS} at 6 months (n=92) significantly associated with PFS (P=0.0011, 0.0090 and 0.0064), whereas ≥2 log reduction at 6 months (n=92) did not significantly associated with PFS (P=0.11) (Figure 1). Both ≤ 1% ^{IS} and ≥2 log reduction of BCR-ABL in PB significantly associated with CCyR in corresponding BM (all P<0.001). For BM samples with CCyR, there were significantly more corresponding PB samples with ≤1% ^{IS} than those with ≥2 log reduction of BCR-ABL (171/185 vs 134/185, 92.4% vs 72.4%, P<0.001). We further compared the molecular responses in our center to those of Germany CML Study IV on the premise of similar cytogenetic response: the proportions of patients with BCR-ABL^{IS} ≤1%, >1-10% and >10% in Germany trial were very similar to those with BCR-ABL^{IS}, but significantly different from those evaluated by log reduction method in our center.
Summary / Conclusion: The molecular response evaluated by BCR-ABL^{IS} has a similar trend in predicting PFS and response, but can better differentiate patients compared to that by log reduction method. Furthermore, the IS makes the molecular response results reported by the different laboratories comparable.

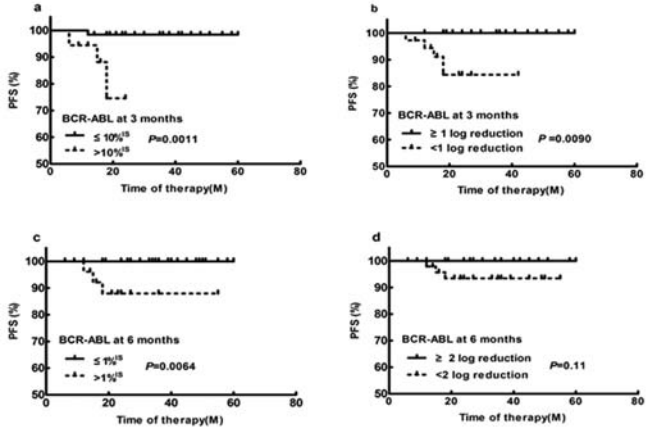

Figure 1.

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DISCONTINUATION OF BCR-ABL1 TYROSINE KINASE INHIBITOR IN CML PATIENTS WITH UNDETECTABLE MOLECULAR RESIDUAL DISEASE FOR AT LEAST 1 YEAR: INCLUDING UPDATED DATA FROM KIDS STUDY

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Background: The recent several reports to assess whether imatinib (IM) can be discontinued in chronic myeloid leukemia (CML) patients have shown that IM discontinuation can be employed based on clinical study in patients who had enough IM therapy and UMRD durations prior to IM discontinuation. However, further validation on precise indications for tyrosine kinase inhibitor (TKI) cessation is needed.
Aims: This study is performed to identify predictor of successful TKI discontinuation for CML patients achieving undetectable molecular residual disease (UMRD) for at least 1 year.
Methods: A total of 74 patients discontinued IM therapy, including 59 patients enrolled on the Korean Imatinib Discontinuation Study (KIDS) and 15 patients with TKI discontinuation due to patient's request (n=11), major surgery (n=2) and drug related adverse event (n=2) after achieving a UMRD. In this study, 23 patients with previous allogeneic stem cell transplantation were included. 71 patients received only IM; upon the intolerance of IM, 3 patients were given a new TKI (DAS = 1, NIL = 1, BOS = 1) before TKI discontinuation with achieving a UMRD for at least 1 year. For the patients enrolled on KIDS, molecular response was monitored using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assay every month up to 6 month follow-up, every 2 months up to 12 month follow-up, and every 3 months thereafter, whereas in real practice qRT-PCR was monitored normally every 3 months. The loss of MMR and UMRD were defined on 2 consecutive assessments, and if loss of MMR occurred, IM treatment was re-introduced.
Results: A total of 74 UMRD patients (including 34 men and 40 women) were analyzed. Their median age was 45 years (range, 19-74). The percentages of patients with low, intermediate and high Sokal risk scores were 38%, 31% and 18%, respectively with unknown Sokal risk scores in 14%. Prior to discontinuation, the median time to UMRD was 26.2 months (range, 0.5-104.6 months) and the median TKI duration was 86.5 months (range, 17.7-129.5 months) including 45.6 months (range, 13.6-105.7 months) of sustained UMRD.
After a median follow-up of 16.2 months since discontinuation of TKI, loss of MMR was observed in 12 non-transplant patients after a median time of 3.6 months (range, 1.9 - 7.6) of treatment discontinuation. Loss of UMRD was detected in 14 non-transplant patients. The 12-month probability of sustained MMR and UMRD were 80.3% and 76.6%, respectively. Probabilities for sustained MMR and UMRD were 62.8% and 68.6% in non-transplant group, respectively. 3 patients received with 2G TKI showed sustained MMR and UMRD. All 13 patients who lost MMR were re-treated with IM for a median of 12.1 months (range, 3.7 - 17.9 months). 11 of these patients re-achieved MMR at a median of 1.8 months (range, 0.9 - 2.8 months) after resuming IM therapy and 7 of these patients re-achieved UMRD at a median of 5.6 months (range, 2.8 - 12.1 months). Univariate analysis of factors affecting loss of MMR showed that TKI duration and UMRD duration before treatment discontinuation had a higher 12-month probability of sustained MMR.
Summary / Conclusion: Although probability of sustained molecular response is relatively lower than those of our previous KIDS, our data suggested TKI may be discontinued in CML patients with undetectable molecular residual disease for at least 1 year, with utilizing increasingly sensitive PCR technology. To make more concrete conclusion, further clinical investigation on a large patient population and much longer follow-up are needed.

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COMBINATION OF EUTOS SCORE AND 3-MONTHS BCR-ABL TRANSCRIPT LEVEL IDENTIFIES A DISTINCT SUBGROUP OF ECP-CML PATIENTS WITH HIGH RISK OF NON OPTIMAL RESPONSE AND IMATINIB DISCONTINUATION

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Background: Response to TKI is considered the strongest predictor of long-term outcome in CML patients. Effective treatment overcomes the negative impact of most prognostic factors, including Sokal and Hasford scores and recent data demonstrated that an early molecular response is strictly related to the outcome. In fact, missing the 10% BCR-ABL landmark at 3 months predicts inferior long-term survival. The 'EUTOS Score' combines baseline spleen size and peripheral blood basophils to foresee the achievement of complete cytogenetic remission (CCyR) and progression-free survival (PFS). Since its efficacy is still matter of debate, the predictive power of EUTOS in discriminating poor risk patients could be improved if associated with early molecular response assessment.
Aims: We tested whether a combination of EUTOS score and 3-months BCR-ABL transcript level identifies a poor prognosis population of CML patients.
Methods: 148 ECP CML patients treated with front-line standard dose imatinib (400 mg daily) at 5 major hematological centres in the north-eastern area of Italy were evaluated. Partial cytogenetic response (PCyR) and complete cytogenetic response (CCyR) were defined as 1–35% and 0% Ph+ metaphases, respectively; major molecular response (MMR) was defined as BCR-ABL <0.1%IS. For the purpose of the analysis patients were stratified into 3 subgroups according to EUTOS and 3-months molecular response ("good": low EUTOS score and ≤10%IS BCR-ABL transcript level; "intermediate": high EUTOS score or >10%IS BCR-ABL; "poor": high EUTOS score and >10%IS BCR-ABL). TTF was measured from the start of imatinib to the date of any of the following events: progression to accelerated or blastic phase, death for any cause at any time, primary or secondary hematologic or cytogenetic resistance leading to imatinib discontinuation. PFS was measured from the start of imatinib to the date of progression to accelerated or blastic phase or death for any cause at any time. Survival probabilities were estimated by the Kaplan-Meier method and compared by log rank test; differences among variables were evaluated by the Chi-squared test or by Cochran–Mantel–Haenszel test.

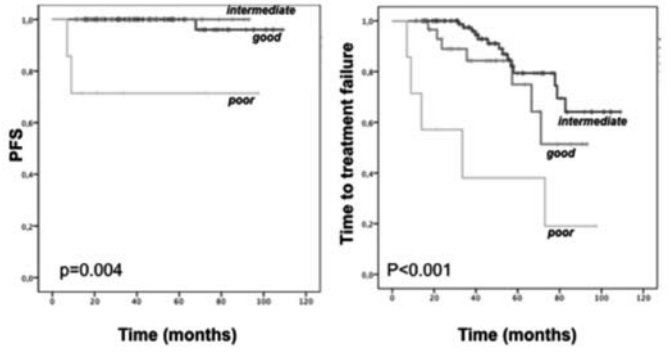

Figure 1. Progression-free survival and time to imatinib treatment failure according to aggregated EUTOS score-3 months molecular response patients stratification.

Results: The median age was 55 years (range 19–84), with 80 males and 68 females. The median follow-up was 42.7 months (range 11–109). The median time from diagnosis to imatinib therapy was 0.9 months (range 0-7.9). The distribution according to the EUTOS score was: 132 patients (89.2%) low risk; 16 patients (10.8%) high risk ; 3-months BCR-ABL transcript was ≤10% in 114 (79.2%) patients, >10% in 30 (20.8%). Patients with "good risk", "intermediate risk" and "poor risk" profile were 72.1%, 22.1% and 4.7%, respectively. The "optimal response" endpoints to imatinib were: 6 months PCyR: 91.7% vs 76.7% vs 42.9%; 12 months CCyR: 91.8% vs 65.4% vs 28.6%; 18 months MMR: 76.7% vs 27.3 vs 25% (differences among groups were significant except for intermediate vs poor profile). Imatinib discontinuation rate for failure was significantly different between poor risk group compared with intermediate and low risk (71.4% vs 21.2% and 13%; P=0.008, P<0.0001 respectively), as well as PFS and TTF (Figure 1). Remarkably, all progressions to blast crisis were observed in "poor risk" group.

Summary / Conclusion: Combination of High EUTOS score and 3-months BCR-ABL transcript level higher than 10% IS identifies a subpopulation of CML patients with significant probability of treatment failure and poorer outcome. Early switch to alternative treatment strategies (including allo-BMT) should be considered for these patients.

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EFFICACY OF NILOTINIB VERSUS HIGH-DOSE IMATINIB IN EARLY CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS WHO HAVE SUBOPTIMAL MOLECULAR RESPONSES TO STANDARD-DOSE IMATINIB: MULTICENTER RE-NICE STUDY

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Background: Achievement of major molecular response (MMR) is a significant prognostic factor in chronic myeloid leukemia (CML) as it has been shown to be associated with longer duration of complete cytogenetic response (CCyR) and long-term progression-free survival (PFS). In IRIS study, patients who achieved both CCyR and MMR showed a higher PFS, compared to those who had CCyR without MMR. Compared to standard dose of imatinib, higher doses of imatinib and second-generation tyrosine kinase inhibitor are expected to yield higher CCyR and MMR rates in patients with CP CML who are resistant to imatinib.
Aims: In this study, the efficacy of nilotinib and high-dose imatinib was investigated in suboptimal molecular response patients who received first line imatinib therapy at a daily dose of 400 mg.
Methods: Early CP CML patients who have achieved CCyR but no MMR after at least 18 months and up to 24 months on first line imatinib therapy at a daily dose of 400 mg were enrolled in this clinical trial, and informed consents were obtained prior to participation. In nilotinib arm, patients received 400 mg BID (800 mg/day), and patients received 400 mg BID (800 mg/day) in high-dose imatinib arm. To assess the drug efficacy, cytogenetic and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assays were performed at regular intervals, and baseline mutational analysis was conducted for every patient with subsequent mutational analyses to demonstrate either lack of response or disease progression. Primary endpoint is to evaluate the cumulative MMR rates by 12 months, and secondary endpoints are to evaluate the cumulative CMR rates and time to and duration of MMR and CMR during further 24 month follow-up. PFS and safety profiles will also be assessed.

Table 1. Characteristics of patients and autcomes.

Data cut-off date: 01 Feb 2013	RE-NICE		
	Total N=49	Nilotinib cohort N = 24	Imatinib cohort N = 25
Characteristics of patients			
Age, median (range)	37 (17-69)	45 (19-65)	36 (17-69)
Sex (F/M)	11/38	9/15	2/23
Transcript type (b3a2/b2a2)	30/19	8/16	11/14
Sokal risk, N (%)			
Low	21/49 (43)	11/24 (46)	10/25 (40)
Intermediate	18/49 (37)	10/24 (42)	8/25 (32)
High	8/49 (16)	2/24 (8)	6/25 (24)
Data not available	2/49 (4)	1/24 (4)	1/25 (4)
Time from Dx. To imatinib initiation (days)	10 (0-36)	10 (0-30)	10 (1-36)
Time from imatinib initiation to enrollment (mos)	18 (17-24)	18(18-22)	18 (17-24)
Time to achieve CCyR with imatinib therapy (mos)	6 (3-13)	8 (3-12)	6 (3-13)
Follow-up duration (mos)	24 (1-50)	24 (1-36)	25 (3-50)
Outcomes			
Cross-over during study†, N (%)	12/49 (24)	0/24 (0)	12/25 (48)
Cumulative incidence of MMR by 12 months*,%	-	43.7	32.7
MMR achievement by 12 months, N (%)	-	10/24 (42)	7/25 (28)
Overall MMR achievement‡, N (%)	-	17/24 (71)	8/25 (32)
Loss of MMR	0/49 (0)	0/24 (0)	0/25 (0)
Loss of CCyR	0/49 (0)	0/24 (0)	0/25 (0)
Progression to AP or BC	0/49 (0)	0/24 (0)	0/25 (0)
Death	0/49 (0)	0/24 (0)	0/25 (0)

‡ Overall 5 patients have achieve MMR.
* P=0.379
† Two patients cross-over before 12 months follow-up, ten patients cross-over after 12 months follow-up.

Results: With a data cut-off date of 01 Feb 2013, a total of 49 patients were randomized into nilotinib arm (n=24) or high-dose imatinib arm (n=25). With a median follow-up of 24 months (range, 1 - 50 months), all patients have maintained CCyR without progression to advanced disease, and decrease in BCR-ABL1 transcript level was observed. Cumulative MMR rates by 12 months were not significantly different between nilotinib arm compared to high-dose imatinib arm (43.7% vs. 32.7%, P=0.379). Four patients in nilotinib arm and two patients in high-dose imatinib arm achievedMR$^{4.0}$ by last follow-up. Twelve patients in high-dose imatinib arm crossed over to the nilotinib arm at a median of 14 months (range, 3-27 months) due to lack of response (n=10) and intolerance (n=2). Post-crossover, three patients have achieved MMR by 12 months at a median of 6 months (range, 3-12 months) and cumulative MMR rates was 27.8%. By last follow-up, five patients have achieved MMR at a median of 18 months (range, 3-33 months).
Overall, the patients treated with high-dose imatinib showed toxicities more frequently, such as leukopenia, thrombocytopenia, edema and decreased phosphate. Although toxicity was observed in both arms, all patients currently maintain the initial dose.
Summary / Conclusion: These preliminary results demonstrate that early switching to nilotinib or high-dose imatinib could be recommended in suboptimal molecular responders. When the tolerability of treatment was considered, switching to nilotinib may be preferred. Through further clinical investigation on a large patient population and longer period of observation, the efficacy of early intervention of suboptimal molecular response using nilotinib or dose escalation of imatinib will be assessed.

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OCT1 GENETIC VARIANTS ARE ASSOCIATED WITH LONG TERM OUTCOMES IN IMATINIB TREATED CML PATIENTS

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Background: Treatment with the tyrosine kinase inhibitor (TKI) imatinib has become the cornerstone of CML therapy and has markedly changed the outcome of this disease. While most patients in early chronic phase started on imatinib achieve major responses, longer follow up of the pivotal IRIS study has shown that only 63% of patients remaining on imatinib at 6 years were still in a CCyR.Currently, the only pre-treatment predictive features for treatment response are disease associated clinical scores including the Sokal and the recently introduced EUTOS scores. The human organic cation transporter 1 (OCT1) effectively mediates the active transport of imatinib into cells (influx pump), and its inhibition decreases the intracellular concentration of imatinib. Single nucleotide polymorphisms (SNP) in hOCT1 were previously reported to predict response and resistance to imatinib therapy.
Aims: To study whether hOCT1 genetic variants are associated with long term outcomes in CML.
Methods: We studied 4 non-synonymous genetic variants in hOCT1, rs683369, rs628031, rs12208357 and rs41267797 using the Sequenom MassARRAY platform (Sequenom, San Diego, CA, USA) in 167 CML patients. Frequency of the genetic variants was associated with clinical outcomes.
Results: Median age at CML diagnosis was 55 years (18-80), F/M 55/112, and most patients were diagnosed (76%) and treated (70%) with imatinib in CP. Overall, 93%, 74%and 56% of evaluable patients achieved CHR, CCyR and MMoR, respectively, during treatment with imatinib. Frequency of the different alleles in the study population was consistent with reported frequency in Caucasians according to the NCBI dbSNP database. There was no difference between the response rates when comparing between the different allele variants for each of the SNP studied. Sixty-six patients were studied for the presence of kinase domain mutation (KD) due to imatinib suboptimal response or failure, resulting in the discovery of KD mutations in 15 (23%). Patients with rs628031 AA/GA genotypes had a higher frequency of KD mutations compared to the GG genotype (8/16 vs. 5/27, P=0.04). With a median follow up of 80 months, the median EFS was 109 months and median OS was not reached for the entire cohort. Median EFS in CP was shorter in patients with the rare genetic variants (comparing heterozygous + homozygous minor allele frequency with homozygous major allele frequency) for all studied SNPs, with statistical significance for rs628031 genotype AA/AG compared with the GG genotype (EFS 61 months and not reached, respectively, P=0.03) and borderline significance for rs12208357 genotype CT compared with CC (EFS 58 and not reached, respectively, P=0.09). In multivariate analysis rs628031 genotype remained an independent predictor for EFS with a hazard ratio of 1.9, 95% CI 1-3.8, P=0.006. There was a trend for lower OS for patients with the AA/AG rs628031 genotypes compared with GG genotype (P=0.09).
Summary / Conclusion: hOCT1 rs628031 AA and AG genotypes are associated with worse long term outcomes in CP CML. Consideration should be given to incorporation of genotypic variables into pretreatment risk stratification of CP CML patients. With availability of several TKIs for first line therapy in CML, this data could potentially assist in the decision to choose one TKI over the other in the specific patient.

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ASSESSMENT OF EARLY CYTOGENETIC RESPONSE AS A PREDICTOR OF LONG-TERM CLINICAL OUTCOMES IN A PHASE 1/2 STUDY OF BOSUTINIB IN CHRONIC PHASE (CP) CHRONIC MYELOID LEUKEMIA (CML)

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Background: Bosutinib (BOS) is an oral, dual Src/Abl tyrosine kinase inhibitor approved for the treatment of Ph+ CML following resistance/intolerance to prior therapy.
Aims: This retrospective landmark analysis investigated early response to BOS as a predictor of long-term outcomes in CP CML patients (pts) receiving BOS as second-line (CP 2L; after imatinib [IM] only) or third/fourth-line (CP 3L; after IM + dasatinib [DAS] and/or nilotinib [NIL]) therapy.
Methods: In this phase 1/2, open-label trial, pts aged ≥18 y with CP CML received oral BOS starting at 500 mg/d. Pts evaluable for cytogenetic response (CyR) had received ≥1 BOS dose and had a valid baseline assessment. Pts evaluable for molecular response (MR) had received ≥1 BOS dose; pts in China, India, Russia, and South Africa were not evaluable due to logistical constraints. MR was not evaluated using the International Scale. *P* values for association of response with cumulative incidence distribution of progression or death (P/D; including lack of efficacy) were based on Gray's test; *P* values for association of response with overall survival (OS) distribution were based on log-rank test. P values >0.05 were considered not statistically significant.

Table 1.

	Cumulative progression/death			Overall survival		
Response	n evaluable	Probability at 24 mo (95% CI)	*P* value	n evaluable	Probability at 24 mo (95% CI)	*P* value
CP 2L; by 3 mo						
MCyR	95	12% (7-21)	0.002	96	98% (92->99)	0.005
No MCyR	157	25% (19-33)		186	88% (82-92)	
CP 2L; by 6 mo						
MCyR	119	14% (9-21)	<0.001	126	97% (92-99)	0.011
No MCyR	101	27% (20-37)		151	88% (82-93)	
CP 2L; by 9 mo						
MCyR	129	13% (9-21)	<0.001	141	96% (92-99)	0.009
No MCyR	80	28% (19-39)		134	89% (82-93)	
CP 3L; by 3 mo						
MCyR	28	21% (11-44)	0.049	28	88% (68-96)	0.232
No MCyR	67	39% (29-53)		87	86% (76-92)	
CP 3L; by 6 mo						
MCyR	36	19% (10-38)	0.018	40	92% (77-97)	0.027
No MCyR	38	42% (29-62)		72	84% (73-91)	
CP 3L; by 9 mo						
MCyR	34	15% (7-33)	0.026	40	95% (80-99)	0.022
No MCyR	28	36% (22-59)		68	88% (77-94)	

Results: A total of 288 CP 2L pts and 119 CP 3L pts were enrolled. Median BOS duration was 22.1 mo (range 0.2-60.8) for CP 2L pts and 8.6 mo (range 0.2-60.8) for CP 3L pts. Time from the last enrolled pt's first dose to the data cutoff was 24 mo; median follow-up duration was 31.8 mo (range 0.6-66.0) for CP 2L pts and 31.4 mo (range 0.3-66.0) for CP 3L pts. Among 266 evaluable CP 2L pts, a major CyR (MCyR) was newly attained or maintained from baseline by 157 (59%) pts, including 128 (48%) pts with a complete CyR (CCyR). A MCyR was attained/maintained by 45/110 evaluable CP 3L pts, including 35 (32%) pts with a CCyR. The Kaplan-Meier probability of maintaining a MCyR at 2 y was 77% (95% CI 69-83) for CP 2L pts and 71% (54-83) for CP 3L pts. A major MR (MMR) was achieved by 69/200 (35%) evaluable CP 2L pts and 17/106 (16%) evaluable CP 3L pts. In CP 2L pts, attaining/maintaining a MCyR by Month3, 6, 9, and 12 on BOS versus no MCyR was significantly associated with a lower cumulative incidence of on-treatment P/D and longer OS (*Table*; Month 12 not shown). Similar results were observed when the analysis evaluated patients attaining/maintaining a CCyR versus partial cytogenetic response versus no MCyR separately. In CP 3L pts, a borderline significant association between attaining/maintaining a MCyR versus no MCyR by Month 3 was found for on-treatment P/D, but not OS; however, both analyses achieved significance at later response time points (Table 1). Achievement of a MMR by Month 3 versus no MMR was not predictive of on-treatment P/D in CP 2L and CP 3L pts, although a numerically lower cumulative incidence was observed (CP 2L, 5% [95% CI 1-34] vs 20% [15-28]; CP 3L, 11% [2-71] vs 35% [26-48]). There was also no significant association between response by Month 3 and OS in the CP 2L (100% [not estimable to 100] vs 90% [84-93]) and CP 3L cohorts

(75% [32-93] vs 88% [79-93]).
Summary / Conclusion: Early attainment or maintenance of a MCyR by CP 2L pts was associated with a decreased likelihood of on-treatment P/D and better OS. Among CP 3L pts, association with long-term outcomes was borderline for MCyR by Month3, but significant for MCyR by Months6, 9, and 12. Achievement of MMR by Month 3 was not predictive of long-term outcomes in either cohort, perhaps due to the fewer number of pts with MMR, thus increasing variability in the long-term estimates.

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DISCONTINUATION OF IMATINIB (IM) IN PEDIATRIC CHRONIC MYELOID LEUKEMIA (CML) PATIENTS WITH SUSTAINED COMPLETE MOLECULAR REMISSION (CMR)

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Background: Results from the Stop IMatinib (STIM) trial suggest that IM may be discontinued in adult CML patients with long-lasting CMR.
Aims: The aim of this study was to assess the possibility of IM discontinuation in pediatric CML patients with sustained CMR.
Methods: From March 2001, 14 pediatric CML patients in chronic phase (CP) (7 M and 7 F), with a median age at diagnosis of 11 years (range: $5^{11/12}$–$17^{10/12}$) were treated with IM at a dosage of 340 mg/m^2/day, according to the local guidelines for children and adolescents with CML. Three patients had an available HLA identical sibling and 6 had previously received alpha-Interferon. Cytogenetic analysis was performed on bone marrow (BM) before and during IM therapy, at planned intervals, while FISH was carried out at complete cytogenetic response (CCyR); quantitative RT-PCR was assessed on peripheral blood (PB) monthly and on BM every 3 months, according to the European LeukemiaNet recommendations for minimal residual disease quantification. Major molecular response (MMR) is defined as ≤0.1% BCR-ABL IS, while CMR is considered as ≤0.01% BCR-ABL IS. Minimum follow-up is 18 months.
Results: The dosage of IM was modulated according to hematologic toxicity and/or appearance of WHO≥2 side-effects, mostly during the first 6 months of treatment (median administered dose: 250 mg/m^2/day). Two patients (14%) stopped IM after 32 days and 2 months, respectively, because of severe extrahematological toxicity. A CCyR was achieved in all 12 evaluable patients after a median time of 6 months (range 3-12 months). As shown in Figure 1, 10/11 evaluable patients (91%) achieved a molecular response (CMR or MMR), including two with a HLA identical sibling, both on PB and BM. Seven out of 11(64%) evaluable patients obtained a CMR both on PB and on BM after a median time of 14 months (range: 8-41) and 15 months (range: 12-44), respectively. Since December 2007, with the aim of reducing the risk of bone alterations and longitudinal growth impairment[1], 6 patients with sustained CMR (≤0.0032% BCR-ABL IS) and 1 adolescent with MMR long-lasting >12 months received IM at a same daily dosage for 3 weeks a month (intermittent IM). After 96, 95 and 93 months of treatment, IM was discontinued in 3 patients (including one with a HLA identical sibling) in CMR (≤0.0032% BCR-ABL IS) for 81, 73 and 72 months, respectively. After IM discontinuation, all patients continued to be monitored by quantitative RT-PCR on PB monthly and on BM every 6 months. As of February 2013, all of them continue to be in CMR (≤0.0032% BCR-ABL IS) without any treatment after 25, 25 and 46 months from IM discontinuation, respectively.
Summary / Conclusion: These results suggest that IM can be safely discontinued in pediatric CML patients with a CMR (≤0.0032% BCR-ABL IS) lasting more than 6 years. International cooperative studies on a lager cohort of pediatric patients are necessary to confirm these promising results which raise the possibility that children with CML might be cured with tyrosine kinase inhibitors alone.

Reference

1. Giona F. *et al*. Bone metabolism, growth rate and pubertal development in children with chronic myeloid leukemia treated with imatinib during puberty. Haematologica. 2013 Mar;98(3):e25-7.

P150

MECHANISM OF IMPAIRED GLUCOSE METABOLISM DURING NILOTINIB THERAPY IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA

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Background: Hyperglycemia represents frequent adverse event reported in chronic myelogenous leukemia (CML) patients treated with nilotinib.
Aims: To determine the major mechanism of glucose metabolism impairment, we performed a metabolic analysis using an oral glucose tolerance test as well as assessment of incretins and adipokines at baseline and after 3 months of nilotinib treatment in patients with CML.
Results: 10 patients were included in our study. 40% received nilotinib as first line, and 60% as second or subsequent line. 50% of patients received 600 mg and 50% received 800 mg of nilotinib/day. The median nilotinib C_{trough} was 1510 (411-2973) ng/ml, median BMI was 27.2 (23.4-32.7) kg/m^2, and median waist circumference was 93.5 (77.0-108.0) cm. Fasting, 1-, and 2-hour plasma glucose concentrations obtained during the OGTT significantly increased after 3 months of nilotinib therapy (Table). 2 patients fulfilled criteria of DM and 2 displayed IGT based on the OGTT. Nilotinib administration significantly increased fasting insulinaemia (Table 1).

Table 1.

	Start median (range)	Month 3 median (range)	p
Fasting glucose [mmol/l]	5.25 (4.8 - 6.1)	5.8 (5.2 - 12.7)	**0.009**
Fasting insulin [µIU/ml]	16.6 (10.8 - 69.0)	27.8 (17.2 - 61.9)	**0.049**
Fasting C-peptide [pmol/ml]	0.87 (0.54 - 2.40)	0.94 (0.55 - 2.21)	0.275
Fasting HbA1c [%]	3.80 (3.4 - 4.2)	3.9 (3.2 - 4.7)	0.725
Incretins			
Fasting GLP-1[pM]	5.4 (4.7 - 6.5)	5.3 (4.6 - 6.4)	0.106
2-hour stimulated GLP-1 [pM]	6.3 (4.7 - 10.5)	6.4 (4.5 - 10.5)	0.160
Fasting GIP [pg/ml]	49.5 (22.9 - 120.5)	58.5 (14.6 - 104.2	0.922
2-hour stimulated GIP [pg/ml]	236.3 (47.2 - 332.2)	150.2 (73.8 - 270.8)	0.232
Adipokines			
Fasting FABP [ng/ml]	15.7 (5.3 - 37.5)	17.3 (5.6 - 55.3)	0.241
Fasting adiponectin [mg/l]	13.8 (0.7 - 45.1)	7.8 (1.0 - 22.3)	**0.027**
Serum lipids			
Total cholesterol [mmol/l]	4.75 (3.5-6.6)	5.35 (4.5-7.0)	**0.013**
Triglycerides [mmol/l]	1.03 (0.58-2.8)	1.29 (0.6-3.0)	0.432
HDL cholesterol [mmol/l]	1.50 (0.7-2.2)	1.45 (0.8-2.3)	0.152
LDL cholesterol [mmol/l]	2.55 (1.6-4.4)	2.80 (2.3-4.8)	**0.020**
Non-HDL cholesterol [mmol/l]	2.90 (1.9-5.3)	3.45 (2.6-6.0)	**0.011**

Moreover, there was also a trend of higher postprandial insulinaemia obtained during OGTT at the 3rd month of treatment (Table 1). C-peptide concentrations (fasting and during OGTT) and fasting HbA1C did not significantly change (Table 1). Insulin resistance as calculated by HOMA2-IR, significantly increased during nilotinib therapy (P=0.008). Moreover, the derived insulin sensitivity index HOMA2
Summary / Conclusion: We proved that rapid insulin resistance, compensatory hyperinsulinaemia, and hypoadiponectinaemia develop after initiation of nilotinib therapy, which clarifies not only the mechanism of impaired glucose metabolism, but also at least partially explains the fast development of dyslipidaemia and peripheral artery occlusion in nilotinib-treated CML patients.

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EVALUATION OF CROSS-INTOLERANCE BETWEEN BOSUTINIB AND PRIOR TYROSINE KINASE INHIBITOR THERAPY IN PATIENTS WITH PHILADELPHIA CHROMOSOME–POSITIVE (PH+) LEUKEMIA

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Background: Bosutinib (BOS) is an orally active, dual Src and Abl tyrosine kinase inhibitor (TKI) approved in the United States for treatment of Ph+ chronic myeloid leukemia (CML) following resistance or intolerance to prior therapy. Prior reports indicated the BOS toxicity profile is primarily characterized by hematologic adverse events (AEs), gastrointestinal AEs, and rash.
Aims: To evaluate the potential for cross-intolerance between BOS and prior TKI therapy.
Methods: In this phase 1/2 study, BOS was evaluated at a starting dose of 500 mg/day in patients (pts) with chronic phase (CP) CML or advanced leukemia (ADV; accelerated/blast phase CML or Ph+ acute lymphoblastic leukemia) after imatinib (IM) only or after IM plus dasatinib (DAS) and/or nilotinib (NIL). Pts were evaluated for cross-intolerance (AEs leading to permanent treatment discontinuation of both BOS and prior TKI therapy) and subsequent occurrence of

these AEs on BOS.
Results: Median (range) duration of BOS treatment was 24.6 (0.2-72.3) mo for 286 2nd-line CP CML pts (prior IM only), 8.6 (0.2-60.8) mo for 119 3rd/4th-line CP CML pts, and 4.0 (0.03-63.8) mo for 165 2nd- to 4th-line ADV pts. The *Table* lists individual AEs leading to discontinuation from prior TKI therapy in ≥5 pts, along with subsequent occurrence of these AEs on BOS (gr 3/4 AE or leading to BOS discontinuation). Intolerance to prior IM was reported for 122 CP CML and 50 ADV 2nd- to 4th-line pts. The most common AEs associated with IM intolerance were cytopenias; 29/57 (51%) CP CML pts and 4/9 (44%) ADV pts with IM intolerance due to cytopenias experienced the same grade 3/4 event on BOS, while cross-intolerance due to cytopenias occurred in 12/57 (21%) CP CML pts and 2/9 (22%) ADV pts. Total numbers are lower for non-hematologic AEs commonly associated with IM intolerance (*Table*). Although diarrhea is the most frequently reported AE on BOS, only 4/10 (40%) CP CML pts and 2/4 (50%) ADV pts with IM intolerance due to diarrhea subsequently experienced grade 3/4 diarrhea on BOS, and 2/10 (20%) CP CML pts and 0/4 ADV pts experienced true cross-intolerance due to diarrhea. Intolerance to prior DAS (after IM) was reported for 21 CP CML and 21 ADV 3rd/4th-line pts. The most common AEs associated with DAS intolerance were pleural effusion (n=19 CP CML, n=6 ADV) and thrombocytopenia (n=8 CP CML, n=5 ADV). In pts with DAS intolerance due to thrombocytopenia, grade 3/4 thrombocytopenia was experienced by all pts on BOS; however, cross-intolerance was observed in 4/8 (50%) CP CML pts and 1/5 (20%) ADV pts. Pleural effusion occurred on BOS; however, there was no cross-intolerance (Table 1). Neither of the 2 pts with DAS intolerance due to diarrhea (both CP CML pts) experienced cross-intolerance. An additional 2 CP CML and 5 ADV 3rd/4th-line pts had intolerance to prior NIL (after IM), including intolerance due to rash (n=3), thrombocytopenia (n=2), neutropenia (n=1), and headache and pleural effusion (n=1); however, none of these patients experienced cross-intolerance and discontinued BOS due to the same AE. No deaths due to the AEs leading to prior TKI discontinuation were reported on BOS in pts with cross-intolerance.
Summary / Conclusion: Hematologic cross-intolerance between BOS and recent IM or DAS therapy was relatively low among both CP CML and ADV pts, although many pts experienced the same grade 3/4 cytopenia on BOS. Cross-intolerance due to nonhematologic AEs, including diarrhea, was rare. These results suggest that most pts intolerant to prior TKI therapy will tolerate long-term therapy with BOS.

Table 1.

Reason for intolerance	n	Experienced same grade 3/4 AE on BOS	Discontinued BOS due to same AE
CP CML – IM intolerance	122	39 (32%)	20 (16%)
Thrombocytopenia	29	19 (66%)	7 (24%)
Neutropenia	19	6 (32%)	3 (16%)
Rash	18	2 (11%)	1 (6%)
Anemia	14	3 (21%)	0
Edema	12	0	0
Diarrhea	10	4 (40%)	2 (20%)
Bone marrow failure	7	5 (71%)	4 (57%)
Fatigue	7	0	1 (14%)
Myalgia	5	1 (20%)	0
Vomiting	5	1 (20%)	1 (20%)
CP CML – DAS intolerance	50	16 (32%)	7 (14%)
Pleural effusion	19	3 (16%)	0
Thrombocytopenia	8	8 (100%)	4 (50%)
Pancytopenia	5	0	0
ADV – IM intolerance	21	6 (29%)	2 (10%)
Thrombocytopenia	5	4 (80%)	2 (40%)
ADV – DAS intolerance	21	10 (48%)	1 (5%)
Pleural effusion	6	2 (33%)	0
Thrombocytopenia	5	5 (100%)	1 (20%)

Hodgkin Lymphoma

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FLOW CYTOMETRY EARLY DETECTION OF CD4+CD26-CD38+ LYMPHOCYTES SUBSET IN LYMPH NODES OF HODGKIN LYMPHOMA

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Background: Hodgkin lymphoma is classically histological diagnosed by a minority of neoplastic cells, the Hodgkin and Reed-Sternberg cells, within a typical inflammatory microenvironment. It is now recognized as the majority of these T CD4 cells are T regulatory and they play an important role in the suppressing effectors and contribute to tumor persistence.
Aims: During this 12-year period our first purpose was to analyze the non-clonal lymphoid, inflammatory and infiltrating cells in HL (108) and to assess the expression of CD26 on the T CD4 total population with other markers of activation such as CD38 with the scope to identify the $CD4^{+}CD26^{dim/neg}CD38^{+}$ subset specific of HL.
Methods: We performed lymphocyte immunophenotyping by flow cytometry from lymph node samples suspected of lymphoma during a 12-year period (2000-2012), to early identify Hodgkin-specific subset and potential biomarkers related to T regulatory cells. We measured CD3, CD19 and the T CD4+CD26-CD38+ subset in lymphocytic infiltrate of 108 lymph nodes, concurrently histological diagnosed as Hodgkin lymphomas and in 43 benign reactive lymphoid hyperplasia. The same subsets were analysed into the peripheral blood of a proportion of both groups and in 20 healthy blood donors as controls.
Results: Compared to benign reactive lymphoid hyperplasia, Hodgkin lymphoma shows statistically significant differences between the two reactive micro environmental populations: decreased CD19+ cells (39% vs 23%; P<0.001), increased CD3+ (58%; vs 74%; P<0.001) and CD4+CD26-CD38+ cells (11,5% vs 38%; P<0.001). Using the co expression markers CD38 and CD26 for ROC curve analysis (area = 0,8639), results confirm that CD4+CD26-CD38+ subset is strongly expresses in HL.
Summary / Conclusion: Although flow cytometry analysis is not routinely applied in lymphomas our findings suggest it may be useful in the characterization of the cellular para-neoplastic inflammatory background of Hodgkin lymphoma. Detection of CD4+CD26-CD38+ cells by flow cytometry seems able to identify in a quick and easy way, different cellular patterns to distinguish Hodgkin lymphoma from benign reactive lymph nodes as well as to explore the activity of T regulatory cells.

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FIRST-LINE TREATMENT OF ADVANCED STAGE HODGKIN LYMPHOMA – FINAL RESULTS OF A SYSTEMATIC REVIEW AND NETWORK META-ANALYSIS

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Background: Hodgkin lymphoma (HL) in advanced stages can nowadays be cured with different combined-modality approaches, but the debate whether $BEACOPP_{escalated}$ (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone) or ABVD (doxorubicin, bleomycin, vincristine, dacarbazine) is superior is still ongoing. ABVD results in a lower progression-free survival, but might be better tolerable than $BEACOPP_{escalated}$. With regard to the most important patient-related outcome overall survival (OS) no high-level evidence supporting one or the other strategy has been generated up to date.
Aims: To assess the efficacy (OS, freedom from treatment failure, FFTF) and safety (secondary neoplasia) of different first-line treatment strategies over standard ABVD and to provide a hierarchy of the regimens for patients with advanced stage HL.
Methods: We developed sensitive search strategies for CENTRAL, MEDLINE, and conference proceedings from 01.1980 to 09.2012, additionally, we obtained missing data from investigators. Two authors independently screened search results, extracted data, and assessed quality of trials. We pooled data using network meta-analysis and combined direct with indirect comparisons with Bayesian random-effects models. Results were reported relative to ABVD, indicating superiority of ABVD if hazard ratio (HR) > 1.
Results: The search resulted in2,229 relevant references, of which 74 publi-

cations with 14 randomised controlled trials evaluating eleven different regimens were included. Overall, we judged the methodological quality of trials as high. Six cycles $BEACOPP_{escalated}$ (HR = 0.38, 95% credible intervals (CrI) 0.20 to 0.75) and eight cycles BEACOPP-14 (HR = 0.43, 95% CrI 0.22 to 0.86) were associated with the lowest risk of mortality and showed a 98% probability to be the best treatment regimens for patients with advanced HL (see forest plot of overall survival). Additional standard meta-regression estimated an 89% five-year survival rate for ABVD, resulting in a five-year survival benefit of 7% for both regimens: six cycles $BEACOPP_{escalated}$ (95% CrI 3% to 10%) and eight cycles BEACOPP-14 (95% CrI 2% to 9%) as compared to ABVD. These results were confirmed by the reconstructed digitised individual patient analysis, that included 10,042 patients and 1,189 deaths over 47,033 patient-years of follow-up. OS was increased by 10% (95% CI 3% to 15%) at five years with six cycles $BEACOPP_{escalated}$ compared to ABVD.

Freedom from treatment failure showed similar results: six cycles $BEACOPP_{escalated}$ have a 66% probability to be the best treatment regimen (HR = 0.37, 95% confidence interval (CI) 0.12 to 1.08) and eight cycles BEACOPP-14 have a probability of 15% to be the best (HR = 0.51, 95%CI 0.16 to 1.48). Overall, the assessment of between-trial heterogeneity was low: τ^2=0.01 for OS and τ^2=0.05 for FFTF.

Data for secondary malignancies was provided by twelve trials with 50,736 patient-years of follow-up. A total amount of 327 secondary malignancies and 109 leukaemias occurred. The low number of included trials led to wide overlapping credible intervals which additionally included the null effect. The low number of events made quantification of the risks associated with each regimen impossible.

Summary / Conclusion: This network analysis of different first-line treatment strategies for patients with advanced stage HL has shown a relevant benefit for FFTF and OS with six cycles $BEACOPP_{esclated}$ and eight cycles BEACOPP-14 as compared to standard ABVD. The OS difference to ABVD is not only highly significant but also relevant for patients with advanced stage HL.

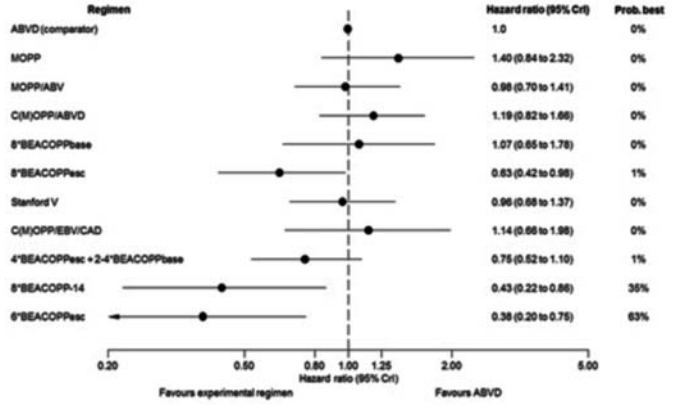

Figure 1.

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UNMANIPULATED HAPLOIDENTICAL BONE MARROW TRANSPLANTATION FOLLOWING NON MYELOABLATIVE CONDITIONING AND POST-TRANSPLANTAT CYCLOPHOSPHAMIDE FOR ADVANCED HODGKIN'S LYMPHOMA

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Background: Patients with Hodgkins Disease (HD) not responding to or relapsing soon after first line therapy, are at high risk of failure: typically these patients receive multiple lines of therapy , including an autograft, and are then considered for an allogeneic transplant. The Baltimore group has described encouraging results in advanced HD patients, using haplo-identical family donors (HAPLO) and high dose post-transplant cyclophosphamide (PT-CY) fro graft versus host disease (GvHD) prophylaxis.

Aims: We have reported 2 years ago our initial experience in advanced HD patients grafted from HAPLO donors: the aim of this study is to update that initial experience with a longer follow up .

Methods: Twentysix patients with advanced HD, received a unmanipulated bone marrow transplant (BMT) from a HAPLO family donor, following a non myeloablative conditioning , including fludarabine, low dose cyclophosphamide and 2 Gy total body irradiation. All patients received PT-CY, mycophenolate and a calcineurin inhibitor for GvHD prophylaxis. All patients had received a previous autograft, and 65% had active disease at the time of BMT.

Results: Sustained engraftment of donor cells occurred in 25 patients (96%), with a median time to neutrophil recovery (>0.5x10^9/L) and platelet recovery (> 20x10^9/L) of +18 and +23 days from BMT. The incidence of grade II – IV acute GVHD and of chronic GVHD was 24% and 8%. With a median follow up of 24 months (range 18 – 44) 21 patients are alive, 20 disease free. The cumulative incidence of transplant related mortality (TRM) and relapse was 4% and 31% The actuarial 3 year survival is 77%, the actuarial 2 year progression free survival is 63%.

Summary / Conclusion: In conclusion we confirm that high dose PT-CY is effective as prophylaxis of GVHD after HLA haploidentical BMT, can prevent rejection and does not appear to eliminate the allogeneic graft versus lymphoma effect.

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A SIMPLE, ACCESSIBLE NEW TOOL TO DEFINE HIGH RISK PATIENTS IN CLASSICAL HODGKIN LYMPHOMA BASED ON VALIDATION OF PRE-EXISTING BIOMARKERS

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Background: Identifying patients with classical Hodgkin lymphoma (CHL) who will fail conventional therapy is essential to guide treatment, but historic scoring systems (e.g. International Prognostic Score/IPS) fail to stratify patients adequately and few patients fall into higher risk categories. The immune microenvironment of a tumour and dysfunctional systemic immunity are key components of cancer pathogenesis, and recent biomarkers of outcome in CHL have confirmed this. Many novel markers have been proposed but fail to enter clinical use due to complexity or expense of technology (e.g. gene expression profiling) or failure to validate markers in independent datasets. The adverse impact of monocytes/macrophages on tumour progression is well described in CHL. A Mayo clinic study (Porrata *et al*. Haematologica 2012; 97(2): 262-9) reported a ratio of absolute lymphocyte and monocyte counts (ALC/AMC) at diagnosis to be associated with poorer clinical outcomes. Validation using their designated cutpoint of 1.1 in independent patient cohorts has not been published to our knowledge.

Aims: We set out to validate routine markers of immune function, some already incorporated into prognostic strategies (albumin, white cell count and ESR), as well as the Mayo ALC/AMC score, and derive optimal cutpoints on other markers (beta-2-microglobulin/B2MG, C-reactive protein/CRP, Ferritin, IgG & IgM) in a new patient cohort. Since interim PET proved to be the most robust early indicator of outcome, this was also assessed in our cohort.

Methods: All HIV negative patients aged >16 years treated at Bart's Hospital London between 2005 and mid-2012 were included, all of whom received ABVD as first line, with standard salvage and intention to treat with BEAM/autologous stem cell rescue in the case of first line treatment failure. The final cohort comprised 88 patients, 48% male, age 16-65 (median 32), median follow up 3.5 years (0.24-8 yrs), 52% advanced stage. Pre-published cutpoints were used for ALC/AMC (1.1) and IPS indices and for ESR (30 & 50mm/hr) for early unfavourable disease according to the German Hodgkin Study Group (GHSG). The xtile software package determined cutpoints for other markers. Survival differences were determined using the Kaplan Meier (KM) method, reported as 3-year overall (OS) and progression-free survival (PFS). Statistically significant differences between groups were determined using the log rank method on pre-determined cutpoints, or corrected based on xtile test/validation methodology.

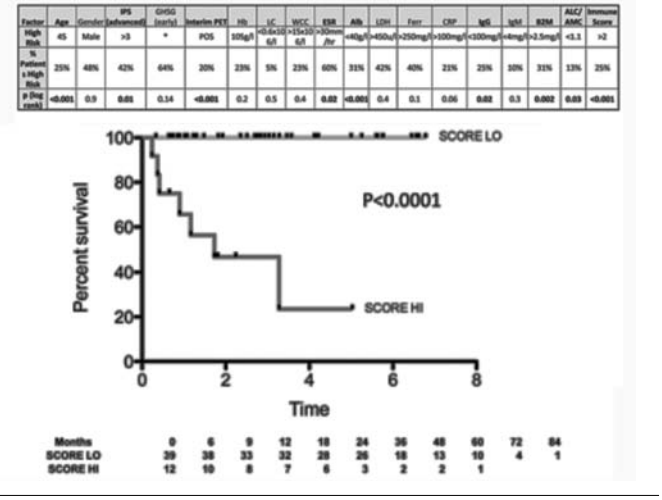

Figure 1. Risk factors assessed with p values according to univariate analysis and resulting "immune score" stratifying all patients.

Results: There were 26 progressions and 14 deaths in our cohort. Patients with early stage disease had 95% OS vs 70% with advanced. Performance of all prognostic scores and biomarkers in the univariate analysis is summarised in the Figure 1. Combining the laboratory biomarkers which significantly discriminated prognostic groups using cutpoints validated in, or derived from this cohort, we developed an 'immune status' prognostic score of adverse results: albumin<40 g/L, ALC/AMC<1.1, B2MG>2.5 mg/L, ESR>30mm/hr & IgG<100 mg/L. 2 groups were defined with 51 patients having a full complement of lab results. In the good risk group (score <3 factors) there were no deaths, and only 5 progressed, 4 of whom were successfully salvaged and are in remission after three years. In the poor risk group (score 3-5) all deaths were captured. KM analysis based on this stratification revealed the good risk group had 3 year PFS 82% and OS 100% and the poor risk group had PFS 39% and OS 43% (P<0.001).
Summary / Conclusion: We confirm limited prognostic ability of the GHSG and IPS systems, and excellent performance of interim PET. The most robust 'lab standard' biomarkers in our cohort were albumin, ESR, IgG, B2M and ALC/AMC ratio which combined into a highly discriminatory score predicting OS/PFS. This cheap, accessible, simple tool may be taken forward and validated in further patient cohorts.

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PHASE 1/2 STUDY OF BRENTUXIMAB VEDOTIN IN PEDIATRIC PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN LYMPHOMA OR SYSTEMIC ANAPLASTIC LARGE-CELL LYMPHOMA: INTERIM PHASE 1 SAFETY AND PHARMACOKINETIC DATA

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Background: Brentuximab vedotin is a CD30-targeted antibody conjugated by a protease-cleavable linker to a microtubule-disrupting agent, monomethyl auristatin E (MMAE). Data for brentuximab vedotin in children are encouraging, but limited. A retrospective analysis of 4 studies allowing enrollment of children with CD30-expressing malignant lymphomas showed that 9 pediatric patients (pts; median age 16 y; 5 Hodgkin lymphoma [HL]; 4 systemic anaplastic large-cell lymphoma [sALCL]) received brentuximab vedotin 0.8 or 1.2 mg/kg weekly for 3 of 4 weeks, or 1.2 or 1.8 mg/kg every 21 days (Q3wk). 6/9 pts (2/5 HL; 4/4 sALCL) had complete remissions (CR); 5/6 pts with CR remained in remission after 6–15+ months of follow-up. Adverse events (AEs) were manageable, with a safety profile similar to that documented in adult pts. No common Gr ≥3 events were observed; there were no discontinuations due to an AE. This study was designed to evaluate safety, pharmacokinetics (PK) and antitumor activity of brentuximab vedotin in pediatric pts.
Aims: The phase 1 portion of this phase 1/2, open-label, multicenter study aims to evaluate the safety, PK, and recommended phase 2 dose (RP2D) of brentuximab vedotin in children with relapsed or refractory (RR) CD30-expressing HL and sALCL (off-label use). Here we report preliminary phase 1 safety and PK data.
Methods: Consenting pts aged 2 to <18 years with any RR CD30-positive hematologic malignancy (5 to <18 years for HL) were eligible for phase 1. Pts received brentuximab vedotin by IV infusion Q3wk. The phase 1 starting dose was 1.4 mg/kg, escalated to 1.8 mg/kg following a 3+3 design. Blood samples for PK analysis were collected immediately before and 5 mins after the infusion on day 1 of all cycles; one sample was drawn on days2,3,5, 14 of cycles 1 and 8, and days2,3, 5 of cycle 2.
Results: 12 pts (median age 14.5 y [range 9–17]; 8M, 4F; 10 HL, 2 sALCL) received brentuximab vedotin (mg/kg/dose: 1.4, n=3; 1.8, n=9). The 1.8 mg/kg cohort was expanded from 6 to 9 pts to increase the total phase 1 pediatric experience to 12 pts before the phase 2 portion. At data cut-off, pts had received a median of 4 cycles (range, 1–10). 11 pts (92%) had ≥1 drug-related adverse event (DRAE): 2 at 1.4 mg/kg, 9 at 1.8 mg/kg. 5 pts (42%) had Gr ≥3 DRAEs: 1 at 1.4 mg/kg, 4 at 1.8 mg/kg. The most frequent (≥15%) treatment-emergent AEs were nausea (50%), abdominal pain, diarrhea (25% each), upper abdominal pain, cough, fatigue, hypoklaemia, leukopenia, decreased lymphocyte count, neutropenia, pain, paresthesia, pyrexia, vomiting, weight loss (17% each). 1 pt (8%) discontinued due to a DRAE of Gr 3 hepatotoxicity. 6 severe AEs were reported in 4 pts at 1.8 mg/kg: Gr 2 supraventricular tachycardia in 1 pt, unrelated to treatment; related Gr 3 febrile neutropenia and related prolonged Gr 3 hepatotoxicity in 1 pt (1.8 mg/kg; both dose-limiting toxicities); Gr 3 bronchospasm and Gr 2 laryngeal edema in 1 pt; 1 cardiac arrest resulting in death, unrelated to treatment. Median concentration-time profiles for brentuximab vedotin and MMAE are presented for the 2 dose cohorts.
Summary / Conclusion: Brentuximab vedotin was generally well tolerated in children with RR CD30-positive HL or sALCL up to 1.8 mg/kg Q3wk. For the majority of pts, toxicities were generally mild to moderate and did not lead to discontinuation. Similar to adults, 1.8 mg/kg is the RP2D for children with CD30-positive RR HL or sALCL. PK analyses showed dose-dependent exposure in these patients. The phase 2 portion of this study is ongoing.

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METABOLIC TUMOR VOLUME BY PET/CT AS A CLINICAL PARAMETER TO DETERMINE THERAPEUTIC MODALITY IN THE EARLY STAGE HODGKIN'S LYMPHOMA.

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Background: stage Hodgkin's lymphoma (HL). Although 70 to 80% of patients were cured with this treatment, radiation related late toxicities such as second cancer and cardiovascular event remained the serious complication. A current treatment standard for the early stage HL is combined modality treatment (CMT) including chemotherapy such as adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) regimen and involved-field radiation therapy (IF-RT). This treatment modality was an attempt to reduce the late toxicity by RT field and doses. However, it is unclear whether long term late toxicities were reduced with IF-RT. Recent several studies have suggested that ABVD therapy alone is a reasonable treatment option for favorable early stage HL. Positron emission tomography/computed tomography (PET/CT) is a promising methology to measure metabolic activity of active tumor burden in HL and Non-Hodgkin's lymphoma (NHL). We showed metabolic tumor volume (MTV) by PET/CT was an important clinical factor to reflect active tumor burden in NHL.
Aims: We investigated whether ABVD alone in early stage HL would lead to control of the disease similar to CMT using metabolic tumor volume (MTV) measured by PET/CT.
Methods: One hundred twenty seven patients with early stage HL between 2006 and 2011 in six medical center underwent PET/CT at diagnosis were enrolled. Treatment schedules were given as follows : 66 patients received six cycles of ABVD regimen. Other 61 patients received IF-RT (the dose, 30Gy) after four to six cycles of ABVD regimen. The median follow-up time was 47.8 months (range, 13.0-78.5 months). PET images were interpreted by nuclear physicians of the each institution. Then, the data were reviewed by a nuclear medicine expert in Pusan National University Hospital. MTV was delineated on the PET images by a pathologic ≥ standard uptake volume (SUV) 2.5 (Syntegra, version 2.1E, Philips Co.).

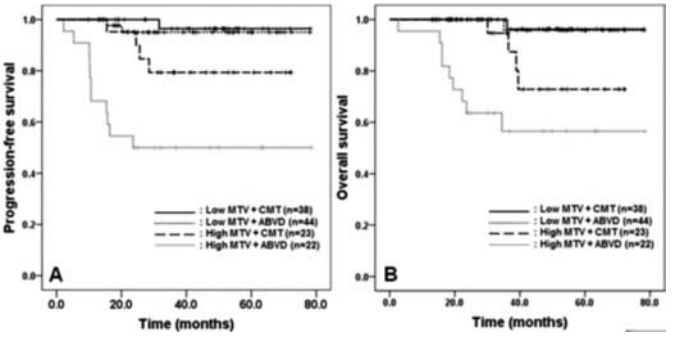

Figure 1.

Results: The male to female ratio was 1.4 : 1. Median age at diagnosis was 42 years (range, 18-78 years). Twenty five patients had B symptoms. Nodular sclerosis subtype was the predominant histology (55 patients), with 41 patients presenting with mixed-cellularity, 20 with lymphocyte-rich and 11 with lymphocyte-depleted subtype. Twenty-seven patients were stage I and 100 patients were stage II. Seventy-eight patients had more than 3 involved sites and 19 patients had mediastinal disease. Bulky disease was present in 27 patients.
The cut-off value of MTV was calculated by ROC curve analysis and the ideal value was 198 cm^3. The clinical outcomes were compared according to several prognostic factors (age ≥ 50 years, male, ECOG PS ≥2, stage II, B symptom, ≥ 3 involved sites, bulky disease, extranodal site, large mediastinal mass, CMT, elevated ESR and high MTV [≥198 cm^3]). Five factors including age ≥ 50 years, B symptom, bulky disease, large mediastinal mass and high MTV) had clinical significant values in univariate analysis (PFS, age ≥50 years [P=0.029], B symptom [P<0.001], bulky disease [P<0.001], large mediastinal mass [P=0.001] and high MTV [P<0.001]; OS, age ≥50 years [0.044], B symptom [P=0.001], bulky disease [P<0.001], large mediastinal mass [P=0.004] and high

MTV [P=0.001]). In multivariate analysis, age ≥50 years (PFS, HR=4.426, 95%CI=1.650-11.802, P=0.003; OS, HR=4.327, 95% CI=1.485-12.607, P=0.007), B symptoms (PFS, HR=4.239, 95%CI=1.510-11.899, P=0.006; OS, HR=3.549, 95% CI=1.089-11.568, P=0.036) and high MTV (PFS, HR=8.733, 95%CI=1.744-3.728, P=0.008; OS, HR=12.586, 95% CI=2.026-78.194, P=0.007) were significant prognostic factors in PFS and OS.

In the analysis of survival according to MTV and therapeutic modality (CMV vs. ABVD only), PFS and OS of high MTV group treated with ABVD only were lower than others groups (PFS, P< 0.001; OS, P<0.001, Figure 1).

Summary / Conclusion: MTV as a clinical parameter of tumor burden was an significant prognostic factor, that would be helpful for deciding therapeutic modality in early stage HL.

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ESHAP AS SALVAGE THERAPY FOR RELAPSED OR REFRACTORY HODGKIN'S LYMPHOMA

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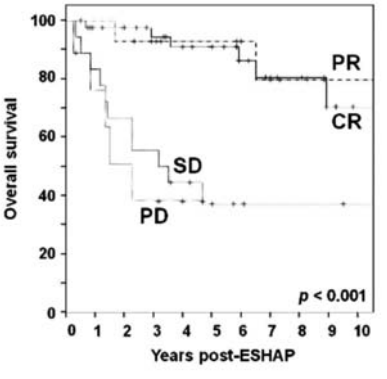
Background: The management of relapsed and/or refractory Hodgkin Lymphoma (RR-HL) remains a current challenge for hematologists, since more than a half of such patients cannot be cured from this typically curable disease. Current standard of care includes a salvage chemotherapy followed by autologous stem cell transplantation (ASCT). However, one of the most debatable key points in this strategy is what could be the best salvage protocol. It should introduce drugs not previously used in the patient in order to induce maximum response, but with a reasonable toxicity profile, including the avoidance of any stem cell impairment. A large number of salvage regimens have been published. However, there are no randomized trials comparing it.

Aims: We report our experience and long-term results with the ESHAP (etoposide, steroid, cytarabine, cisplatin) regimen in RR-HL patients with the intention to proceed to ASCT.

Methods: We conducted a retrospective study with 82 consecutive patients with RR-HL who received ESHAP as salvage therapy in order to achieve effective cytoreduction and proceed to ASCT with the BEAM conditioning in CR or with low residual disease. Forty-one out of 82 patients (50%) were considered to be refractory to front-line therapy.



CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease.

Figure 1. Overall survival in RR-HL according to the response to salvage treatment with ESHAP.

Results: This regimen provided an overall response rate (ORR) of 67% with a 50% complete response (CR) rate, with a median of two cycles of ESHAP. Three disease characteristics at ESHAP retained their adverse influence on CR rate on multivariate analysis: refractory disease or ≥2nd relapse, B symptoms and mediastinal involvement. Twenty eight out of 82 patients (34%) received additional salvage therapy after ESHAP treatment in order to proceed with the ASCT and 26/28 patients finally underwent SCT. In total, 75/82 patients (91%) received hematopoietic SCT: 71 ASCT and 4 allogeneic SCT from related donor. The response rate after the transplant among patients who received autologous or allo-SCT was improved to an ORR of 84% (63/75) and CR rate of 82.6% (62/75). The mean follow-up after ESHAP administration was 70.2±41months. The median progression-free survival (PFS) was 52 months (95% CI 7.2-96.7) and the time to progression (TTP) was 56 months (95% CI 11.5-100.5). Overall survival (OS) at 2 and 5 years were 80.5% and 72.6%, respectively. At the time of reporting, 70.7% patients are still alive. On multivariate analysis, CR achievement after ESHAP was the most important prognostic factor influencing PFS (78% *vs.* 15.5% at 5 years) and TTP (80% *vs.* 19% at 5 years). Patients who achieved PR or CR in pre-transplant evaluation also retained its favorable prognostic value in both, PFS and TTP. The response to ESHAP was the most important prognostic factor for OS (91 *vs.* 36% at 5 years) in the multivariate analysis, without significant differences between CR and PR patients (Figure 1). Forty one percent of patients experienced hematological toxicity ≥3, but only 10% had grade 4 neutropenia and <10% of patients developed neutropenic fever, with no toxic deaths. In addition, most of our patients (94%) scheduled to receive ASCT could be adequately mobilized for stem cell collection under the ESHAP regimen. In the present series, after a long time follow-up, the incidence of second malignancies was 7.3%, mostly myelodysplastic syndromes.

Summary / Conclusion: ESHAP regimen is a safe and efficient therapeutic option for patients with RR-HL who are candidates to ASCT, since it combines a high response rate and a high mobilizing potential with low toxicity profile. However, further studies to refine this regimen with the addition of new drugs or small dose/density changes should be considered in selected patients who have less possibility with the protocol as it is currently defined.

P159

SOLUBLE CD30 LEVELS UNDER BRENTUXIMAB VEDOTIN THERAPY

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Background: Targeted therapy with the anti-CD30 antibody Brentuximab Vedotin (BV) has shown promising results in patients with relapsed or refractory CD30 positive neoplasms such as Hodgkin´s disease (HD) or anaplastic large cell lymphoma (ALCL). However, many patients relapse during or early after treatment with BV. The soluble form of the target surface receptor (sCD30) can be measured in serum and may therefore serve as biomarker for prediction of response and monitoring during therapy. No data are currently available on serum kinetics of sCD30 during treatment with BV.

Aims: To determine sCD30 serum levels during treatment with BV and to correlate levels with clinical outcome.

Methods: Eight HD and 2 ALCL patients, all refractory to prior treatments, received BV and were included in this study. Soluble CD30 levels, measured by ELISA, were determined during BV treatment. Therapy response was determined by clinical signs (B-symptoms, lymph node size) and radiological techniques (PET, PET-CT, CT and/or sonography). Kaplan Meier survival analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, USA). P-values <0.05 were considered significant.

Results: All HD patients received at least 6 cycles of BV (range: 6-12 cycles), ALCL patients received 4 cycles each. All patients showed rapidly decreasing sCD30 levels already after the first two cycles. This matched clinical signs of therapy response. Among HD patients, 3 showed sCD30 levels less than 40 ng/ml already after the first cycle and had durable remissions. One of these 3 patients relapsed after 31 weeks. The other 2 patients, still being in complete remission (CR) after 24 and 50 weeks, underwent successful autologous stem cell transplantation and are still in CR to date (43 and 78 weeks, respectively). The other patients (5/8) did not reach sCD30 levels < 40 ng/ml and had shorter remission durations (6, 10, 11, 14 and 30 weeks; P=0.019) (Figure 1). Continuously increasing sCD30 levels were observed about two months before first clinical signs of relapse. Notably, therapy response was independent of histologic subtypes. In 2/5 patients who repeatedly relapsed after BV treatment, re-administration of BV was started after 7 and 8 months and unsuccessful treatments with other regimens. However, due to lack of response to BV, treatment was abandoned after 3 and 2 cycles, respectively. Compatible with this clinical course, sCD30 serum levels did not decrease. Unlike CD20 expression of tumor cells after Rituximab administration in other lymphomas, CD30 expression in tumor samples remained unaffected by BV as determined by immunohistochemistry. In comparison, both ALCL patients had much higher initial sCD30 levels than HD patients (median 6854 ng/mL vs. 122,2 ng/mL). One patient responded to BV with a sCD30<50 ng/mL five days after the first cycle and was in remission for 40 weeks. The second ALCL patient showed the lowest sCD30 level (81,48 ng/mL) after the second cycle of BV. However, this patient relapsed two weeks later.

Summary / Conclusion: We report first data on sCD30 serum levels in patients with HD or ALCL under treatment with BV. Although investigated in a small cohort, our data suggest that i) MH patients under BV treatment with sCD30 levels rapidly approaching less than 40 ng/ml) have durable clinical remissions; ii) continuously increasing sCD30 levels occur shortly before relapse; and iii)

CD30 expression persists in tumor tissue samples during and after BV treatment when patients have relapsed. Soluble CD30 may therefore serve as biomarker for monitoring under anti-CD30 directed therapy.

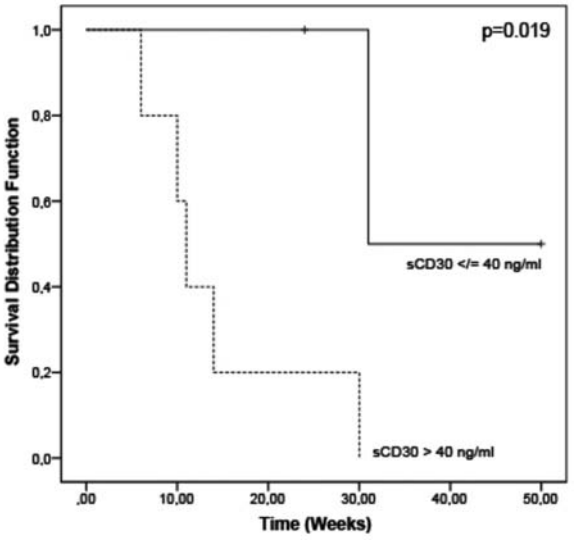

Figure 1. Time to relapse of Hodgkin's Disease patients during/after treatment with Brentuximab Vedotin. Patients not reaching soluble CD30 serum levels <40 ng/mL during treatment (broken line) had shorter remission times (P=0.019).

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THE LYMPHOCYTIC HISTOPATHOLOGIC SUBTYPES OF HODGKIN LYMPHOMA (HL): CLINICAL PRESENTATION AND LONG-TERM OUTCOME IN A CASE SERIES OF 61 PATIENTS

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Background: Nodular lymphocyte predominant HL (NLPHL) and lymphocyte rich classical HL (LRCHL) are rare subtypes of HL. Relevant data derived from homogeneously treated patient populations are scarce.
Aims: The evaluation of clinical manifestations and long-term prognosis of patients with (NLPHL) and (LRCHL), compared with other histologic subtypes of HL under ABVD or equivalent chemotherapy with or without radiotherapy (RT).

Table 1.

	HISTOPATHOLOGIC SUBTYPES			
Patients	NLPHL	LRC	Others	p
	(n=28)	(n=33)	(n=719)	
Age (median, range)	38.5 (17-73)	43 (15-76)	30 (14-82)	0.002
Males	75%	72%	61%	0.003
Stage I/II/III/IV	6%/26%/14%/	42%/33%/6%	20%/30%/18%/	<0.001
B-symptoms	11%	3%	34%	<0.001
Marrow involvement	0	3%	6%	0.37
Pure infradiaphragmatic disease (for stages I/II)	38%	15%	8%	<0.001
Anemia (Hb<10.5 g/dl)	11%	18%	42%	<0.001
WBC≥10x10⁹/L	14%	22%	44%	<0.001
Lymphocytopenia (IPS definition)	4%	3%	9%	0.39
PLT >400x10⁹/L	0	6%	27%	<0.001
ESR ≥50 mm/h	8%	7%	50%	<0.001
↑ LDH	8%	6%	28%	0.002
Albumin <4 g/dL	4%	20%	41%	<0.001
↑ CRP (>5 mg/L)	39%	29%	75%	<0.001
CRP ≥30 mg/L	0	6%	51%	<0.001
5-year tumor control	80%	88%	79%	0.34
5-year event free survival	76%	85%	77%	0.39
10-year overall survival	77%	89%	85%	0.93
10-year disease specific survival	95%	100%	90%	0.24

Methods: Retrospective study of 780 patients with histologicaly-confirmed diagnosis of HL, treated at our center. Histologic material was evaluated or reviewed at major Haematopathology referral centers according to current classification criteria. With respect to histopathologic subtype, the distribution of patients was: NLPHL 28 (3.6%), LRCHL 33 (4.2%), nodular sclerosis 545 (69.9%), mixed cellularity 158 (20.3%), lymphocyte depleted 3 (0.4%). The patients were assessed according to their clinical-laboratory features at presentation, and their course after treatment with ABVD or equivalent regimens ±RT.
Results: Patients with NLPHL and LRCHL were clinically similar, with the exception of slightly more frequent pure infradiaphragmatic localization in those with NLPHL (P=0.07). Compared with the others, patients with NLPHL and LRCHL tended to be male and older, asymptomatic, with more limited disease and higher incidence of pure infradiaphragmatic involvement (Table). Moreover, NLPHL and LRCHL very rarely had markedly abnormal laboratory features compared with the more common forms of classical HL (with the exception of the rather infrequent severe lymphocytopenia) (Table 1). Despite those differences, disease control and overall survival were similar among all histologic subtypes, as shown in the table.
Summary / Conclusion: NLPHL and LRCHL are rare subtypes of HL with distinct demographic and clinical features, that favor their separation from the common subtypes of classical HL. At present, in our series, the outcome of patients with NLPHL and LRCHL resembles that of patients with the other subtypes of HL.

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LONG TERM RESULTS OF MONOCENTRIC PROSPECTIVE STUDY OF VEBEP PLUS RADIOTHERAPY REGIMEN IN NEWLY DIAGNOSED EARLY-UNFAVORABLE STAGE HODGKIN LYMPHOMA

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Background: The gold standard therapy for early unfavorable Hodgkin Lymphoma (HL) has not reached a consensus yet. At present, ABVD followed by 30 Gy Involved Field Radiotherapy (IFRT) is the most emploied strategy in such patients. More aggressive regimens, such as BEACOPP escalated and/or BEACOPP baseline, in association with IFRT, may improve disease control in comparison with ABVD. Data from GHSG HD11 trial in early unfavorable HL showed that 5-year Freedom From Treatment Failure (FFTF) and Overall survival (OS) following BEACOPP baseline (4 cycles) plus 20 Gy IFRT were not significantly better than ABVD (4 cycles) plus 30 Gy IFTR: 87% *vs.* 85% and 94% *vs.* 95%, respectively. In addition, short- and long-term toxicity, as well as incidence of secondary neoplasms, were higher in BEACOPP *vs.* ABVD arm.
Aims: We propose mature results of this monocentric prospective study in order to define an alternative intensive and low toxic regimen for early unfavorable HL. Analysis of survival rates was focused on Disease Free Survival (DFS), as primary endpoint and OS, as secondary endpoint. Complete Remission Rate (CRR) and early and late toxicity rates were also estimated.
Methods: In this study, patients 18-65 year-old with newly diagnosed early unfavorable stage HL (according with GHGS criteria) were treated with six courses of VEBEP regimen (vinblastine 6 mg/m^2 dd 1 and 15 *i.v.*, epirubicine 40 mg/m^2 dd 1 and 15 *i.v.*, bleomicine 10 mg/m^2 dd 1 and 15 *i.v.*, etoposide 80 mg/m^2 dd 1-3 and 15-17 *i.v.*, and prednisone 40 mg/m^2 dd 1-5 and 15-19 orally; twice week G-CSF prophylaxis). Chemotherapy was followed by 32 Gy IFRT. Complete remission was defined according to revised criteria of the International Harmonization Group and the assessment of response was ^{18}FDG-PET/TC based. The follow up assessment concerned a clinical, laboratoristical and imaging-instrumental screening. Estimation of response and survival rates was conducted by Kaplan and Mayer analysis
Results: From 2001 to 2009, 117 patients were enrolled in the study. All patients completed therapeutic schedule and were included into the analysis. Median follow up was 86 months (11-135 months). CRR was 94% (109/117 patients achieved CR at the end of treatment and 8/12 patients had persistent/progressive disease). According to WHO Toxicity Grading System no grade 3-4 extra-hematological and hematological acute toxicity occurred. Five cases of pulmonary infection were diagnosed during the treatment, all successfully managed by antibiotics. 10-year DFS rate was 97% (4/109 had relapsed disease). All relapsed/refractory patients received salvage treatment and autologous stem cell transplantation. Grade 3-4 chronic toxicity profile of VEBEP plus radiotherapy regimen was very low. Only 2 patients had late cardiac toxicity (1%) and 2 patients developed restrictive respiratory disease (1%) during the follow-up. Three secondary neoplasms were diagnosed (2%) (1 Lymphoblastic Acute Leukemia, 1 Promyelocitic Acute Leukemia, 1 Nasopharyngeal cancer). 10-year OS was 96% (5/117 patients died: 3 for persistent disease and 2 for secondary leukemias).
Summary / Conclusion: VEBEP regimen plus IFRT could be considered an effective therapy for early unfavorable HL patients in order to its low toxicity and its efficacy.

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DEVELOPMENT OF A PROGNOSTIC SCORING SYSTEM FOR PRIMARY REFRACTORY HODGKIN'S LYMPHOMA TREATED WITH HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: Management of primary refractory Hodgkin's lymphoma represents a major therapeutic challenge in malignant haematology. Several authors have identified prognostic factors in cohorts of patients with relapsed and/or refractory disease who have undergone subsequent salvage chemotherapy followed by conditioning therapy and autologous stem cell rescue (ASCT). In these publications relapsed and refractory Hodgkin's lymphoma patients have usually been analyzed together as one group but there are significant survival differences in previously responding and never responding patients.
Aims: Thirteen prognostic factors have been analyzed in 45 patients with primary refractory Hodgkin's lymphoma treated with salvage chemotherapy and subsequent high dose therapy and ASCT to create a prognostic scoring system specific for primary refractory Hodgkin's lymphoma.
Methods: The ASCTs were performed between January 2000 and April 2012. The sex distribution of the patients showed a slight male predominance (21 females, 24 males), their mean age at the time of their ASCT was 30.31 years (range: 17-60 years). The most common histological subtype was nodular sclerosis in 65% of patients. 56% of them could be categorized as having Ann Arbor stage III-IV disease with B symptoms in 70% and with bulky disease in 55%.
The analyzed prognostic factors were the following: Age, Sex, Ann Arbor stage, Histologic subtype, Presence of B symptoms, Bulky disease, Radiotherapy, Type of conditioning regimen, Number of previous treatment lines, Response before ASCT, Time interval between diagnosis and ASCT, LDH and Serum albumin. Statistical analysis: Univariate analysis has been performed according to Kaplan-Meier estimates, multivariate analysis according to Cox regression model. P<0.05 was considered as significant.
Results: The outcome of the ASCTs was the following: overall response rate 84% (complete response in 52%, partial response in 32%), stable disease in 7%, 9% of the patients turned out to be non-responders to ASCT. Overall survival according to Kaplan- Meier estimates at 3 years was 68%, at 5 years 28%. The ASCTs were well tolerated (non-relapse mortality (<100 days): 1 of 45 patients - 2.2%). Five adverse prognostic factors have been identified as significant (P<0.05) in both statistical analyses: 1. Ann Arbor stage (stage 4 disease), 2. Number of previous treatment lines (≤3, >3), 3. Response before ASCT (progression, stable disease, response), 4. Time interval between diagnosis and ASCT (≤18, >18 months), 5. Serum albumin (≤35, >35 g/L).
A prognostic scoring system has been constructed from these five significant prognostic factors: 0 or 1 adverse prognostic factor: 'low' risk group; 2 or 3 adverse prognostic factors: medium risk group; >3 adverse prognostic factors: 'ultra high' risk group. There was a highly significant difference (P=0.000) in the overall survival of patients belonged to the three different prognostic categories (Figure 1).
Summary / Conclusion: A new prognostic scoring system has been constructed specifically for primary refractory Hodgkin's lymphoma that can be useful in driving treatment decisions for patients within this desperate disease category. There were significant overall survival differences between the three prognostic categories (Figure 1) constructed on the bases of the five adverse prognostic factors that were significant with both statistical analyses. Our results should further be approved on a larger cohorts of patients with primary refractory Hodgkin's lymphoma.

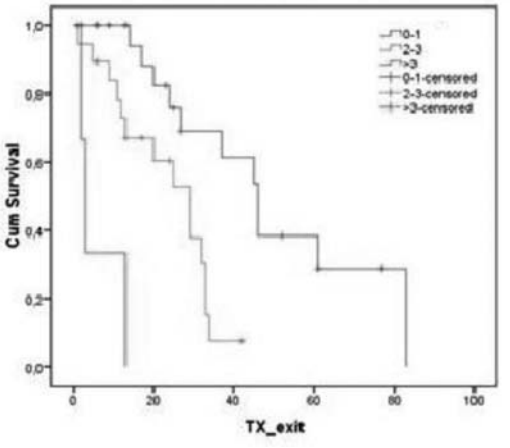

Figure 1. Kaplan-Meier estimate of OS according to risk categories (P=0.000).

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HETEROGENEOUS MANAGEMENT OF ADOLESCENTS AND YOUNG ADULTS WITH HODGKIN LYMPHOMA AND INCONSTANT CONFORMITY OF MEDICAL PRACTICE TO CLINICAL GUIDELINES: EXHAUSTIVE COHORT FROM RHÔNE-ALPES REGION

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Background: Management of adolescents and young adults (AYAs) with cancer remains unsatisfying and carelessness, as indicated by data from the SEER registry. Overall outcome of Hodgkin lymphoma (HL), the most frequent cancer of AYAs, is favorable, but the question of the optimal treatment is far from being resolved, in particular for the impact of the therapeutic choice on the long-term secondary effects. In an attempt to measure quality of AYAs management, evaluation of physicians' decisions using conformity rate of successive procedures with clinical practice guidelines (CPG's) was observed.
Aims: The aim of this study was to describe the primary management of HL among AYAs and the compliance's rates with CPG's used at time of management.
Methods: All institutional patients' records of 13-25y AYAs with classical HL diagnosed in the Rhône-Alpes region between 2000 and 2005 were retrospectively analyzed. Data from diagnosis to last follow-up were collected until 2011. Five successive sequences were assessed: staging, histology, chemotherapy, radiotherapy and follow-up. The overall sequence of treatment was considered to be conform when each assessable part of the procedure was respected.
Results: One hundred and ninety eight patients were included, 148 (75%) managed in adult units (A) and 50 (25%) in pediatric units (P). Median age was 21y (range 13-25) in (A) and 14y (range 13-24) in (P). Thirty three patients (17%) were included in clinical trials, 13 (7%) were managed apart from any recommendation and 97 (49%) had their medical case reviewed in multidisciplinary staff. Treatment delay, once diagnosis made, was longer in (A) than in (P) (median 18 days (range 0-62) versus 9 days (range 1-33), P<0.01). All patients received initially chemotherapy; 63% in (A) received after radiotherapy, 100% in (P). Among irradiated patients, average dose received was lower in (P) than in (A) (23 Grays versus 34 for supradiaphragmatic, 21 Grays versus 31 for infradiaphragmatic irradiation). Differences were notified between (A) and (P) for chemotherapy protocols used: larger use of anthracyclins and bleomycin in (A), of corticosteroids and alkylants in (P). End of treatment evaluation for response was similar in both groups. At the last follow-up, staging was conform to CPG's in 88% for all patients (86% and 92% respectively for (A) and (P)), chemotherapy in 82% (78% and 94%), radiotherapy in 81% (79% and 88%), follow-up in 87% (90% and 78%), global conformity in 56% (55% and 60%). In the 15-18y subgroup (*n*=60, among whose 40 managed in (A), 20 in (P)), conformity was found to be significantly higher in (P) for global conformity (P=0.02) and for chemotherapy conformity (P=0.05). Ten patients (5%) died, 9 in (A) and 1 in (P), all from HL disease except 1 from treatment toxicity (managed in (A)). Six patients (3%) were lost to follow-up. Among the 182 alive patients (64 months median follow-up), 1 managed in (A) developed a thyroid carcinoma.
Summary / Conclusion: Our study emphasizes the heterogeneity of the management of AYAs with HL as well as the absence of standard of care. The too short follow-up does not allow to compare (A) and (P) management about long term toxicities, particularly for second malignancies occurrence. We encourage increased cooperation and communication between adult and pediatric oncologists to develop dedicated clinical trials for this population. Improving compliance with CPG's should lead to improve specific cares for AYAs, to induce more homogeneity in their management and to facilitate the development of new drugs.

Table1.

	Adult (*n*=148)	Pediatric (*n*=50)
	n= (%)	*n*= (%)
Ratio F/M	1,3	1,0
Histological subtype		
nodular sclerosis	131 (89%)	46 (92%)
mixed cellularity	4 (3%)	0 (0%)
lymphocyte-rich	13 (9%)	3 (6%)
lymphocytic depleted	0 (0%)	1 (2%)
Ann Arbor stage		
I	15 (10%)	1 (2%)
II	75 (51%)	29 (58%)
III	26 (18%)	10 (20%)
IV	32 (22%)	10 (20%)
B symptoms	49 (33%)	25 (50%)
Elevated ESR (>50)	39 (26%)	19 (38%)
5-years OS (95% CI)	95% (93-97)	98% (96-100)
5-years PFS (95% CI)	87% (84-90)	76% (70-82)

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THERAPEUTIC STRATEGIES FOR RELAPSED OR REFRACTORY HODGKIN'S LYMPHOMA (RR-HL): A SINGLE CENTER FDG-PET-ADAPTED STRATEGY

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Background: The current standard of care for relapse and refractory RR-HL consist of salvage chemotherapy (SLT) followed by high dose chemotherapy and autologous stem cell transplantation (HDT/ASCT).
Aims: FDG-PET scan has consistently been identified as a critical predictor of outcome. As a consequence, we have adopted at our center a PET-adapted strategy for the treatment of RR-HL. We report here the outcome of patients (pts) treated according to this strategy.
Methods: Pts < 65 years old with RR-HL were treated with 1 or 2 lines of SLT. The response was then evaluated by PET scan before a planned HDT/ASCT. Pts with a negative pretransplant PET scan (PETneg) underwent HDT/ASCT after a BEAM regimen. If the pretransplant PET remained positive (PETpos), patients underwent HDT/ASCT followed by an additional treatment consisting of tandem allogeneic stem cell transplant (Allo-SCT), a second HDT or radiotherapy (RT).
Results: From 2006 to 2012, 61 pts were included with a median time between end of first-line therapy and relapse of 6 months (range, 0,5-167,5 months). The median age was 38 years (range, 17-66 years). The first line chemotherapy was ABVD in 58 pts (95%) completed by RT in 16 pts. Our RR-HL cohort was defined as follow: 22 pts (36%) had primary-refractory (PR) disease defined as the persistence of active disease at the end of therapy or recurring within 3 months, while 23 (38%) and 16 (26%) have early (< 1 year) or late (> 1 year) relapse. Thirty-one pts (51%) were PETneg before HDT/ASCT: 6 had PR disease (out of 22, 27%); 16 had early relapse (out of 23, 70%); and 9 had a late relapse (out of 16, 56%). All PETneg patients underwent HDT/ASCT as planned. Thirty pts had PETpos before HDT/ASCT. We performed HDT/ASCT in 25 of these pts, among whom 12 subsequently underwent a reduced-intensity (RIC) allo-SCT, 1 a second HDT, and 7 a RT. Considering the entire cohort 11 pts died, 9 because of progression of disease and 2 of toxicity. With a median follow-up of 37 months (range, 4-88) the 5-y OS was 75% and EFS was 59%. Pretransplant PETscan a critical predictor of outcome: for PETneg pts, 5-y OS and EFS were 87% and 82 % respectively while it was 61% and 33% for PETpos pts (P=0.009 and P=0.0001 respectively). For PETpos pts3y-OS was 57% if they underwent tandem allo-SCT after HDT/ASCT versus 19% if they received a RT or no treatment (P=ns). For relapse pts 3-y EFS is 95% when TEP is negative and 65% when TEP is positive (P<0.00001) regardless of time of relapse (early or late). For PR pts, 3-y EFS is 37% when TEP is negative and 10% when TEP is positive. Otherwise, the 3-y OS is 32% for primary refractory pts with TEPpos while it is 100% for PR pts with TEPneg, 90% for relapsed pts with TEPpos and 95% for relapsed pts with TEPneg (P=0.002).
Summary / Conclusion: In our cohort, patients with a negative PET after SLT had a very good outcome as compared to pts with a positive PET. For these PETpos pts, alloSCT in tandem after HDT/ASCT seems to be a good therapeutic option even if our results have to be interpreted with caution because of the limited number of pts. Patients with a PR have poor EFS despite a negative PET before HDT/ASCT. However, despite a significant relapse rate for patients with PR disease, the salvage treatment after ASCT was effective enough to allow good survival especially for pts with TEPneg before HDT/ASCT. In sharp contrast, PR pts with a positive PET had a very poor outcome.

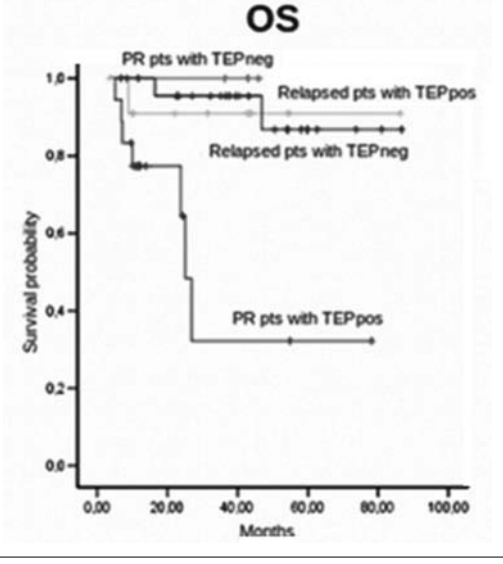

Figure 1.

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HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION FOR ADULTS WITH HODGKINS LYMPHOMA IN BELARUS: OUTCOME IN 214 PATIENTS OF A SINGLE CENTRE.

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Background: Relapsed or refractory Hodgkin lymphoma is a challenging problem for clinicians. Hodgkin's lymphoma has high rates of cure, but in 15% to 20% of general patients and between 35% and 40% of those in advanced stages, the disease will progress or will relapse after initial treatment. The standard management of these patients should include the use of salvage chemotherapy followed by autologous stem cell transplant (ASCT).
Aims: We retrospectively analyzed efficacy in 214 adult patients with relapsed and refractory Hodgkin's lymphoma who were consecutively submitted to high-dose chemotherapy followed by ASCT in a single centre between November 1996 and December 2010. Induction failure was defined as failure to achieve a partial remission (PR), complete remission (CR) after initial treatment with combination chemotherapy, using one or more regimens.
Methods: 109 (51%) patients were male and 105 (49%) were female, with a median age of 29,3 years. All patients received dexaBEAM and DHAP as salvage. Responses to salvage therapy were defined as PR in 38 (18%), CR in 146 (38%) and stable/minimally responsive disease (SD/MR) in 30 (14%). OS and PFS rates were determined , and prognostic factors were investigated using univariate analyses.
Results: Responses to high-dose therapy and ASCT were complete response 141 (66%), partial response 39 (18%), stable disease 19 (9%), progressive 10 (5%), and toxic death 4 (2%). Actuarial 5-year OS rates were 63% and 10-year OS rates were 46%; 5-year PFS rates were 53% and 10-year PFS rates were 32%, respectively. Univariate analysis shows that sensitivity to pre-transplant treatment (5-year OS 73% (CR) vs 67% (PR) vs 34% (SD), PFS 72% (CR) vs 12.
Summary / Conclusion: High-dose therapy and ASCT is an effective treatment strategy for patients with Hodgkin's disease for relapse and refractory Hodgkin's lymphoma for cases that were responsive to pre-transplant chemotherapy. Additionally, using radiotherapy before ASCT has a negative impact on the survival of patients after transplant.

Myelodysplastic syndromes and bone marrow failure syndromes incl. PNH - Biology

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RECURRENT SETBP1 MUTATIONS IN ATYPICAL CHRONIC MYELOID LEUKEMIA

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Background: Atypical Chronic Myeloid Leukemia (aCML) is a heterogeneous disorder which belongs to the group of myelodysplastic/myeloproliferative (MDS/MPN) syndromes. Since no specific recurrent genomic or karyotypic abnormalities have been identified in aCML, the molecular pathogenesis of this disease has remained elusive and the outcome dismal. This sharply contrasts with the outcome of CML, for which the prognosis has been dramatically improved by the development of imatinib.
Aims: To identify recurrent genetic lesions specifically present in aCML patients.
Methods: Analysis of exome and transcriptome was performed using Illumina Genome Analyzer IIx according to standard procedure for the preparation of the libraries.
Results: By applying whole-exome and transcriptome sequencing on aCML samples, we show for the first time the presence of recurrent somatic mutations of SETBP1 in approximately 25% of aCML cases. Surprisingly, the mutations identified in aCML, albeit somatic, are virtually identical to the germline variants identified in the Schinzel-Giedion syndrome (SGS), which suggests a common link between the two disorders. SGS is a rare genetic disease characterized by midface retraction, a prominent forehead and a higher incidence of tumors, which suggest a critical role for mutated SETBP1 in activating yet unknown pro-oncogenic pathways. Very little is known about the biological function of SETBP1, besides that it binds SET protein, a physiological inhibitor of protein phosphatase 2A (PP2A), protecting it from proteolytic cleavage. Interaction of SETBP1 with the HOXA9 and HOXA10 promoters has been also recently described. By screening the region where aCML and SGS mutations cluster (aa858-871) with the ELM server (http://elm.eu.org/), we identify a virtually perfect degron, containing the consensus binding region (DpSGXXpS/pT) for beta-TrCP1, the substrate recognition subunit of the E3 ubiquitin ligase (aa868-873). This degron lies inside a PEST domain (HSEETIPSDSGIGTDNNSTSDQAEK), a sequence associated with proteins that have a short intracellular half-life, suggesting that this region may be critical for ubiquitin binding and for subsequent protein degradation. This hypothesis was experimentally confirmed using biotinylated, phosphorylated peptides encompassing aa859-879: while the WT peptide could efficiently bind beta-TrCP1, no binding could be detected when peptides carrying mutations D868N, S869G and G870S, were used as a bait. In line with this model, we demonstrated that the mutated SETBP1 is overexpressed at protein but not at mRNA level, protecting SET from proteolytic cleavage and leading to SET accumulation, which causes phosphorylation of PP2A in Y307 (a well-known marker of PP2A inactivation), inhibition of PP2A phosphatase activity and increase in the proliferation rate
Summary / Conclusion: In summary, mutated SETBP1 represents a newly discovered oncogene recurrently present in aCML.

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A NEW RECURRENT CHROMOSOMAL ABERRATION OF MYELODYSPLASTIC SYNDROME WITH COMPLEX KARYOTYPE: T(5;17)(Q11~Q13;Q11~Q13)

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Background: A complex karyotype, detected in approximately 10%>15% of patients with myelodysplastic syndrome (MDS), is associated with a very short median survival and a high risk of transformation into AML. The most frequent chromosome aberrations in complex karyotypes are deletion of 5q (del(5q)) and deletion of 17p (del(17p)) harboring the tumor suppressor gene *TP53*. It is still unclear, how complex karyotypes develop.
Aims: We have identified an unbalanced translocation der(5)t(5;17)(q11~q13;q11~q13) in 20 patients with MDS or secondary AML after MDS. The cohort consists of 9 male and 11 female patients between 1 and 74 years of age (median age 68 years). It was our aim to better understand the underlying pathomechanism of this aberration and we have investigated these cases in greater detail.
Methods: For all patients, we performed cytogenetic banding analysis, fluorescence in-situ hybridization (FISH) and for 7 of the 20 patients multicolor-FISH.
Results: In all patients, a complex aberrant karyotype with a median of 10 aberrations was observed, indicating high chromosomal instability. In 8 patients, a clonal evolution was identified. To identify the breakpoints in 5q and 17q more precisely, array-CGH was performed in 7 of the 20 patients. The breakpoints in 5q and 17p were located between 5p15 and between 17q22, respectively, indicating that no fusion transcript evolved from the translocation. Notably, the breaking points were all very close to the centromeric region and heterochromatin. It is known that an altered methylation of heterochromatic regions plays an important role in tumor development. Therefore, alterations of DNA methylation or histone modifications may be involved in the generation of the unbalanced translocation t(5;17). By performing whole exome sequencing, we aimed to define the mutation spectrum of complex karyotypes with t(5;17). In one patient we were able to analyse bone marrow cells from different time points: complex karyotype at diagnosis – complete remission – relapse with complex karyotype again. As possible candidate genes for driver mutations we identified mutations in the genes *NF1*, *TEL* and *MLL3*. Especially the identification of a mutation in *NF1*, a negative regulator in the *RAS* pathway, is of great significance. *NF1* is encoded on 17q11.2. A possible underlying mechanism for the induction of a complex karyotype could be a downregulation of *NF1* by a mutation of one allele and by a deletion evolved from the unbalanced translocation t(5;17) of the second allele.
Summary / Conclusion: These data provide further evidence that the inactivation of *NF1* seems to play an important role in clonal evolution and leukemic progression.

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ABERRANT SPLICING DURING ERYTHROID DIFFERENTIATION IN SF3B1 MUTATED SIDEROBLASTIC ANEMIA

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Background: Refractory anemia with ring sideroblasts (RARS) is a myelodysplastic syndrome (MDS) subgroup distinguished by anemia, erythroid apoptosis and mitochondrial ferritin accumulation in erythroblasts, which fail to mature into erythrocytes. It is poorly understood at which stage and by which mechanism erythroid failure occurs; hence possible targets for therapy are yet to be discovered. Recently, heterozygous mutations in SF3B1, a core component of the spliceosome, were identified in >70% of RARS cases. This discovery may provide clues to how the failure of terminal differentiation is executed, and how the hematopoietic stem cells (HSC) gain their clonal advantage.
Aims: To explore the molecular and cellular mechanisms behind the clonal expansion of immature progenitors and the defective erythroid maturation, we investigated transcriptome characteristics, splice patterns and SF3B1 allelic burden during erythroid differentiation in 11 RARS patients SF3B1 mutated, of which the majority also carried other driver mutations.
Methods: Initially, normal and RARS transcriptomes from two time points during early erythroid differentiation were analyzed and compared, and in a second phase, transcriptome findings were validated using Taqman Low Density Array (TLDA) analysis in an extended cohort.
Results: We observed the activation of genes involved in defense against oxidative stress in $CD34^+$ progenitors. By contrast, erythroblasts were characterized by a failure to up-regulate genes in the autophagy pathway, essential for terminal maturation to erythrocytes, and by a maintained expression over time of regulators of transcription, apoptosis and adhesion, indicating that transcription in general is not hampered by SF3B1 mutation. We also report marked alterations in gene expression during RARS differentiation (FDR <5%) and altered splicing of genes involved in hematopoiesis. The exon usage pattern was very heterogeneous within the RARS population, while showing limited variation in the NBM cohort. Finally, we demonstrated that SF3B1 mutation does not confer a growth disadvantage to the erythroid cells until final maturation to reticulocytes, the stage at which autophagy becomes essential for erythroid differentiation.

Summary / Conclusion: Our study suggests oxidative stress defense, potentially caused by mitochondrial iron accumulation, as an underlying mechanism for the clonal advantage of RARS stem and early progenitor cells and provides novel insights into the erythroid differentiation process. By showing that anemia develops during terminal maturation into erythrocytes, we provide important insights into how the severe anemia in RARS patients may be addressed.

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RESISTANCE TO APOPTOSIS IN HIGH-RISK MYELODYSPLASTIC SYNDROME CAN BE OVERCOME BY PRO-APOPTOTIC DRUG TREATMENT USING ABT-737

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Background: Increased apoptosis in the bone marrow (BM) is an identifying feature of low/intermediate-risk myelodysplastic syndrome (MDS). Upon disease progression to high-risk MDS/sAML, bone marrow cells acquire a resistance to apoptosis and exhibit increased survival. These findings are consistent with an elevated expression of anti-apoptotic Bcl-2 family proteins relative to their pro-apoptotic family members in MDS cells. The lack of mitochondrial priming for cell death is an identifying feature of leukemic blasts and correlates with resistance to therapy. We therefore hypothesize that restoring the balance between pro- and anti-apoptotic Bcl-2 family members overcomes acquired apoptotic resistance in high-risk MDS, reduces the survival of the malignant clone and decreases the probability of transformation into sAML.
Aims: Pharmacologic inhibition of pro-survival Bcl-2 family members using specific BH3-mimetic compounds (e.g. ABT-737) potently induces apoptosis in hematopoietic cells *in vitro* and in clinical trials for hematologic malignancies including chronic lymphocytic leukemia *in vivo*. Using ABT-737, we want to induce apoptosis specifically in high-risk MDS/sAML and quantify the toxicity on healthy age-matched bone marrow controls.
Methods: We subjected primary bone marrow mononuclear cells (BMMNCs) from patients diagnosed with various MDS risk groups (WHO / WPSS) or sAML in the presence of protective cytokines to ABT-737 treatment. A large cohort of primary BMMNCs of healthy, age-matched donors was used as controls. Induction of apoptosis of cellular subpopulations including $CD34^+$ BMMNCs was measured by flow cytometry. Individual BMMNC samples were cultured with or without protection by stromal cells using the murine cell line EL08-1D2, which protects human hematopoietic $CD34^+$ cells.
Results: ABT-737 specifically killed MDS/sAML cells in a time and dose-dependent manner, whereas $CD34^+$ age-matched healthy control cells remained largely unaffected. Induction of apoptosis in low/intermediate-risk MDS cells was minimal, whereas high risk MDS/sAML underwent apoptosis very efficiently in the presence of ABT-737 (1 uM) (Figure 1; low-risk vs. high-risk (P=0,0042); low risk vs. sAML (P=0,001); intermediate-risk vs high-risk (P=0,001) and intermediate-risk vs. sAML (P<0,0001)). We found a clear correlation between accelerated induction of apoptosis by ABT-737 and increasing risk group of patients in MDS cells. In representative MDS BMMNCs cultured in the presence of stromal cells, induction of apoptosis was similar to BMMNcs without stromal cell support, indicating the potent cell death-inducing potential of ABT-737. FICOLL-purified BMMNC from primary bone marrow aspirate from 11 healthy donors and 2 patients with low-risk, 5 intermediate-risk, 7 high-risk or very high-risk MDS patients and 7 patients with sAML were treated with ABT-737 (1 μM) or vehicle control for 72h. Each circle represents the ratio between viable cells after ABT-737 treatment and viable cells after vehicle treatment. Cells were stained with an anti-CD34 antibody and Annexin V/7-AAD for viability and measured by flow cytometry.
Summary / Conclusion: These results support the notion that pharmacologic intervention with the pro-apoptotic compound ABT-737 potently induces apoptosis in high-risk MDS/sAML bone marrow cells. Therefore, ABT-737 may be a powerful tool to overcome resistance to apoptosis in high-risk MDS and sAML and delay progression into accelerated disease.

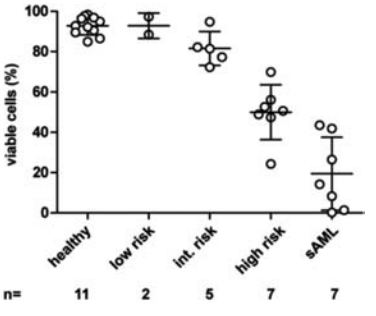

Figure 1. Accelerated induction of apoptosis in high-risk CD34+ MDS cells.

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DNA METHYLATION PROFILING IN PATIENTS WITH MYELODYSPLASTIC SYNDROME TREATED WITH 5-AZACYTIDINE

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Background: Myelodysplasticsyndromes (MDS) are clonal disorders of hematopoietic stem cells characterized by ineffective hematopoiesis. Aberrant DNA methylation in MDS was documented in several studies. Hypermethylation in the promoter regions may reduce the expression of tumor suppressor genes, whereas hypomethylation may lead to an increased expression of oncogenes. The DNA-hypomethylating drug 5-azacytidine is in clinical use for the treatment of MDS.
Aims: To date, correlation between DNA methylation and the clinical response to hypomethylating agents has not been found.Therefore, we investigated whether DNA methylation status might predict response to azacytidine treatment.We also analyzed different methylation levels between patients and controls.
Methods: We assessed methylation status in 31 samples from MDSpatients before 5-azacytidine treatment using the Illumina Infinium HumanMethylation27 BeadChip, which interrogates 27,578 CpG sites, selected predominantly from the promoter regions of 14,000 annotated genes. DNA was isolated from CD34+ cells separated by magnetic bead from bone marrow. DNA was subsequently modified by sodium bisulfite. The nonparametric Mann-Whitney test was used for comparison of β-values between responders and nonresponders and between patients and control group.
Results: We found significant promoter hypermethylation in CD34+ cells in 10 genes with transcription regulator activity (e.g. *GATA4, WT1, EGR3, MAPK15, MYF6* and *PAX3*) *and* 10 genes involved in embryonic organ morphogenesis (e.g.*HOXA2, HOXA5, HOXB6* and *TCF21*) (P<0.001) in MDS patients compared to controls. The contrary, we detected promoter hypomethylation in genes participating in regulation of programmed cell death (*CASP3, IL3, MAPK, PRAME* and *TNFSF14*) and genes are involved in cellular response to stress (e.g. *DDB1, MBD4, MAPK1 and PARP3*) (P<0.005). To determine whether DNA methylation could predict response to 5-azacytidine treatment, we compared DNA methylation at baseline with clinical responses in 31 MDSpatients. Fourteen patients of 31 (45 %) achieved complete remission or partial remission, 10 had stable disease (32%), and 7 showed progression (23%). We found significant differences in methylation status in 45 genes (P<0.05) between groups of responders and nonresponders. The affected genes were functionally annotated with the David database. 20 genes (e.g. *HMP19, NOS, PDCD, 1LG21, VPS53* and *GPR92*) from these were integral to membrane.Furthermore, among the genes with the largest methylation difference between responders and nonresponders belonged *HSD17B4* (0.29 vs 0.75, p <0.001), *HMP19* (0.37 vs 0.77, P<0.01) and *DAAM2* (0.51 vs 0.15, P<0.001).
Summary / Conclusion: Our study shows aberrant promoter methylation in MDS patientscompared to controls and different levels of methylation between groups of patients with unlike response to therapy. We suppose that the success of 5-azacytidine therapy may depend on its transport into cells. Drug must be transported into cells before its incorporation into DNA and subsequently inactivates DNA methyltransferases. Cellular transport across membranes is crucial for uptake of this highly polar hydrophilic molecule. We detected significantly altered levels of methylation in large number of genes for proteins, which are integrated into the membrane in responders vs nonresponders. These changes of methylation level may influence the absorption of the active substance.
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FUNCTIONAL ANALYSIS OF COHESIN MUTATIONS IN MYELOID NEOPLASMS

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Background: Cohesin is a multimeric protein complex, which is involved in

cohesion of sister chromatids, post-replicative DNA repair and transcriptional regulation. Using high-throughput target resequencing and SNP array-based copy number analysis, we found that the core components of cohesin, including STAG2, RAD21, SMC1A and SMC3, were mutated or deleted in a variety of myeloid neoplasms, including AML, CMML, MDS and CML, in a mutually exclusive manner. However, the functional role of cohesin mutations in myeloid leukemogenesis is largely unknown.

Aims: The purpose of this study is to obtain an insight into the functional role of cohesin mutations in myeloid leukemogenesis.

Results: First, we investigated the possible impact of mutations on cohesin function. The expression of several cohesin components was examined in 17 myeloid leukemia cell lines with (N=4) or without (N=13) known cohesin mutations, as well as in the chromatin-bound fractions of 13 cell lines. Although a significant reduction in RAD21 was observed in Kasumi-1 harboring RAD21 p.K330PfsX6, P31FUJ, CMY and MOLM-7 were not accompanied by significant decreases in the corresponding proteins compared with the wild-type cell lines. In contrast, severely reduced expression of one or more cohesin components was observed in KG1 (STAG2) and MOLM-13 (STAG1/2 and RAD21) without any accompanying mutations in the relevant genes. No significant differences in protein expression of the cohesin components in cohesin-mutated and non-mutated cell lines were noted in whole cell lysates. However, several components of the cohesin in chromatin-bound fractions, including SMC1, SMC3, RAD21, and STAG2 were significantly reduced in cell lines with mutated or reduced cohesin components compared with the cell lines with no known cohesin mutations or abnormal cohesin expression. These data indicate that mutation or reduction of one or more cohesin components compromise the formation of integrated cohesin complex on chromatin. Next, we examined the effect of forced expression of wild-type cohesin component on cell proliferation of cohesin-defective cell lines. Forced expression of the wild-type RAD21, but not a truncated RAD21 allele, induced significant growth suppression of the Kasumi-1 cells harboring RAD21 p.K330PfsX6. No growth suppression was observed in K562 and TF1 cells harboring wild-type RAD21 alleles by forced expression of RAD21. We also observed that forced expression of RAD21 or STAG2 induced the growth suppression of MOLM-13 cell lines which showed severely reduced RAD21/STAG2 expression. These results support a leukemogenic role for compromised RAD21 and STAG2 functions. Expression microarray analysis of RAD21- and mock-transduced Kasumi-1 cells revealed that a total of 63 genes reproducibly and significantly showed more than 1.2 fold increase or decrease in gene expression, which was confirmed by quantitative PCR and RNA sequencing. We are now examining the relationship of cohesin localization and transcription by ChIP sequencing analysis using antibody against cohesin components.

Summary / Conclusion: A mutated cohesin component could induce compromised recruitment of the cohesin complex onto chromatin. Defective cohesin function is implicated in leukemic proliferation possibly through deregulated gene expression.

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CLINICAL AND BIOLOGICAL RELEVANCE OF SRSF2 MUTATION IN CHRONIC MYELOMONOCYTIC LEUKEMIA AND THEIR COOPERATION WITH ADDITIONAL GENE MUTATIONS IN THE PROGRESSION TO SECONDARY ACUTE MYELOID LEUKEMIA

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Background: Gene mutations involving splicing machinery have recently been described in myelodysplastic syndromes (MDS) and chronic myelomonocytic leukemia (CMML). *SRSF2* mutation is one of the most frequent spliceosome component mutations and occurred exclusively at Pro95. The role of *SRSF2* mutation in the progression of CMML to secondary acute myeloid leukemia (sAML) is not clear.

Aims: We aimed to investigate the clinical relevance of *SRSF2* mutation in CMML and its cooperation with other genetic alterations at initial diagnosis and during sAML progression.

Methods: Bone marrow cells from 103 CMML patients: 34 dysplastic form (CMML-MD) and 69 proliferative form (CMML-MP), were analyzed for *SRSF2* mutation, 36 of them had matched paired CMML/sAML samples available for comparative analysis. The mutation status and relative allelic frequency of *SRSF2* mutants were measured by pyrosequencing. Additional 20 known mutated genes associated with myeloid neoplasms were also analyzed in *SRSF2*-mutated patients by using PCR-based analysis followed by direct sequencing.

Results: Thirty-six (7 CMML-MD and 29 CMML-MP) of 103 patients (35%) with CMML had *SRSF2* mutations (22 P95H, 9 P95L, and 5 P95R, with one of P95H mutated patient combined with P95R102del) at initial diagnosis. CMML-MP patients had a significant higher frequency of *SRSF2* mutation than CMML-MD patients (42.0% vs. 20.6%, P=0.047). There was no correlation between the mutation status of *SRSF2* and age, gender, hemoglobin, platelet count, bone marrowblasts, cytogenetics, WHO subtype, or IPSS-R; whereas *SRSF2* mutation was significantly associated with higher WBC count (P=0.038). Forty-five patients progressed to sAML. *SRSF2* mutation had no impact on risk to sAML progression (P=0.678), overall survival (P=0.899) or sAML-free survival (P=0.840). All of the 36 *SRSF2* mutation-positive patients harbored at least one additional gene mutations at diagnosis (16 *TET2*, 14 *ASXL1,* 13 *RUNX1*, 8 *RAS*, 7 *C-CBL*, 5 *DNMT3A*, 3 *PTPN11*, 3 *IDH2*, 2 *FLT3*-ITD, 2 *FLT3*-TKD, 2 *CEBPa, 1 JAK2*V617F, and *1 EZH2*). Of the 36 paired CMML/sAML samples, 13 were positive for *SRSF2* mutation at both CMML/sAML phases, their relative allelic frequency of *SRSF2* mutants remained unchanged at both phases (40.1±12.4% vs. 41.0±11.2%, P=0.469). One *SRSF2* wild-type patient harboring *TET2, ASXL1* and *JAK2*V617F mutations at diagnosis of CMML acquired *SRSF2* and *RUNX1* mutations but lost *JAK2*V617F mutation at sAML phase. Together, progression to sAML was accompanied by evolution of a new clone in 5 patients (1 *CEBPα,* 1 *NPM1*, 1 *FLT3*-TKD, 1 *SRSF2* and *RUNX1*, and 1 *IDH2* with *FLT3*-ITD and *RUNX1*) during sAML transformation.

Summary / Conclusion: Our results showed that *SRSF2* mutation was frequently detected in CMML and inevitably cooperated with other gene mutations at initial diagnosis. Progression of *SRSF2*-mutated CMML to sAML was frequently accompanied by acquisition of additional genetic alterations.

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A CASE OF TWO MONOZYGOTIC TWINS WITH CONCORDANT JUVENILE MYELOMONOCYTIC LEUKEMIA: INSIGHT IN THE COURSE OF THE DISEASE

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Background: Juvenile myelomonocytic leukemia (JMML) is a rare early childhood disorder characterized by excessive proliferation of myelomonocytic cells, mutations in the RAS signaling pathway (85% of patients) and monosomy of chromosome 7 (25% of patients). The incidence of leukemia in monozygotic twins is high and this has been related to a common clonal origin of the disease; studies reporting cases of monozygotic twins with JMML have been also reported but they are lacking information concerning the chorionic/amniotic type and the presence of genomic aberrations

Aims: Taking advantages of the newest molecular biology technologies we investigated a pair of monoplacental monozygotic twins with concordant JMML, *PTPN11* mutation and abnormal chromosome 7 in order to better understand the origin of the disease.

Methods: *PTPN11* mutations were analyzed using 454 Roche Next Generation Sequencing and validated by Sanger sequencing in bone marrow (BM), peripheral blood (PB) fibroblast, oral swabs and hair bulbs. In the same samples abnormalities of chromosome 7 were analyzed using FISH, aCGH and STR. To detect mutations and chromosome 7 abnormalities in different BM and PB lineage, sorted cell populations were analyzed. Gene expression profiling (GEP) analysis at diagnosis of both twins was performed using Affymetrix HG U133 Plus 2.0 arrays.

Results: A common *PTPN11* mutation (E76G) was found in both twins in BM and PB. Both twins share the same abnormal chromosome 7. Moreover, STR analysis revealed loss of chromosome 7 material of maternal origin. The germline origin of the disease was excluded as no *PTPN11* mutations or loss of maternal chromosome 7 were detected in fibroblast, hair bulbs, mesenchymal cells and in the PB of patients' parents. *PTPN11* mutations and abnormal chromosome 7 in oral swabs were detected only in the active phase of the disease attributed to infiltration by mutated cells. To show that JMML is a clonal disease that originated from a pluripotent stem cells we screened *PTPN11* mutations and STR markers in different cell lineages of BM and PB. Notably, all cell lineages harbored different percentages of the mutation and the chromosome 7 abnormality.

Furthermore, GEP analysis of the twins identified two signatures: an AML-like signature in twin_01 and non AML-like signature in twin_02. The distinct GEP signatures predicted a different course of disease for the twins. Indeed, twin_01 presented a more aggressive disease, relapsed at 8 months after HSCT and died; whereas twin_02 had a less aggressive disease and is in remission after HSCT.

Summary / Conclusion: We showed that mutation of *PTPN11* and maternal der 7 occurred as two concurrent prenatal events and concordance of the leukemia in both monoplacental twins may be attributed to the high probability of vascular anastomoses within the common placenta. Remarkably, the different GEP signatures at diagnosis predicted the large divergence in the clinical course of the disease in twins sharing most biological features. Further studies are in course to discover possible second hits that may explain the large discordance in the clinical course of disease in the two twins.

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FAMILIAL, NON-ZINC FINGER REGION, GATA2 MUTATED MYELODYSPLASTIC SYNDROME; ASSOCIATED WITH SHORTENED TELOMERE LENGTHS, VIRAL WARTS, PROFOUND MONOCYTE/B/NK CELL DEFICIENCY

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Background: GATA-2 is a transcription factor vital for stem cell homeostasis. Haploinsufficiency of *GATA2* results in familial immunodeficiency and myelodysplastic syndromes (MDS)/ acute myeloid leukemia (AML). Some of the well characterized familial *GATA2* mutated phenotypes include: MonoMAC syndrome (monocytopenia with *Mycobacterium avium* complex infections), DCML (dendritic cell, monocyte, B and NK lymphoid deficiency) and Emberger syndrome (primary lymphedema with or without deafness and risk for AML). The GATA-2 protein contains two highly conserved zinc finger domains mediating protein-DNA and protein-protein interactions. Mutations affecting these regions produce clinical syndromes as described above.
Aims: To characterize the genetic defect in a family that presented with an extensive history of MDS/AML, viral warts, and B/NK lymphoid immunodeficiency.
Methods: The propositus is a 37 year old male who was diagnosed with AML-MRC (myelodysplastic related changes) with trisomy 8. He had an extensive history of HPV driven warts, and reported a family history with multiple maternal relatives with warts, cytopenias and formal diagnoses of MDS/AML (2 maternal aunts, 1 maternal uncle and 1 maternal nephew who died of MDS/AML). He received standard induction chemotherapy with idarubicin and cytarabine and developed profound bone marrow (BM) aplasia. His course was complicated with an *Absidia* invasive fungal infection. He was given 12 granulocyte transfusions along with antifungal therapy and underwent extensive sinus debridement. At D+70 BM was hyoplastic and demonstrated recurrent disease. Due to the lack of a sibling donor, a matched unrelated donor was identified, salvage chemotherapy was administered, and the patient is currently undergoing a reduced intensity conditioning allogeneic stem cell transplant. At day 70, after due IRB approval and patient consent, blood samples, including DNA, were obtained. Tests performed included quantitative and qualitative, B/T/NK lymphoid cell assessment, gene sequencing for *GATA-2* (National Institute of Health), *RUNX-1*, *CEBPA*, *JAK2*, *CXCR4* mutations and flow-FISH for telomere length (TL) [Repeat Diagnostics, Vancouver, Canada]. His 16 year old daughter, who has multiple warts, underwent a BM biopsy that demonstrated MDS with diploid cytogenetics. His 9 year old son, who also has extensive warts and unilateral lymphedema was found to have a markedly hypoplastic BM without dysplasia. Both children had severe monocytopenia and profound monocyte/B/NK lymphoid deficiency.
Results: Gene sequencing identified a heterozygous *GATA2* mutation; c1339A>C, p.S447R. This mutation is located outside the second zinc finger and is predicted to be deleterious. Telomere length assessment revealed reduced TL among the granulocyte (< 1st percentile) and NK cell population (< 10th percentile). Among the lymphocytes, there were two populations of distinct TL. 35% had TL < 1st percentile while 65% had normal TL. These results are confounded by the recent exposure to chemotherapy (70 days) and TL results on the children are awaited. Total number of B cells and NK cells were reduced at 0 and 2% respectively and NK cytotoxicity was low at 1%. Neither the propositus, nor affected family members had MAC infections or defects involving T lymphocytes.
Summary / Conclusion: We describe a *GATA-2* mutation located outside the zinc finger region, associated with familial MDS/AML with high penetrance. Affected members have palmoplantar warts, reduced monocyte, NK and B lymphoid numbers and function. None of the affected patients had MAC infections. Based on preliminary reports there could be an association with shortened telomere lengths, resulting in extended cytopenias from standard chemotherapy and possible bone marrow failure. The final results will be available before presentation at the meeting.

MDS and bone marrow failure syndromes incl. PNH - Biology

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EVALUATION OF PRO-SURVIVAL PATHWAYS, INCLUDED AUTOPHAGY, IN HIGH-RISK MDS BONE MARROW AFTER TREATMENT WITH 5-AZACITIDINE TO RESTORE NORMAL HEMATOPOIESIS

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Background: Myelodysplastic Syndromes (MDS) are clonal diseases due to an uncoupling of proliferation and differentiation at the level of the hemopoietic stem cell with consequent ineffective hematopoiesis, peripheral cytopenias and variable risk of transformation to acute leukemia. Recently, 5-azacytidine (AZA) has been used to improve clinical outcomes, but despite an initial response obtained within first nine months of treatment, many patients progress to leukaemia and die within two years from the therapy start.
Aims: Evaluation of autophagy as a pro-survival compensatory pathway induced by long-term exposure to AZA in-vivo.
Methods: By reverse-phase protein microarray (RPMA), we analyzed bone marrow mononuclear cells (BMC) from 19 patients affected of high risk MDS, treated for 4 months with 5-azacytidine (median age 71 years, M/F=12/5). Treatment consisted of 4 cycles of 100mg flat dose for 7 days+21 days of wash-out. In 7 cases the sample after 4 cycles of therapy was matched to the sample collected before the therapy start. RPMA was used to quantitatively map 45 cell signaling pathway endpoints, including survival, proliferation, drug resistance, apoptosis, and autophagy. In additional 4 patients, BMC have been exposed to AZA 5uM every 24 hours for three days, then washed and treated with 1-2.5-5uM chloroquine (CQ, an autophagy inhibitor) for 12-24 hours to evaluate cell viability using a luminescence assay, changes in protein expression and cell-cycle by flow cytometry. Dose-response curve was generated for each drug and each condition, including pre-treatment and wash of each investigated drug; combination index was detected using the Chou`s method.
Results: All patients were evaluable for response one month after the 4th cycle. Three patients were refractory, progressing to AML under treatment and 1 was a late responder (documented response after 7cycles). All other patients experienced hematologic improvement. Autophagy was induced independently form the clinical response observed: ATG5, Beclin 1 and LC3B were significantly elevated after treatment (p values respectively <0.0001, 0.0056 and 0.0124), at downstream of mTOR, since mTORSer2448, AktSer473, AktThr308, ERKThr202Tyr204 (and in general proliferation markers) were not affected. After three days of 5uM AZA, CQ inhibited cell growth in a dosage and time-dependent manner, via G1 *cell cycle* arrest. Combination index of AZA and CQ was found to be >1, thereby indicating antagonistic drug interaction, sustained by AZA-mediated autophagy induction.
Summary / Conclusion: Taken together our data confirm the ability of AZA to activate pro-survival pathways after long term exposure.

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IDENTIFICATION OF CAMPATH-1 (CD52) AS A DRUG TARGET IN NEOPLASTIC STEM CELLS IN 5Q- PATIENTS WITH MDS OR AML

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Background: The anti-CD52 antibody alemtuzumab (MabCampath) induces major responses in a group of patients with myelodysplastic syndromes (MDS). The mechanisms underlying this drug effect remain unknown.
Aims: In the present study, we asked whether CD34+/CD38− neoplastic stem cells (NSC) express CD52 in patients with MDS (n=29) or acute myeloid leukemia (AML, n=62).
Results: As assessed by flow cytometry, CD52 was expressed on NSC in 7/10 patients with MDS and isolated 5q-. In most other patients with MDS, CD52 was weakly expressed or not detectable on NSC. In AML, NSC displayed CD52 in 23/62 patients, namely 4 with complex karyotype including 5q-, one with 5q- and t(1;17;X), 2 with inv(3), 2 with t(8;21), one with inv(16), one with isolated del(13q), 3 with trisomy 8, one with monosomy7, and 8 with normal karyotype. In highly enriched NSC of 5q- patients, qPCR confirmed expression of CD52 mRNA, and clonality of NSC/LSC was confirmed by FISH. Consecutive studies revealed that CD52-expression correlates with EVI1 mRNA-expression-levels in NSC, but not with expression of CD300a mRNA expression. The CD52 antibody alemtuzumab induced complement-dependent lysis of CD34+/CD38−/CD52+ NSC/LSC in all patients examined, but did not induce lysis in CD52− NSC/LSC. Finally, alemtuzumab inhibited AML-formation in NOD-SCID-IL-2Rg$^{-/-}$ (NSG) mice.
Summary / Conclusion: In conclusion, our data show that the target-antigen CD52 is expressed abundantly in NSC/LSC in a group of patients with MDS and AML, including 5q- patients. These observations may have clinical implications and may explain clinical effects seen with alemtuzumab in these patients.

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FLOW CYTOMETRIC CLASSIFICATION OF 350 TYPE III PNH CLONES IN ITALY: A MULTI-CETRIC STUDY

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Background: So far , three different types of PNH have been described, based upon the evidence of bone marrow failure (BMF) and hemolysis: a) florid PNH (hemolysis+/BMF-), b) PNH within a BMF syndrome (hemolysis+/BMF+), c) subclinical PNH (hemolysis-/BMF+). In any case, the presence of a PNH clone detected by Flow Cytometry is a prerequisite for PNH diagnosis. In 2010, an Italian archive of FCM-detected PNH clones (HYPERLINK "http://int.clonepnh.com/" http://int.clonepnh.com/) was created on a multi-laboratory basis.
Aims: We had two main goals: a) to provide a large dictionary of Italian PNH clones; b) based upon stringent rules regarding the compilation of records, to obtain an auto-educational effect on participating laboratories.
Methods: Ninety-one laboratories participated in the project. We analyzed data from *Clone*PNH Archive to evaluate: a) how many PNH clones have been identified so far and how they are classifiable on the basis of the Reason for Testing (RFT) reported in clinical request; b) how many clones received more than one FCM determinations; c) among these, which was the initial RFT as well as the fate of the clone. Data refer to analysis registered in *Clone*PNH Archive up to 02/20/2013.
Results: Here, we describe the cellular composition and the clonal evolution of 350 type III (complete defect of GPI-linked proteins) PNH clones identified during the study. Forty-nine of these clones (16.6%) were accompanied by a PNH-II clone (partial defect of GPI-linked proteins). Hemoglobinuria was the most frequent (45.4%) reason for testing (RFT), followed by aplastic anemia (AA, 17.7%), MDS (11.1%), unexplained cytopenia (UC, 8%), hemolytic anemia (5.7%), , BMF (4.6%), atypical venous thrombosis (3.1%), other (8%). Fluorescent Aerolysin (FLAER) was used since 2007, with an increasing % of utilization, from 4% to 60% of cases. CD24 utilization also progressively increased. CD59 was the most used antigen for RBC typing. The most used gating strategies were based upon physical parameters for RBC, CD45 and/or CD33 *vs* side scatter for granulocytes and monocytes. The 350 clones were categorized into 3 classes according to their size, determined as the percent of PNH cells in peripheral granulocytes: 0.01-10% (134 clones, or 38.3%, defined as "small"), 10.1%>70% (74 clones, or 21.1%, defined as "intermediate"), 70.1%>100% (131 clones, or 37.4%, defined as "large"). This distribution was significantly different from that expected on the basis of a random distribution within the three classes (*i.e.* 10%, 60% and 30%): *chi* square was 51 with a p value <0.0001. Ninety-six clones were sequentially studied (with a follow up ranging from 3 to 74 months): Twenty-nine of them (30%) showed a change in category (19 increased and 10 decreased). Just 5 clones jumped from "smallest" to "biggest" category or *vice versa* (three increased ant 2 decreased).
Summary / Conclusion: This is the first multi-laboratory relational database of FCM-detected PNH clones described so far. An auto-educational goal was reached, since general sensitivity increased progressively and reagent choice significantly changed, leading to a stable FCM protocol, consisting of FLAER and CD24 for granulocytes, FLAER and CD14 for monocytes, CD59 for erythrocytes. As regards clonal evolution, the rarity of migration between extreme categories suggests that the belonging to these ones could be sustained by different backgrounds and pressures, as suggested by different association with HLA genotypes.

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CYTOGENETIC CHARACTERIZATION OF CD34+ HEMATOPOIETIC PROGENITOR CELLS AND MESENCHYMAL STROMAL CELLS IN MYELODYSPLASTIC SYNDROME (MDS)

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Background: Chromosomal abnormalities play an important role in understanding biology of the disease, predicting prognosis and choosing therapeutical approach to MDS patients. It is known that MDS is caused by clonal impairment of hematopoietic stem cell. Moreover dysfunction of bone marrow stroma could also contribute the pathogenesis of the disease.
Aims: To investigate and compare cytogenetic changes in bone marrow (BM) and peripheral blood (PB) CD34+ hematopoietic progenitor cells and mesenchymal stromal cells (MSC) in MDS.
Methods: The study comprised 42 patients: 35 MDS (RA-4, RCMD-15, RARS-2, RAEB-11, 5q-syndrome-3), 7 transformed to acute myeloid leukemia (AML) and 7 healthy individuals. Male/female-19/23. Age median was 60 (range 19-77). Cytogenetic analysis was performed by G-banding and FISH.
Results: BM karyotype was abnormal in 21 (50%) out of 42 pts. We confirmed the presence of the same abnormalities in magnetically separated BM and PB CD34+ cells using FISH DNA probes LSI(5q33-q34), LSI(7q31)/CEP7, CEP8, LSI AML1/ETO, CEPX/CEPY, LSI(20q12) (Vysis, USA), EVI t(3;3),inv(3)(3q26) Break (Kreatech Diagnostics, Netherlands). The percentage of abnormal BM cells, BM CD34+ and PB CD34+ cells had no statistical difference (means 62.7%, 69.2% and 70.5% respectively). However, in 3 pts we detected significant difference between clone sizes in BM cells and CD34+ cells. In 2 pts with isolated del(5q) (27% clone) and -21 (25% clone) the percentage of abnormal nuclei in BM cells was much lower than in CD34+ cells, in which it constituted 75% and 85% respectively, and in 1 pt with -7 clone=60% in BM there was only 26% abnormal CD34+ cells. We didn't reveal any cytogenetic abnormality in CD34+ cells from pts with normal BM karyotype. We performed flow cytometry (FACS Canto II, BD) to calculate the percentage of CD34 cells in BM and PB in 8 pts (RCMD-1, RAEB-6, AML-1) using monoclonal antibodies CD34-FITS and CD45-PE (BD, USA). According to ISHAGE protocol 100,000 events were counted. In all pts CD34+ cells showed an abnormal granularity and a large proportion of non-viable cells (mean 6.7%). The mean percentage of BM CD34+ cells was 4.65% in RAEB pts. There were 0.9% of BM CD34+ cells in patient with RCMD and 15% in AML patient. The mean percentage of PB CD34+ cells was 0.4% in RAEB pts. We didn't detect PB CD34+ cells in RCMD and AML pts. MSC karyotype was carried out in 26 pts (22 MDS and 4 AML). We didn't obtain the growth of MSC culture in 38% pts (16 out of 42) due to a low proliferation ability of these cells. MSC karyotype from AML pts was normal. There were structural abnormalities in 2 (9%) out of 22 MDS pts: in one pt with constitutional inv(9)(p13q21) we detected non-clonal translocation-46XY,t(2;22)(p10;q11),inv(9)(p13q21)[1]/46XY,inv(9)(p13q21)[19] and in the other pt–clonal abnormality 46XY,add(2q)[7]/46XY[13]. The 1st pt had only constitutional inv(9) in BM cells and the 2nd one had a complex karyotype. We performed FISH in MSC from 12 pts with cytogenetic abnormalities in BM with appropriate DNA probes, which didn't show any of those abnormalities in MSC. MCS karyotype from all healthy individuals was normal.
Summary / Conclusion: Hematopoietic and mesenchymal progenitor cells in MDS have different cytogenetic changes. CD34+ cells from BM and PB display the same abnormalities as whole population of BM cells. Cytogenetic analysis in MSC revealed structural clonal and non-clonal aberrations in MDS pts, which were different from BM and CD34+ cells abnormalities in these patients. We didn't obtain any cytogenetic abnormality in MSC from AML pts. Our results suggest that the stroma in MDS is not a part of the malignant clone, but it shows genetic instability and may play an important role in the pathogenesis of MDS.

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POLYMORPHISMS IN GENES INVOLVED IN DNA REPAIR AND FOLATE PATHWAY SIGNIFICANTLY IMPACT ON SURVIVAL IN LOW-RISK, UNTREATED MYELODYSPLASIA

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Background: the analysis of polymorphisms in DNA repair genes and folate pathway could help to identify patients with possible different prognosis and survival. Single nucleotide polymorphisms (SNPs) and insertion/deletion polymorphisms have been described in association with prevalence, progression-free (PFS) and overall survival (OS) in both solid and hematological cancers. However, up to now, correlations between polymorphisms of genes involved in DNA repair and folate pathways have not been demonstrated in patients with myelodisplastic syndromes (MDS) and low or intermediate-1 IPSS.

Aims: to prospectively evaluate the frequencies of the studied polymorphisms (XRCC1, XRCC3, TS, MTHFR and APE1), not previously evaluated in MDS; to investigate the impact of the studied polymorphisms on survival of patients with low/Int-1 IPSS MDS.

Methods: We prospectively genotyped 54 MDS patients (median age 75 years) with IPSS low (n=23) or intermediate-1 (n=31) treated with best supportive care only. Genomic DNA was isolated from 1ml of peripheral blood by means of commercially available kits. Polymorphisms were determined by PCR-HRM (High Resolution Melting) assay and restriction digests of PCR products. All samples were analyzed for the following polymorphisms: XRCC1 194 (rs1799782 C/T, Arg/Trp) and 399 (rs25487 G/A, Arg/Gln), XRCC3 241 (rs861539 C/T, Thr/Met), TS 5'-UTR (2R/3R and rs183205964 G/C) and 3'-UTR Ins/Del (rs11280056 6bp+/6bp-), MTHFR 677 (rs1801133 C/T, Ala/Val) and 1298 (rs801131 A/C, Gln/Ala), APE1 148 (rs1130409 T/G, Asp/Glu). The characteristics and laboratory features of MDS patients with each polymorphisms were compared using X^2(chi square)-test and Mann-Whitney test.

Results: The frequencies of genotypes of studied gene polymorphism in patients with low/Int-1 risk were as follows: Arg/Arg 91%, Arg/Trp 9% for XRCC1 194; Gln/Gln 11%, Arg/Gln 43%, Arg/Arg 46% for XRCC1 399; Met/Met 41%, Thr/Met 23%, Met/Thr 23%, Thr/Thr 13% for XRCC3 241; 2R/3G, 3C/3G and 3G/3G 50%, 2R/2R, 2R/3C and 3C/3C 50% for TS5'-UTR; Ins/Ins 26%, Ins/Del 50%, Del/Del 24% for TS3'-UTR; Ala/Ala 30%, Ala/Val 46%, Val/Val 24% for MTHFR 677; Ala/Ala 48%, Gln/Ala 52% for MTHFR 1298 and Glu/Glu 13%, Glu/Asp 50%, Asp/Asp 37% for APE1 148. No significant associations between polymorphisms and demographic, clinical or prognostic characteristics were observed. When comparing all the allele and genotype frequencies according to OS, the groups of patients with XRCC1 399 Arg/Arg, TS5'-UTR 2R/3G, 3C/3G, 3G/3G, TS3'-UTR Del/Del and MTHFR 677 Val/Val genotypes showed longer OS (XRCC1 399 Arg/Arg vs non- Arg/Arg P=0.015; TS5'-UTR 2R/3G, 3C/3G, 3G/3G vs 2R/2R, 2R/3C, 3C/3C P=0.03; TS3'-UTR Del/Del vs non-Del/Del P=0.04 and MTHFR 677 Val/Val vs non-Val/Val P<0.001). On the other hand, no statistically significant association between XRCC1 Arg194Trp, XRCC3 Thr241Met, MTHFR Gln1298Ala polymorphisms and OS was found. In multivariate analysis XRCC1 399 Arg/Arg (P=0.007), TS5'-UTR 2R/3G, 3C/3G, 3G/3G (P=0.038), TS3'-UTR Del/Del (P=0.041) and MTHFR 677 Val/Val (P=0.001) genotypes were independent prognostic factors, significantly associated with longer survival.

Summary / Conclusion: BER gene and folate pathway gene polymorphisms may affect the prognosis and survival of patients with low-Int-1 myelodisplastic syndrome treated with best supportive care only. If confirmed in larger series, these polymorphisms could help to identify a subset of patients with short survival, who could benefit from an early treatment with hypometilating agents which are, at present, not indicated for the treatment of patients with MDS and low/Int-1 IPSS.

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HIGHER LEVELS OF CIRCULATING MYELOID DERIVED SUPPRESSOR CELLS (MDSC) IN MYELOID DYSPLASTIC SYNDROMES (MDS)

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Background: MDSC is a group of immature cells that can differentiate into dendritic cells (DC), granulocytes or macrophages. An expansion of these cells has been described in many cancers and in autoimmune diseases. Arginase-1- and ROS-activity, Interleukin (IL)-10 and Transforming Growth Factor (TGF)-β, take part in direct and indirect inhibition of T cells.

Aims: Our hypothesis is that immunosuppressive effects of MDSC participate in MDS immunopathogenesis. Chemokine receptors direct leukocyte migration, and we therefore investigated the levels of CX3CR1, CXCR3 and CXCR4 on MDSC from MDS patients, to see if this expression could be involved in MDSC accumulation in MDS bone marrow.

Methods: We investigated the percentages of MDSC in fresh peripheral blood (PB) from 24 MDS patients and fresh bone marrow (BM) samples from 12 MDS patients from the out-patient clinic at Haukeland University Hospital. 17 of the patients had either RA, RARS or RCMD, 7 patients had MDS with excess of blasts. We also analysed fresh peripheral blood samples from 5 age-matched healthy controls. Flow cytometry of PB and BM was performed after red cell-lysis using the following markers: Live/dead, Lineage (CD3, CD16, CD19, CD20, CD34, CD56, HLA-DR), CD33, CD11b, CD15, CD66b, CD14, IL-10, TGF-beta, CX3CR1, CXCR3 and CXCR4. Internal staining was performed with and without stimulation with IL-6 and IL-1beta.

Results: We found that cells with MDSC phenotype exist in PB of MDS patients in higher percentages than in healthy controls, both when comparing healthy controls (N=5, median 0.7 % (0.4-1.6%) and MDS with less than 5 % blasts (N=17, median 1.75 % (0.3-3.9%) P=0.031), and when comparing the same healthy controls with patients with MDS RAEB-1 and RAEB-2 (N=7, median 2.6 % (2.1-17.9%) P=0.003). There was also a significant difference when comparing MDS RA, RARS, RCMD and RAEB-1 and -2 (P=0.031). We found a significant correlation between MDSC and blasts in RAEB (Pearsons correlation significant at the 0.01 level, R^2 Linear 0.837). There was no correlation between MDSC percentages and bone marrow blast percentages in RA, RARS and RCMD. No correlation was found between subsets of granulocytic and monocytic MDSC and levels of neutrophils or monocytes in blood for neither group. In peripheral blood CX3CR1 was expressed on a higher level on M-MDSC than G-MDSC P=0.08. There was higher expression of CXCR3 on G-MDSCs than M-MDSC (P=0.046), but CXCR4 was higher expressed on M-MDSC than G-MDSC (P=0.011). In bone marrow, levels of G-MDSC were significantly higher than of M-MDSC (N=12, P=0.002), and the same chemokine receptor pattern was observed as in peripheral blood MDSC; CX3CR1 and CXCR4 significantly higher on M-MDSC (N=10, P=0.013and N=9, P=0.007, respectively).

Summary / Conclusion: Circulating MDSCs are increased in peripheral blood of MDS patients, indicating a possible role for these cells in MDS immunopathogenesis. Higher expression of chemokine receptors in subtypes of MDSCs may lead to redistribution of these cells to the bone marrow.

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DOWNREGLULATION OF BNIP3 AND NIX, TARGETS OF HIF-1 ALPHA, IN HIGH-RISK MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA

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Background: HIF-1 alpha is a key component of hypoxia-responsive regulatory pathways and is a transcription factor that regulates several signaling pathways involved in cell differentiation, angiogenesis, apoptosis and autophagy. Expression of HIF-1 alpha protein levels has already been correlated with poor overall survival and disease progression in myelodysplatic syndromes (MDS). Among other proteins, BNIP3, NIX and GLUT-1 are targets of HIF-1 alpha. *BNIP3* and *NIX* are two members of the Bcl-2 family of proteins and regulate both apoptotic and autophagic pathways. The glucose transporter GLUT-1 participates in the control of cell energy metabolism and its activation has been related to the energy imbalance found in several malignancies.
Aims: We aimed to characterize the expression of *BNIP3, NIX, GLUT-1* and *HIF-1 alpha* in bone marrow samples from healthy controls and in patients with MDS and AML.
Methods: We studied total bone marrow cells from 24 healthy controls, 47 patients at diagnosis of MDS and 41 patients at diagnosis of AML. MDS patients were grouped in low-risk and high-risk according to WHO (RAUD/RCMD/del5q/=34; RAEB1/RAEB2=13) and IPSS (low/INT1=41; INT2/high=6). *BNIP3*, *NIX*, *GLUT-1,* and *HIF-1 alpha* expression were evaluated by quantitative PCR. Appropriated statistical analysis was performed and the data were showed as median.
Results: *BNIP3* and *NIX* expression were significantly decreased in high-risk MDS and AML patients compared to healthy controls (BNIP3: 5.42 *vs.* 0.67 *vs.* 2.8 and NIX: 1.95 *vs.* 1.03 *vs.* 0.89 for controls, high risk MDS and AML [WHO], respectively; P<0.05). When MDS patients were stratified by IPSS, similar results were found. *HIF-1 alpha* expression trended to decrease in high risk MDS and AML compared to healthy controls, although the differences were not statistically significant (*HIF-1 alpha*: 0.37 *vs.* 0.15 *vs.* 0.24 for controls, high risk MDS and AML [WHO], respectively; *P*>0.05). *GLUT-1* expression was not modulated between normal, MDS and AML samples. Interestingly, a positive correlation was observed between *NIX* and *HIF-1 alpha* expression (P=0.0001; r=0.46). No correlation was observed between *HIF-1 aplha* and *BNIP3* or *GLUT-1* expression.
Summary / Conclusion: We demonstrated that *BNIP3* and *NIX* expression are significantly decreased in bone marrow samples from high-risk MDS and AML patients, and a positive correlation between *NIX* and *HIF-1 alpha*. It is well know that HIF-1 alpha protein levels are controlled by oxygen availability resulting in the regulation of numerous targeting genes. We then provide evidence that *HIF-1 alpha* mRNA levels may be important for the control of *NIX* expression. These data point out to a possible role of HIF-1 alpha, NIX and BNIP3 in MDS pathophysiology.
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EVALUATION OF A HIGH THROUGHPUT MUTATION-SCREENING STRATEGY IN MYELODYSPLASTIC SYNDROME PATIENTS AND ACUTE MYELOID LEUKEMIA USING TARGETED-GENE ENRICHMENT TECHNOLOGY

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Background: A number of recurrent mutations are implicated in the disease biology of myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Clinically, there is a continuum between *de novo*, therapy-related and secondary AML (t/sAML), and MDS of the various risk categories, but the molecular characteristics of these conditions have not been compared.
Aims: By applying targeted sequencing of a selection of candidate genes with relevance for both MDS and AML we hypothesized that we would be able to identify molecular patterns with relevance for the diagnostic and prognostic process.Sequencing data was validated using the Sequenom methodology, which potentially could develop into an inexpensive and quick clinical tool.
Methods: We analyzed 200 MDS and AML patients diagnosed according to the WHO classifications for MDS, MDS/MPN and AML. The cohort included 100 MDS and 100 AML patients and were grouped as follows; 23 RARS/RCMD-RS/RARS-T, 19 lower-risk MDS with del (5q), 19 RAEB-1 / RCMD, 24 RAEB-2, 15 CMML, 25 AML following MDS, 11 AML following MPN, 17 therapy related AML, and 47 *de novo* AML.Diagnostic samples were investigated for mutations in 22 genes using Halogenomics™ targeted-gene enrichment technology followed by high-throughput sequencing using a pooling strategy of 10 samples per pool.
Results: Analysis of sequencing data relieved 223 single nucleotide variations affecting protein sequences. All mutations found by Halogenomics enrichment, as well as 9 hot spot mutations in SF3B1, SRSF2 and U2AF35 genes, were validated in individual patients by the Sequenom™ genotyping system. Sequenom analysis confirmed 100 SNVs, of which 23 were SNPs (according to 1000 Genome) and 77 were defined as mutations. Based on Sequenom data 61/100 (61%) of MDS and 51/100 (51%) of AML samples carried mutations at least in one gene while 22% and 16% showed more than one mutation, respectively. *TET2*, *SRSF2*, *SF3B1*, *U2AF35* and *IDH2* were the most mutated genes while no mutation was detected in 7 genes *ETV6*, *KDM6A*, *KIT*, *MPL*, *NPM1*, *SH2B3*, and *WT1*. The most common mutated genes in the respective subgroups are shown in Table 1. Gene function analysis revealed that gene mutations in MDS patients was mainly in Splicing machinary and Epigenetics factors while in AML patints gene mutations occured more in cell signaling and Oncogenes.
Summary / Conclusion: We conclude that next generation sequencing of candidate genes may help to understand the spectrum of disorders on the boarder between MDS and AML. Moreover, genotyping by Sequenom may, in the future, develop into a rapid and cost-effective method for clinical detection of hot spot mutations in a high troughput manner.

Table 1. Most frequent mutated genes in each subgroup of patients.

Subgroups	No. Patients	Gene 1 (%)	Gene 2 (%)
RARS	23	SF3B1 (61%)	TET2 (17%)
Del 5q	19	TET2 (11%)	SRSF2, ASXL1 (5%)
RAEBI +RCMD	19	IDH1 (21%)	U2AF35 (21%)
RAEBII	24	SRSF2 (21%)	IDH2 (17%)
CMML	15	TET2 (60%)	SRSF2 (40%)
MDS-AML	25	U2AF35 (21%)	TET2 (17%)
MPN-AML	11	ASXL1 (27%)	TP53 (18%)
t-AML	17	SRSF2 (18%)	DNMT3A (18%)
De novo AML	47	DNMT3A (33%)	IDH1, SRSF2 (33%)

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IMPACT OF 5Q BREAKPOINTS ON CLINICAL OUTCOMES IN PATIENTS WITH IPSS LOW-/INT-1-RISK MYELODYSPLASTIC SYNDROMES (MDS) AND ISOLATED DEL(5Q) TREATED WITH LENALIDOMIDE IN THE MDS-004 STUDY

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Background: Deletions of the long arm of chromosome 5 [del(5q)] are the most frequent abnormalities observed in MDS (Solé F, *et al*. Br J Haematol. 2000;108:346-56). Proximal and distal del(5q) breakpoints can vary considerably. Breakpoints at the proximal and distal extremes of 5q may result in a more aggressive disease phenotype (Jerez A, *et al*. J Clin Oncol. 2012;30:1343-9). The exact clinical impact of affected 5q regions on overall survival (OS), acute myeloid leukemia (AML) progression, and response remain largely undefined.
Aims: This retrospective study analyzed OS, AML progression, and the rate of RBC-transfusion independence (TI) ≥26 weeks according to the 5q breakpoints in lower-risk MDS patients with isolated del(5q) treated with lenalidomide, in an attempt to ascertain if the proximal level of the breakpoints influences OS, AML progression, or the RBC-TI response.
Methods: Of 205 MDS patients with International Prognostic Scoring System (IPSS) Low-/Int-1-risk del(5q) included in a multicenter phase 3 study (MDS-004; Fenaux P, *et al*. Blood. 2011;118:3765-76), 137 had isolated del(5q) with available data on the proximal and distal breakpoints and were included in the current analysis. Fluorescence R banding cytogenetics were used to determine 5q breakpoints by central cytogenetics review. OS, time to AML progression, and rate of RBC-TI ≥ 26 weeks were analyzed according to the most commonly occurring 5q breakpoints in patients who received lenalidomide from study start (n=91); i.e. excluding those who were randomized to placebo.
Results: Overall, the identified 5q breakpoints were q13q34 (6.6%), q14q34 (64.2%), q15q34 (2.2%), q21q34 to q31q34 (26.3%), and q31q35 (0.7%). Clinical outcomes were analyzed according to the most frequent q14q34 breakpoint versus all other 5q breakpoints. Among lenalidomide-treated patients with breakpoints at q14q34, baseline characteristics such as age, hemoglobin and platelet levels, absolute neutrophil counts, and time from diagnosis were similar to those patients with other breakpoints. OS was similar for patients with breakpoints at q14q34 (n=59) versus other positions (n=32) (logrank test P=0.6533); median OS was 3.8 years (95% confidence interval [CI]: 2.5–not evaluable [NE]) and 4.4 years (95% CI: 2.3–NE), respectively. Time to AML progression was comparable for patients with q14q34 versus other breakpoints (logrank test P=0.2662); 5-year rates were 37.3% (95% CI: 23.7–50.9%) and 34.5% (95% CI: 13.6–55.3%), respectively. Results for time to AML progression remained similar when analyzed with death as competing risk (Gray's test P=0.1442). Rates of RBC-TI ≥ 26 weeks were similar across patients with q14q34 versus other breakpoints (P=0.830).
Summary / Conclusion: The 5q breakpoint q14q34 was the most frequent breakpoint among patients with IPSS Low-/Int-1-risk MDS with isolated del(5q), and was not associated with altered probabilities for AML progression and OS, or altered rates for RBC-TI ≥ 26 weeks compared with breakpoints occurring at other positions. These findings suggest that there are unlikely to be any genes involved in the very proximal portions of the isolated deletion 5q chromosome that may influence response, OS, or progression to AML.

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PROGNOSTIC IMPACT OF DER(1;7) IN MDS IS DIFFERENT FROM DEL(7Q)

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Background: Der (1;7) (p10;q10) is a rare abnormality in MDS, occurring in less than 1% of patients with primary MDS (Schanz *et al*. JCO 2012). The unbalanced translocation results in a deletion of 7q and a trisomy of 1q. As chromosome 7 is involved in this aberration, der(1;7) was suspected to be associated with an adverse prognosis. However, less is known about the real prognostic impact of der(1;7) in MDS.
Aims: The present study was performed to analyze the prognostic impact of der(1;7) as compared to a partial or total monosomy 7 in a multicentric cohort of patients with MDS.
Methods: In total, 35 patients with der(1;7) were coalesced from 8 European centers and retrospectively analyzed. The patients were dervived from the Munich leukemia Lab (n=20), the University of Leuven (n=5), the University of Nice (n=3), the Institut de Recerca contra la Leucèmia Josep Carreras (n=3), the University of Goettingen (n=2), the University of Hamburg (n=1) and the University of Dresden (n=1). Additionally, 107 patients with del(7q) (n=45) and -7 (n=62), originating from a monosomy 7 database (Schanz *et al*., EHA 2012) were analyzed. Time-to event analyses were performed by the method of Kaplan and Meier.
Results: Among the 142 patients with der(1;7), del(7q) and -7, 129 patients (90.8%) showed a primary MDS. A disease-modifying treatment was applied in 7 (5%) of all patients. In the group of der(1;7), 31 patients (88.6%) showed the abnormality as an isolated aberration and 4 (11.4%) had one additional abnormality. As compared to del(7q)/-7, a significantly lower (P=0.01) mean hemoglobin level was observed (der(1;7): 9.6 g/dl; del(7q)/-7: 10.3 g/dl). Furthermore, the lactate dehydrogenase (LDH) was significantly lower in these patients (251 U/l in der(1;7); 741 U/l in del(7q)/-7, P<0.001). The bone marrow blast count, platelet count and ANC count did not show a significant difference. The median survival was 53.4 months in der(1;7), 23.5 months in del(7q) and 13.8 months in -7 (P<0.01) and the AML-free survival was not reached in der(1;7), 9.6 months in del(7q) and 6.3 months in -7.
Summary / Conclusion: The prognosis of der(1;7) is better as compared to del(7q) and patients with der(1;7) show a very low risk of transformation to AML. Hence, der(1;7)(q10;p10) needs to be prognostically classified independently from the deletion of 7q that results from this unbalanced translocation.

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CLINICAL SIGNIFICANCE OF ASXL1 MUTATION IN PATIENTS WITH PRIMARY MYELODYSPLASTIC SYNDROME AND ITS STABILITY DURING DISEASE PROGRESSION

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Background: Mutations of the additional sex comb-like 1 (*ASXL1*) gene were recently identified in a substantial portion of patients with myelodysplastic syndrome (MDS), but the interaction of this mutation with other genetic alterations and the stability of this mutation during disease progression remain to be determined.
Aims: We correlated the *ASXL1* mutation with clinical characteristics and other genetic alterations in 455 *de novo* MDS patients. Paired samples during disease progression were also analyzed in 108 patients to evaluate the stability of *ASXL1* mutation.
Methods: According to French-American-British (FAB) classificationfour hundred and fifty fiveadult MDS patients with adequate cryopreserved bone marrow cells for study were recruited for gene mutation analyses. The *ASXL1* exon 12 until the stop codon was amplified by 3 pairs of primers and sequenced by another 6 internal primers. Detection of mutations in other genes, including *IDH1/2*, *JAK2*, *MLL*-PTD, *AML1/RUNX1*, *FLT3*-ITD, *WT1*, *NRAS*, *KRAS*, *EZH2*, and *DNMT3A* was performed.
Results: *ASXL1* mutations were identified in 106 (23.3%) of the 455 patients with primary MDS based on the FAB classification and 63 (17.9%) of the 351 patients based on the 2008 WHO classification. *ASXL1* mutation was closely associated with male sex, older age, normal karyotype, chronic myelomonocytic leukemia and refractory anemia with excess blasts. The patients with *ASXL1* mutation had significantly higher incidences of concurrent *AML1/RUNX1* mutation (32.2% *versus*. 7.8%, P<0.001), *EZH2* mutation (23.6% *versus*. 0.9%, P<0.001), *IDH* mutation (11.4% *versus.* 2.3%, P<0.001), *NRAS* mutation (8.6% *versus*. 3.2%, P=0.028), and *JAK2 mutation (4.4% versus*. 0%, P=0.019) than those with wild type *ASXL1*. With a median follow-up of 58.2 months, there was a significant correlation between *ASXL1* mutations and acute leukemia transformation (36.3% *versus.* 17.9%; P<0.001). Patients with *ASXL1* mutation had a significantly poorer overall survival than those without the mutation (median, 18.7 months *versus.* 32.5 months, P<0.001). *ASXL1* mutation was an independent poor prognostic factor in all MDS patients (HR=1.496, CI 95%, 1.040-2.153, P=0.030) and those with normal karyotype (HR=2.217, CI 95%, 1.253-3.925, P=0.006). To investigate the role of *ASXL1* mutation in disease progression, sequential analysis of the gene mutation was performed in 299 samples from 108 patients, including 32 patients with *ASXL1* mutation and 76 patients without the mutation at the beginning. Among the 32 *ASXL1*-mutated patients,two patientslost the original *ASXL1* mutation following transplantation; all of the remaining 30 *ASXL1*-mutated patients retained

the same mutation during follow-ups, including 17 with acute leukemic transformation. Among the 76 *ASXL1*-wild patients who were sequentially studied, only one patient acquired *ASXL1* mutation when the disease progressed to acute myeloid leukemia. Using a more sensitive TA cloning technique, no *ASXL1* mutation could be detected in the initial sample of this patient.
Summary / Conclusion: In conclusion, *ASXL1* mutations were associated with distinct clinical-biologic characteristics and correlated with shorter survival in MDS patients. Further, sequential analysis showed that *ASXL1* mutations were in general stable during disease progression, but may contribute to disease progression in few patients.

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A LONGITUDINAL OBSERVATIONAL STUDY ON THE HEALTH-RELATED QUALITY OF LIFE IN IPSS LOW-RISK MDS - IMPACT OF TIME COURSE AND TRANSFUSION NEED

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Background: The EuropeanLeukemiaNet has initiated a prospective, multicenter European Registry (EUMDS) for newly diagnosed IPSS low and intermediate-1 MDS. In MDS health-related quality of life (HR-QoL) represents an important parameter in patient reported outcome as well as in individualized therapy planning. Thus, the EQ-5D (European quality group 5 dimensions) descriptive system was used in this registry to evaluate HR-QoL at initial diagnosis and at follow-up visits every six months.
Aims: To describe the time course as well as the impact of blood transfusions and hemoglobin (Hb)-levels on HR-QoL in this cohort of MDS-patients.
Methods: Results from EQ-5D Visual analog scale (VAS) were analyzed. A multiple linear regression model incorporating random effects (allowing both the baseline score and the rate of change over time to vary between patients) was performed. Adjusting factors included in the model are age at diagnosis, sex, WHO-subgroup, country and haemoglobin measured at each follow-up visit. The effect of transfusions was assessed in two ways: First, all patients that received any transfusion during follow-up were compared to all patients who received no transfusions. Second, the transfusion intensity (defined as the average number of units received per month) recorded for the previous six months at each visit was included. Interactions between the trend in VAS over time and the other factors were analysed.
Results: Median age among the first 1000 patients in the registry is 74.2 years (range 18.7-95.3) with 60.3% male patients and a median follow-up of two years. 936 patients with a definable follow-up period were eligible for this analysis. The mean VAS at the first visit was 69 (±20 SD). In both regression models, in the absence of any interacting effects, there was a moderate average decrease in VAS of approximately 0.2 units per month (95% CI: -0.28, -0.15) identified. Analyses of the random effects indicated that the baseline VAS varied between patients as did the rate of decrease over time. Older patients had a lower average VAS score (0.46 lower per life year; [-0.56; - 0.35] 95% CI; P<0.001). There was no evidence of difference in VAS between men and women (-2.23 lower among women; [-4.51; 0.04] 95% CI; P=0.055). VAS varied between the diagnostic groups and varied between countries. VAS was varied by WHO-subtype with RAEB-2 displaying the lowest levels and 5q-syndrome the highest (P=0.045). Marked differences between different countries were also observed (P<0.0001) and there was some evidence that the rate of decrease in VAS over time varied by country. VAS was positively associated with Hb values over time: VAS increased by 1.6 for each 1 g/dL increase in Hb ([1.2; 2.01] 95% CI; P<0.001). These effects were consistent regardless of which of the regression models were examined. Transfused patients had, on average, lower VAS scores (3.06 units lower [-5.52; -0.59] 95% CI; P=0.015) than untransfused patients. In addition, there was evidence that the rate of change in VAS over time was different for transfused and untransfused patients: no significant change over time among untransfused patients (-0.09 units/months; 95%CI: -0.19, 0.01) but a decline of 0.32 units/month (95% CI: -0.41, -0.23) in transfused patients. When transfusion intensity that varies over time is incorporated into the model, higher rates of transfusion are associated with lower VAS scores (-1.64 lower VAS for each additional unit transfused per month, 95% CI: -2.84, -0.44). There was no interaction detected between this effect and the change in VAS over time.
Summary / Conclusion: HR-QoL in MDS is associated with diagnostic subgroups, Hb-values and transfusion need. Analyses of time course reveal a faster decrease in VAS over time in transfused as compared to untransfused patients. Moreover, high rates of transfusions are associated with lower HR-QoL VAS. Prevention of transfusion need might sustain and improve the QoL in MDS patients.

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HIGH DOSES OF ELTROMBOPAG ARE WELL-TOLERATED IN CONJUNCTION WITH AZACITIDINE AND THE COMBINATION DEMONSTRATES ENCOURAGING ACTIVITY IN PATIENTS WITH MDS AND AML.

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Background: Pre-existing thrombocytopenia in MDS/AML is exacerbated during the initial cycles of azacitidine (AZA) therapy, resulting in bleeding risk and possible platelet transfusion. Eltrombopag is an oral TPO-receptor agonist. *In vitro*, it has anti-proliferative effects on AML blasts. Hence, it may have clinical utility in MDS/AML as a treatment for thrombocytopenia as well as the leukemic process.
Aims: We wished to assess the safety and initial signal of efficacy of the combination of azacitidine and high doses of eltrombopag in MDS/AML.
Methods: This is a phase-II, single arm, dual-centre, open-label study of escalating doses of eltrombopag with AZA. Inclusion required: a diagnosis of relapsed or *de-novo* MDS/CMML/AML with marrow blasts 5-30%; or symptomatic cytopenia; or blasts 31-50% if either ≥65 years *or* previously-treated disease; and platelets ≤150x10^9/L. The primary endpoint was the rate of grade III/IV non-haematological events related to therapy. Secondary endpoints were AE rates, overall response rates, and survival. Eltrombopag 50 mg/day continuously was begun with AZA 75 mg/m^2 d1-5, & 8-9 on a 28d cycle. For patients with platelets ≤100 there was a 14d pre-treatment with eltrombopag monotherapy. Intrapatient dose-escalation of eltrombopag to 100 mg, 200 mg and 300mg fortnightly occurred in those with cycle start platelet counts <75 or nadir ≤50. Eltrombopag was ceased after 6 months but could be restarted for severe thrombocytopenia at investigator discretion.
Results: 17 of a planned 25 patients have been recruited; 6 had received prior therapy for MDS/AML, 11 had baseline marrow blasts ≥10%. Median baseline platelet count was 37.5 (range 17-127). A median of 8 (2-18) cycles of AZA and 5(1-11) cycles of eltrombopag have been delivered.
One patient developed grade-III eltrombopag-related LFT abnormalities (resolved). There were no other grade III/IV AEs attributable to the combination. Thrombocytosis resulting in eltrombopag cessation occurred in 4 patients (at 50, 50, 150 and 200mg), without complications. 10 patients experienced reversible skin yellowing. Activity was seen in 76%: 4CR, 3CR-marrow, 3 HI-P (2 unconfirmed), 1 HI-E (unconfirmed), 2 with >50% blast reduction from >20% (1 also with Hi-P unconfirmed). 2 patients with previously-treated AML progressed at cycle 2 and one with untreated AML progressed at cycle 10. Platelet improvement was seen in 53% (8/15) of patients with baseline platelets <100 at median (range) at 63d (21-106) following commencement of AZA. One patient (7%) had an improvement in platelets following the monotherapy phase.
Summary / Conclusion: A strategy of intra-patient dose escalation of eltrombopag from 50-300mg with AZA has promising response rates, is well-tolerated and has an acceptable rate of AE. However, the 14d 50mg eltrombopag monotherapy pre-phase was insufficient to lead to consistent haematological improvement.

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OUTCOMES OF INTERMEDIATE OR HIGH RISK MYELODYSPLASTIC SYNDROMES (MDS) PATIENTS POST AZACITIDINE AND/OR DECITABINE TREATMENT FAILURES WITH SGI-110, A NOVEL SECOND GENERATION HYPOMETHYLATING AGENT (HMA)

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Background: SGI-110 is second generation HMA formulated as a dinucleotide of decitabine (DAC) and deoxyguanosine delivered as a low volume and pharmaceutically stable subcutaneous injection allowing longer half-life and more extended decitabine exposure than DAC intravenous infusion. As previously reported, the differentiated pharmacokinetic profile offers the potential of improved biological and clinical activity and safety over currently available HMAs (Kantarjain *et al*. ASH, 2012).
Aims: Determine the maximum tolerated dose (MTD), biological effective dose (BED), the overall safety profile and preliminary efficacy of SGI-110 in relapsed/refractory MDS patients.
Methods: We report here the clinical characteristics of MDS patients treated in a randomized Phase 1 Dose Escalation study with two different regimens of SGI-110 (QDx5 or QWx3). Responses were based on the International Working Group 2006 MDS Criteria and adverse events were based on CTCAE v4. Biological activity was assessed by SGI-110 effects on LINE1 methylation in blood.
Results: Fifteen patients with Intermediate-1 (3), Intermediate-2 (5), High Risk (6) MDS and 1 with CMML, median age 74 years (range, 46–82), ECOG PS 0/1/2 in 5/8/2 patients respectively, with a median number of prior regimens of 2 (range, 2–6) were enrolled (9 and 6 patients in the QDx5 and QWx3, respectively). All patients enrolled (100%) had prior treatment with decitabine and/or azacitidine; 87% had prior azacitidine, 53% had prior decitabine, and 40% had both azacitidine and decitabine as prior treatment. Patients were treated with doses ranging from 3-125 mg/m^2. The BED was determined as 60 mg/m^2 QDx5. Responses were observed in 5 patients for an overall response rate of 33% with reported response duration of 28–224 days. Details of each of the 5 responders are shown in Table 1. The median bone marrow blast count at baseline for the responders was 16.5% while the non-responders were 5%. Median LINE1 demethylation in responders showed a decrease by -19.3% compared to -12.0% in non-responders. Marrow CRs were reported in 2 of 8 patients who demonstrated LINE1 demethylation ≥10%. Treatment was well tolerated with the most common reported non-hematological adverse events being injection site pain and diarrhea mostly Grade1, and anticipated hematological adverse events of neutropenia/febrile neutropenia, thrombocytopenia, and anemia. The MTD was determined at 90 mg/m^2 QDx5. Currently the trial is enrolling treatment naïve MDS patients in the Phase 2 Dose Expansion Segment randomized to either 60 or 90 mg/m^2 QDx5. Updated results from the Phase 2 will be presented.
Summary / Conclusion: SGI-110 given as subcutaneous injection was well tolerated and clinical responses were observed in heavily-pretreated MDS patients, particularly High Risk patients, who had received prior treatment with decitabine and/or azacitidine.

Table 1. SGI-110 dose and regimen for MDS patient response outcomes.

Patient # (Risk category)	SGI-110 Dose (mg/m^2)	Response Status	Duration of Response (days)	Prior Treatments
QD x 5				
120 (HR)	18	mCR/HI-E	224	azacitidine, decitabine, lenalidomide/decitabine
188 (HR)	125	mCR	28	azacitidine, lenalidomide, arsenic trioxide/decitabine, hydroxyurea
QW x 3				
108 (HR)	6	HI-E/HI-N	100	azacitidine, decitabine
158 (HR)	90	HI-E	84	azacitidine/entinostat, azacitidine
160 (Int-1)	90	HI-P	126	azacytidine, ezatiostat HCL (TLK-199)

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MULTICENTER STUDY EVALUATING THE IMPACT OF HYPOMETHYLATING AGENTS AS BRIDGING THERAPY TO HEMATOPOIETIC STEM CELL TRANSPLANTATION IN MYELODYSPLASTIC SYNDROMES

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Background: Allogeneic hematopoietic stem cell transplantation (alloHSCT) is the only curative modality for myelodysplastic syndromes (MDS). Recently, treatment paradigm has changed since the introduction of hypomethylating agent (HMA) to treatment of MDS. There is little known about effect of HMA to transplant outcome and appropriate dose and schedule when used as bridging therapy to alloHCT.
Aims: Therefore this retrospective multicenter study aimed to assess the effect of pre-transplant HMA on transplant outcome and aimed to determinate the patient who would benefit from pre-transplant HMA therapy.
Methods: Medical record of 113 patients (male n=69; female n=44) were reviewed. Patients who received HSCT from 2007 to 2010 were enrolled regardless of pre-transplant HMA therapy. Five institutions participated. Primary endpoint event-free survival (EFS) after HSCT. Second endpoint was engraftment after HSCT. Analysis was done by HMA versus non-HMA.
Results: Eighty-five of the 113 patients were treated with HMA before HSCT (51 with Azacitidine (AZA), 30 with Decitabine (DCT) and 4 with both alternatively). Twenty-eight patients received HSCT without HMA bridging. The median age 47 (range 20-69) for HMA group and 42 (range 17-64) for non-HMA group (P=0.035). Distribution of WHO classification group and IPSS score were similar criteria (P=0.230 and P=0.328), For HMA group, median number of HMA administration was 5 cycles (range 1~20). Among HMA treated patients, 21 (18.5 %) achieved CR or marrow CR (mCR) and 4 (3.5 %) achieved PR. For all patients, median EFS was 29±2 months. IPSS score at diagnosis (Low/Intermediate (INT)-1 vs. INT-2/ High) affected OS after HSCT (32±3 vs. 25±4 months, respectively; P=0.020). Pre-transplant HMA didn't affect OS (P=0.771) and there was also no difference between AZA and DEC (P=0.60). However, for patient with high blast count (>5% of bone marrow at diagnosis) pre-transplant HMA therapy had a benefit of 1-yr EFS (16.7 % for non-HMA vs. 67.9 % for HMA, P=0.126) Median time to neutrophil engraftment was 28 (range, 2~380 days) for HMA and 12 (range, 7~18 days) for non-HMA (P=0.031). Median time for platelet engraftment was 35 for HMA group and 19 for non-HMA group (P=0.052). Effect of HMA to GVHD or graft failure was uncertain.
Summary / Conclusion: Benefit of bridging therapy of HMA before HSCT was not definite by this retrospective study. However for a proportion of patients with high leukemic burden, HMA tended to have benefit for post-HSCT EFS. There was considerable delay of engraftment among HMA treated patients. Therefore, pre-HSCT bridging HMA therapy may not be appropriate for all MDS patients. Prospective trial is required to confirm the benefit and effect of HMA bridging therapy to HSCT.

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AZACITIDINE PRETREATMENT FOLLOWED BY NON-MYELOABLATIVE STEM CELL TRANSPLATION IN PATIENTS WITH HIGH RISK MYELODYSPLASTIC SYNDROME : PROSPECTIVE, OPEN LABEL, MULTICENTER, PHASE II TRIAL

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Background: Hypomethylating agents(HMA) are commonly used as " bridging" therapy to facilitate allogeneic stem cell transplanataion (allo-SCT) preventing progression during the period while donor is identified and the patients undergoes pre-transplant screening. However, it is largely unknown whether treatment with HMA before allo-SCT will affect the results of the transplant and no prospective data are available.
Aims: In phase II study, we explored the efficacy and safety of azacitidine in patients with transplant eligible high risk MDS and we analyzed the data to identify the predictor of survival.
Methods: Patients were enrolled from all participating institutions prospectively. Subjects could have an IPSS score of intermediate- 2 (1.5-2.0) or high (≥2.5), the age was < 65-year and performance status was 0-2 without serious comorbid disease. Response was assessed using 2006 IWG MDS response criteria after 4 cycles of therapy.
Results: A total of 30 subjects were enrolled at the 13 centers from Feb. 2008 to Dec, 2010 and 27 patients could be collected clinical data at analysis. Six out of 27 patients were excluded because they were diagnosed as intermediate-1. Among 21 patients, median age was 50 years (range 18-63) and median follow-up was 25.4 months (range, 21.7-52.9). IPSS categories included intermediate-2 (11) and high (10). The overall response rate of azacitidine pretreatment was 62%: 2 CR, 1 PR and 10 hematologic improvement. During azacitidine therapy, three (14.3%) patient showed the disease progression (2: leukemic conversion, 1: died of infection). Fourteen (66.7%) out of 21 patients received planed non-myeloablative SCT (7: sibling, 7: unrelated) and donor was not available in 4 (19%) patient. After transplantation, two (9.5%) patients died as a result of GVHD and colitis, respectively. Disease progression was observed in 8/14 (57%) transplant patients and 6 of them died of disease progression. Five patients were alive at last follow-up. The two-year overall survival and progression free survival was 47.6±10.9% and 32.5±10.8%, respec-

tively. In univariate analysis, the karyotype and WPSS at diagnosis is the only predictable factor for overall survival in intention to treat analysis (P=0.016, 0.030).
Summary / Conclusion: Even though high risk MDS patients received azacitidine pretreatment followed by allo-SCT, the most common cause of treatment failure is the disease progression especially in high risk karyotype. So, more intensive consolidative treatment after transplantation may be considered in high risk patient.

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CLINICAL SPECTRUM AND OUTCOMES OF PATIENTS WITH INHERITED BONE MARROW FAILURE SYNDROMES: A MAYO CLINIC SERIES OF 55 PATIENTS

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Background: Bone marrow failure syndromes (BMFS) are clonal, heterogenous, stem cell disorders, characterized by progressive cytopenias and hypocellular bone marrows. While most cases are acquired, resulting from myelodysplastic syndromes (MDS) and aplastic anemia (AA), there are a spectrum of Inherited BMFS (IBMFS) associated with multi system abnormalities, immunodeficiency and an inherent predisposition for clonal evolution to MDS and acute myeloid leukemia (AML). Early recognition and multi-disciplinary evaluations are critical for optimal outcomes.
Aims: To identify and describe the spectrum of inherited BMFS at our institution, with special focus on demographics, clinical characteristics, treatment strategies and over all outcomes.
Methods: After an approval by the Institutional Review Board, the Mayo Clinic BMFS database (1990-2012) was evaluated to identify all patients with inherited BMFS. Clinical data was retrospectively abstracted and pathological data was reviewed. Information analyzed included; presenting features, genetic aberrations, telomere lengths, presence and magnitude of PNH clone sizes, details on clonal evolution, treatment strategies and overall outcomes.

Table 1. Clinical characteristics, treatment and outcomes of patients with IBMFS.

IBMFS	#	Med Age	Sex (Male)	CE	SCT	Solid tumors	Death	Cause	Other Features
FA	12	13 0-32	5 (42%)	3 (25%)	7 58.3%	Vulvar 25%, BCC 8%, HNSCC 8%, Anal 8%, Cervix 8%	2 (17%)	AML, vulvar Ca	Skeletal abnormalities 30%, CHD 8%, Infertility 8%, Single kidney 8%, Undescended testes 8%
DC	14	16 2-61	8 (57%)	N	4 28.5%	N	3 (21%)	Pul Fibrosis, NRM	Nail 64%, Deafness 14%, Pulmonary fibrosis 21%, Cirrhosis 14%, Esophageal strictures 14%
DBA	12	0 0-2	6 (50%)	1 (8%)	2 16.7%	N	1 (8%)	TRM	Fe overload 100%, Growth retardation 67%, Skeletal abnormalities 25%, FSGS 8%
SDA	3	0 0-1	3 100%	N	N	N	0%		Malabsorption 100%, Growth retardation 100%, Hepatitis 33%
TAR	4	0	2(50%)	N	N	N	0%		Growth retardation 50%
CAMT	5	1	1(20%)	N	2(40%)	N	0%		
Pearson syndrome	1	1	1 (100%)	N	N	N	1 (100%)	Sepsis	CHD, recurrent pancreatitis, ataxia, malabsorption, Fanconi Syndrome
IBMFS NOS	4	20 16-38	1 (25%)	1 (25%)	N	Vulvar ca 25%	1 25%	Vulvar Ca	Polycystic ovaries 25%
Total	**55**		**27 (49%)**	**5 (9%)**			**8 (15%)**		

IBMFS=inherited bone marrow failure syndromes, CE=clonal evolution, SCT=allogeneic stem cell transplantation, FA=Fanconi Anemia, DC=Dyskeratosis Congenital, DBA= Diamond-Blackfan Anemia, SDA=Shwachman-Diamond Anemia, TAR=Thrombocytopenia with Absent Radii, CAMT=Congenital Amegakaryocytic Thrombocytopenia, CDA=Congenital Dyserythropoietic Anemia, NOS=not otherwise specified, BCC=basal cell carcinoma, HNSCC=head and neck squamous cell carcinoma, AML=acute myeloid leukemia, TRM=transplant related mortality, CHD=congenital heart disease, FSGS=focal segmented glomerulosclerosis, NRM = non-relapse mortality

Results: Three hundred and seventy five patients were diagnosed with an IBMFS or Severe AA between 1990 and 2012. Of these, 55 (15%) had an inherited BMFS, with the median age of presentation being 13 years (range, 0-61 years). The specific diagnostic categories included; Fanconi Anemia (FA) in 12 (21.8%), Dyskeratosis Congenita (DC) in 14 (25.4%), Diamond Blackfan Anemia (DBA) in 12 (21.8%), Shwachman Diamond Anemia (SDA) in 3 (5.5%), Thrombocytopenia with Absent Radii (TAR) in 4 (7.3%), Congenital Amegakaryocytic Thrombocytopenia (CAMT) in 5 (9.1%), and Pearson syndrome in 1(1.8%). Four patients (7.3%) had a familial BMFS, without a formal diagnostic categorization [IBMFS, not otherwise specified (nos)]. Twenty-seven (49%) were males. All patients had genetic defects and multi-system abnormalities as outlined in Table 1. While the majority presented during the first decade of life, 16 (29%) patients were diagnosed with an IBMFS in adulthood [5 with FA (31.3%), 7 with DC (43.7%), and 4 with IBMFS-nos (25%)]. Fifteen (27%) patients underwent reduced intensity conditioning (RIC) allogeneic stem cell transplantation (allo-SCT); 7 with FA, 4 with DC, 2 with DBA, and 2 with CAMT. Stem cell sources included; 6 (38 %) matched sibling donors, 7 (47%) matched unrelated donors and 2 (13%) umbilical cord blood units. The 100 day and 5 year, non-relapse mortality rates were 13% and 16% respectively. There was one patient with DC, who in spite of receiving a RIC conditioning, died from progressive pulmonary fibrosis. Five patients (9%) had clonal evolution to MDS or AML. Of these, 3 had FA, one had DBA and one had IBMFS-nos. Cytogenetic alterations at time of evolution included trisomy 8, complex karyotype, 20q deletion, and monosomy 7. Three of these 5 patients underwent allo-SCT, while the other two were transplant ineligible (concomitant metastatic vulvar cancer and active infections). The 5 year overall mortality in the entire cohort of patients with IBMFS was 15%.
Summary / Conclusion: Approximately 30% of IBMFS can present in adulthood, indicating that this is not a disease restricted to the pediatric age group. Early identification, followed by a multidisciplinary diagnostic and therapeutic approach, does result in good outcomes. RIC Allo transplant is associated with low non relapse mortality rates (5 year NRM 16%) and excellent overall and disease free survivals.

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IMPROVED FATIGUE AND QUALITY-OF-LIFE IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA DURING TREATMENT WITH ECULIZUMAB: DATA FROM THE GLOBAL PNH REGISTRY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, progressive, life-threatening disease characterized by chronic intravascular hemolysis caused by uncontrolled complement activation. PNH can present in all ages; the median age is approximately 34 yrs. Patients have a highly increased risk of thrombosis and mortality with approximately 35% of patients dying within 5 yrs, despite best supportive care. PNH symptoms and sequelae of vascular events decrease patient's quality of life (QOL) with fatigue as one of the most common and burdensome problems. The terminal complement inhibitor eculizumab has been shown to provide a rapid, sustained, and clinically meaningful reduction in intravascular hemolysis and thrombotic events, alleviate common PNH-related symptoms, and improve survival.
Aims: Assessment of changes in patient-reported QOL and fatigue after 6 and 12 months of treatment with eculizumab.
Methods: As of January 2013, 2013 patients were entered into PNH Registry, of whom 144 had started eculizumab treatment on or after entry into the Registry. Completed pretreatment and follow-up study questionnaires were available for 62 patients with a median age of 41 (10-86) yrs. The questionnaires were based on Functional Assessment of Chronic Illness Therapy (FACIT) fatigue evaluations and the European Organization for Research and Treatment of Cancer (EORTC) QOL and symptom assessments. Mean changes from baseline were assessed at 6- and 12-month follow-up intervals. Two-sided paired t-tests were used to assess statistical significance. All patients provided written informed consent prior to enrollment in the registry.
Results: At baseline patients had a median granulocyte PNH clone size of 86.3%, with median LDH level 5× the upper limit of normal. Median time from diagnosis to start of treatment was 4 years. After 6 months of treatment, statistically significant improvements over baseline were reported for FACIT-Fatigue (+6.8, P<0.001) and EORTC assessments of global health/QOL (+12.0, P=0.002), role (+24.0, P<0.001), social (+17.0, P<0.001), and physical functioning (+11.9, P<0.001), fatigue (-14.7, P<0.001), dyspnea (-15.6, P=0.004), pain (-8.8, P=0.019), and nausea/vomiting (-6.3, P=0.029). At 12 months these improvements were maintained, with the majority of assessments showing further improvement, with significant changes over baseline also seen in emotional functioning (+12.9, P=0.003) and insomnia (-16.3, P=0.015). At both 6- and 12-months >60% of patients had a ≥3-point improvement in FACIT-Fatigue score and, with the exception of cognitive functioning, the majority of patients had a ≥5-point improvement in EORTC functioning scores. At least 40% of patients had a ≥10-point improvement in all EORTC functioning scores and symptom assessments of fatigue, pain, and dyspnea at 6 and 12 months with a ≥10-point improvement in insomnia reported for >40% of patients at 12 months.
Summary / Conclusion: PNH patients in the Global Registry on eculizumab

reported a significant improvement in fatigue and other PNH-related symptoms following commencement of eculizumab therapy. These improvements were associated with a significant increase in patient reported global health and QOL. All effects were maintained over the 12 months of assessment. Alleviation of symptoms, such as pain and dyspnea, is consistent with the reduction of hemolysis and maintenance of circulating nitric oxide levels. Improvements in fatigue and QOL are very important additional effects of eculizumab in patients with PNH.

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APPLICABILITY OF A REPRODUCIBLE FLOW CYTOMETRY SCORING SYSTEM IN THE DIAGNOSIS OF REFRACTORY CYTOPENIA OF CHILDHOOD

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Background: Refractory cytopenia of childhood (RCC), defined as myelodysplasia without an increased blast count, is the most common subtype of childhood myelodysplastic syndrome (MDS). Karyotype is normal in the majority of patients with RCC, and, in contrast to adults with MDS-refractory anemia (RA), about 80% of children have a hypocellular bone marrow. Although morphologic criteria for diagnosing RCC are strictly defined, differentiating RCC from the immune mediated bone marrow failure syndrome aplastic anemia can be challenging. Flow cytometry immunophenotyping has been suggested to be a valuable addition to morphology in the diagnosis of MDS in adults. Recently, a reproducible flow cytometry scoring system has been described as diagnostic tool by Ogata and others in adult low-grade MDS, with a sensitivity of 70% and a specificity of 93% (Ogata *et al*. Haematologica 2009 and Della Porta *et al*. Haematologica 2012).
Aims: We investigated whether the flow cytometry scoring system described by Ogata and others is applicable in childhood MDS, with emphasis on RCC.

Table 1.

	0	1	2	3	4	Cases positive (%)
Children						
RCC	29	14	3	1	0	4 of 47 (8.5)
RAEB	2	0	3	3	2	8 of 10 (80)
Cytopenic controls						
FA/ITP/TEC	2	3	0	0	0	0 of 5 (0)
(v)SAA	3	0	0	0	0	0 of 3 (0)
Adults						
Low-grade MDS	1	0	5	1	1	7 of 8 (88)
Cytopenic controls	20	6	2	0	0	2 of 28 (7.1)
Healthy controls	9	0	0	0	0	0 of 9 (0)

Methods: Bone marrow samples, obtained from 82 previously untreated primary RCC patients, who were included in the prospective studies EWOG-MDS 2006 and EWOG-MDS RC06 and diagnosed between June 2005 and December 2011, were analyzed by flow cytometry. Diagnosis of RCC was based on WHO criteria for pediatric MDS and confirmed by central review of bone marrow morphology and histology. Pediatric MDS-RAEB or RAEB-t patients, low-grade adult MDS patients, healthy individuals, and cytopenic non-MDS adult and pediatric patients were used as controls. Samples were evaluated based on the published criteria and cut-offs: CD34+ myeloid blast cells (parameter 1) ≥2%, CD34+ B-cell progenitors within CD34+ blast cells (parameter 2) ≤5%, lymphocyte/myeloid blast cell CD45 MFI ratio (parameter 3) ≤4 or ≥7.5, granulocyte/lymphocyte SSC peak channel ratio (parameter 4) ≤6. Patients scored 1 point for each fulfilled criteria; patients scoring 2 or more points were considered likely to have MDS.
Results: The median age of included RCC patients was 10.5 years (range: 1-18 years). Bone marrow was hypocellular in 81% of patients; cytogenetic analysis was normal in 76% of patients, monosomy 7 was present in 6%, and other cytogenetic aberrations in 6%. In 35 of 82 patients (43%), insufficient blast cells and/or granulocytes were present for reliable analysis of all four parameters. Of the patients who could be evaluated completely, 4 of 47 patients (8.5%) scored ≥2 points, and were thus likely to have MDS according to the previously published criteria. The patients who scored positive were slightly older than the total RCC cohort (median age at diagnosis: 13 years); 2 patients carried a monosomy7, and in 2 patients no cytogenetic result was obtained due to insufficient metaphases. Results obtained in evaluable RCC patients and controls are summarized in Table 1.
Summary / Conclusion: The scoring system proposed by Ogata and others is not usefully applicable in RCC due to a limited number of patients that can be evaluated for all four parameters, and due to a low sensitivity in those patients that can be evaluated. To evaluate whether other immunophenotypic abnormalities are present and might aid in diagnosing RCC, more detailed analyses of myeloid maturation patterns are currently underway.

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IMPROVED PREDICTIVE PROGNOSTIC POWER OF REVISED-IPSS (IPSS-R) IN A SERIES OF 301 PATIENTS WITH MYELODYSPLASTIC SYNDROME FROM A SINGLE CENTER.

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Background: Background: The International Prognostic Scoring System (IPSS) (Greenberg *et al*, 1997) has recently been revised (IPSS-R) (Greenberg *et al*, 2012) in order to better define the prognostic impact on overall survival (OS) and risk of transformation to acute leukemia (PFS) in patients diagnosed with MDS. The variables considered are the same but include five cytogenetic categories (Schanz *et al*, JCO,2011), new cut off values for cytopenias and bone marrow blasts and different weighing of these parameters.
Aims: Aims: The purpose of this study was to validate the prognostic value of IPSS-R respect to IPSS, in a series of 682 patients with MDS.
Methods: Material and Methods: Between 1987 and 2012, 682 patients with MDS were identified in our database. Among the 682 MDS patients registered, cytogenetic analysis at diagnosis was available in 301 patients (47%). We evaluated the prognostic power of IPSS-R respect to IPSS by Harrell's C and Somers' D statistics, analyzing as endpoints overall survival (OS) and leukemia-free survival (LFS).

Table 1.

		IPSS-R					
		Very Low	Low	Intermediate	High	Very High	TOTAL
IPSS	Low	83	73	3	0	0	159
	Intermediate-1	2	63	36	7	0	108
	Intermediate-2	0	3	10	10	5	28
	High	0	0	0	1	5	6
	TOTAL	85	139	49	18	10	301

Results: Median age at diagnosis was 71 years (range 29-101). 209 (69%) were male. WHO diagnosis was: 1% CRDU, 7% RA, 35% RCMD, 20% RAEB-1, 10% RAEB-2, 25% CMML, the remaining 2% were MDS-U and isolated 5q deletion. Median follow up was 46.8 months (1.2-213.84). Classification of the 301 patients by IPSS versus IPSS-R is shown in the table. In this comparative classification, IPSS-R refined the MDS in five prognostic risk groups. Patients with low-risk IPSS remained on groups of very low (52%) and low risk (46%) of IPSS-R, while all high risk IPSS spread between high (16%) and very high risk (84%). However, those classified in Int-1 and Int-2 IPSS categories are reclassified in the IPSS-R in very low (2% / 0%), low (58% / 10%), intermediate (34% / 36%), high (6% / 36%) and very high risk (0/36%) respectively. Kendall's tau was 0.68 showing a good correlation between IPSS-R and IPSS. The 71% (5/7) of patients with IPSS INT-1 who were reclassified as high risk in the IPSS-R had an OS ≤ 30 months, 43% progressed to acute leukemia and 86% of them were transfusion-dependent. OS from 66% (2/3) of IPSS INT-2 group patients who were reclassified as low risk in IPSS-R index was about 60 months, the other patient died for a cause not related with MDS. OS and LFS were estimated using the Kaplan-Meier method. Both prognostic scores systems allowed the identification of curves with statistically significant differences

among the different risk categories (All P<0.001). Harrel's C and Somers' D statistics analysis demonstrated that IPSS-R exhibit a better predictive power and discriminated prognostic risk more effectively than IPSS (C= 0.656 vs 0.624; D=0.312 vs 0.247).
Summary / Conclusion: Conclusion: In our series, the IPSS-R score demonstrated a strong prognostic value for OS and LFS and a greater prognostic power compared to IPSS. As the decisions on therapy are based on this initial prognostic risk assessment, the improvement in the definition of risk categories will directly impact on therapeutic approach.

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PREDICTIVE ROLE OF WT1 AND RPS14 EXPRESSION IN PATIENTS AFFECTED BY MYELODYSPLASTIC SYNDROMES RECEIVING AZACITIDINE

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Background: Demetylating agents, including azacitidine, represent a valid therapeutic approach for patients affected by intermediate-2/high risk myelodysplastic syndromes (MDS). Different groups previously reported that WT1 expression levels at diagnosis and during follow-up could significantly condition the prognosis of high-risk MDS patients. Moreover, in the recent years, many efforts have been spent in order to understand the pathogenesis of the 5q- syndrome; low levels of RPS14 were previously reported also in MDS intermediate-1 patients without 5q- aberration, thus supporting the hypothesis that the defective ribosomal biogenesis could represent a more general pathogenetic mechanism.
Aims: 1) to evaluate the WT1 expression levels at diagnosis and during follow-up of high-risk MDS patients treated by azacitidine; 2) to evaluate the RPS14 expression at diagnosis and its modulation during demethylating therapy; 3) to correlate these molecular findings with clinical outcome (quality of response, PFS, OS).
Methods: WT1 and RPS14 mRNA levels have been measured by quantitative real-time PCR on bone marrow from 33 high-risk MDS patients at diagnosis, and then after 8, 12, 16, and 24 weeks of azacitidine treatment. Bone marrow samples from 8 healthy donors (patients who underwent femur surgery) were used as controls; the stratification in 2 categories (low and high) was defined according to the median value measured in the healthy donors. Patients' characteristics (age, sex, IPSS, WPSS, blast count) did not differ between cases with high or low RPS14 and WT1 levels. At the start of treatment, WPSS was high or very high in 77% of cases and 49% of patients were transfusion-dependent. When mRNA expression was monitored during the follow-up, increases ≥2.5 folds or decreases ≥0.5 folds were considered as significant.
Results: For the entire series, 2-year overall survival (OS) was 63,2% and 2-year leukemia-free-survival (LFS) 53,7%. In univariate analysis, LFS was significantly affected by the quality of response after 24 weeks (P=0.001) and by the persistence of erythroid transfusion dependence at 24 weeks (P=0.03). OS was significantly affected by quality of response at 24 weeks of treatment (P=0.001) and by the erythroid transfusion dependence at 24 weeks (P=0.002). For patients with low RPS14 levels, 2-year LFS was significantly shorter (39% for cases with low RPS14 versus 100%; P=0.018). Also the OS appeared negatively affected by low RPS14 levels (2-year OS of 51% for cases with low RPS14 expression versus 100% for cases with high levels), but without statistical significance (P=0.08). At diagnosis, WT1 was over-expressed in 63% of cases; its quantitative expression before treatment did not significantly influence the quality of response or the long-term outcome. After 16 weeks of therapy, 49% of cases showed a partial or complete response, and only 18% did not respond at all. At that time-point, WT1 was reduced in the 74% of cases (percentage comparable to that of responding cases), with a significant impact on the 2-year PFS: indeed, 56% of patients with WT1 decreased levels remained progression-free versus 21% of cases with stable/increased WT1 levels. On the contrary, 66% of cases had stable, 21% decreased, and 13% increased RPS14 levels. RPS14 levels during the follow-up did not correlate with clinical response or outcome, predicting the disease status in less than half of patients.
Summary / Conclusion: This study shows that RPS14 and WT1 would be useful molecular markers in the management of high-risk MDS patients receiving azacitidine. Nevertheless, their role could be different in the different phases of the disease: RPS14 could further stratify patients at diagnosis, whereas WT1 would be more useful during the follow-up. On this basis, a patient-tailored approach could be designed: 1) cases with low RPS14 expression at diagnosis or with increasing WT1 levels during treatment could be probably treated for longer periods or, when possible, considered for alternative treatments (allogeneic transplantation, lenalidomide).

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HIGH LEVELS OF CEREBLON MESSENGER RNA ARE THE CHARACTERISTIC FEATURE OF LOWER RISK MYELODYSPLASTIC SYNDROMES WITH 5Q DELETION AND ARE CONNECTED WITH THE EFFICACY OF LENALIDOMIDE

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Background: Cereblon (CRBN) was named according to its putative role in cerebral development, especially in memory and learning. CRBN forms an E3 ubiquitin ligase complex with damaged DNA binding protein 1 (DDB1), cullin-4 (CUL4) and regulator of cullin 1 (ROC1). This complex regulates DNA repair, DNA replication and transcription. CRBN is a primary target of thalidomide teratogenicity (Ito *et al*., *Science* 2010; 327:1345-1350). The binding of immunomodulatory drugs (IMiDs), including lenalidomide, to CRBN is associated with cytotoxicity of IMiDs and is used to treat multiple myeloma, myelodysplastic syndromes (MDS) and lymphomas. Down-regulation of the *CRBN* expression is associated with the development of marked IMiDs resistance in human multiple myeloma cells (Zhu *et al*., *Blood* 2011; 118: 4771-4779; Lopez Girona *et al*., *Leukemia* 2012; 26: 2326-2335). Therefore, the *CRBN* expression is required for the antimyeloma activity of IMiDs.
Aims: To gain insight into the role of CRBN in lower risk MDS with 5q deletion or with normal chromosome 5 and into the mechanisms of lenalidomide action, we studied the *CRBN* expression in these two groups of lower risk MDS patients and in healthy controls.
Methods: Informed consent was obtained from all patients and healthy controls. Mononuclear cells were isolated from bone marrow [19 MDS patients with 5q- syndrome, 28 patients with low-risk MDS with normal chromosome 5 (non5q-) and 15 healthy controls] and from peripheral blood [23 MDS patients with 5q- syndrome, 19 non5q-patients and 11 healthy controls] by Ficoll-Paque PLUS gradient separation, washed with phosphate-buffered saline and rest red cells were lysed. Total RNA was isolated using RNA isolation solvent and complementary DNA was synthesized from total RNA using SuperScript II reverse transcriptase. Relative levels of the CRBN mRNA were determined by Taq-Man-based quantitative real-time PCR and by calculation to the level of housekeeping glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. The experiments were performed in duplicate. Evaluation of 2^{-ddCt} indicates the fold change in gene expression relative to the control.
Results: The median of CRBN mRNA levels was the highest (3.6) in total RNA isolated from peripheral blood mononuclear cells of lower risk myelodysplastic syndromes with 5q deletion, the lower (2.2) in non5q- MDS and the lowest (1.0) in healthy controls. The similar results were obtained in bone marrow blood mononuclear cells (medians 3.2 in 5q- syndrome, 2.2 in non5q- and 1.2 in healthy controls). The differences between all groups were statistically significant (P<0.05, Mann-Whitney test). We analysed CRBN mRNA levels before and in the course of the treatment of 5q- syndrome with lenalidomide. High level of CRBN mRNA before and during the treatment correlated with sustained response to lenalidomide. In one 5q- syndrome patient who relapsed after the discontinuation of successful treatment the level of CRBN mRNA sharply decreased. This patient did not respond to the second course of lenalidomide and progressed to RAEB II.
Summary / Conclusion: Low risk MDS patients with 5q deletion have the highest levels of CRBN mRNA in comparison to lower risk MDS with normal chromosome 5 or healthy controls. High levels of CRBN mRNA in 5q- syndrome are necessary for the efficacy of treatment by lenalidomide. Great decrease of CRBN levels during the treatment by lenalidomide is connected with the absence of response and the disease progression.
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ECULIZUMAB PROTECTS AGAINST TE AND PROLONGS SURVIVAL IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: AN INTERNATIONAL PNH REGISTRY STUDY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a chronic and life-threatening hematopoietic stem-cell disorder characterized by uncontrolled complement-mediated hemolysis. Thromboembolism (TE) is one of the leading causes of mortality in PNH, in large part due to chronic hemolysis and platelet hyperactivation. Eculizumab, a monoclonal antibody that inhibits terminal complement activation, has been shown in clinical trials to reduce hemolysis and the incidence of TE.
Aims: To assess characteristics associated with TE and mortality and the effectiveness of eculizumab in reducing the occurrence of these outcomes in PNH patients enrolled in the International PNH Registry.
Methods: Patients are eligible for the Registry if they have a detectable PNH clone, regardless of disease severity or treatment status. The cumulative incidence of TE was determined using competing risks (to account for bone marrow transplantation [BMT] and death), while Kaplan-Meier methods were used for the cumulative incidence of mortality. Characteristics associated with TE and mortality were explored using a Cox proportional hazards model with stepwise selection. Variables examined included: demographics, medical history, symptoms, Karnofsky performance score, granulocyte clone size, lactate dehydrogenase (LDH), red blood cell (RBC) transfusions in the 6 months prior to enrollment, anticoagulant use, BMT (for mortality model), and use of eculizumab during follow-up as a time-varying covariate.
Results: As of June 30, 2011, 1047 patients were evaluable: mean age 45 years, 51% were female. Anticoagulants (heparin/warfarin) were used by 28% of patients and eculizumab was used by 51% during follow-up (18% used both). During a mean follow-up of 22.5 months, there were 16 patients with TE and 51 deaths. Patients treated with eculizumab during follow-up had a cumulative incidence of TE at 2 years of 1.35%, while those not treated with eculizumab had TE incidence of 2.61% at 2 years. In the multivariate Cox model, the greatest associations with TE were recent RBC transfusion (adjusted hazard ratio [HR]=8.42, 95% CI 2.38-29.79), history of impaired hepatic function (HR=5.22, 95% CI 1.17-23.34), and headache (HR=2.72, 95% CI 0.98-7.51). While controlling for these variables, eculizumab had a protective effect (HR=0.52, 95% CI=0.18-1.51). The cumulative incidence of mortality in eculizumab-treated patients was 4.21% at 2 years, while in untreated patients it was 7.01%. The top 5 reported causes of death (accounting for 68% of cases) were aplastic anemia, cardiovascular disease, PNH, BMT, and AML. In the multivariate Cox model of mortality, the greatest associations were age 60+ years (HR=6.32, 95% CI 2.35-17.00), BMT (HR=5.43, 95% CI 2.06-14.32), and Karnofsky score <80 (HR=2.47, 95% CI 1.24-4.92). While controlling for these variables, eculizumab had a protective effect (HR=0.40, 95% CI=0.21-0.77).
Summary / Conclusion: This analysis of a large international cohort of 'real world' PNH patients receiving a variety of treatments (or no treatment) showed that eculizumab is associated with a reduced risk of TE and mortality, consistent with prior research. Recent RBC transfusion, a surrogate marker for hemolysis, was associated with increased risk of TE. As might be expected, older age, lower performance status, and BMT were associated with mortality. These data are limited due to small number of TE and mortality outcomes and should be interpreted in the context of a contemporary cohort of PNH patients who may or may not be treated (with either eculizumab and/or anticoagulation).

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MORTALITY BURDEN OF TRANSFUSION DEPENDENCY AND IRON OVERLOAD IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES.

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Background: Transfusion dependency (TD) is associated with iron overload (IO) and a decreased probability of overall survival (OS) in patients with MDS (*Malcovati L et al. J Clin Oncol 2007 25:3505*).
Aims: The purpose of this study was to validate the prognostic value of TD and IO and their impact on life expectancy in our single-centre series of 635 MDS patients.
Results: Median age at diagnosis 71y (27-101); 411 male; median follow-up 60 months (0-228). Karyotype available at diagnosis in 263 patients (41%) in whom IPSS-R could be assessed (26% very low risk, 47% low, 17% intermediate, 7% high and 3% very high. 31% were TD, as defined by the WPSS, requiring a median of 4 packed red blood cell (PRBC) monthly transfusions. Our results confirm that patient age, gender, FAB and WHO subtype, karyotype risk according to IPSS and IPSS-R, percentage of BM blasts at diagnosis, number of cytopenias, IPSS, IPSS-R, WPSS, IO at diagnosis and TD were all associated with overall survival (OS) in this series (P< 0.001 in all cases). Furthermore, TD and the intensity of TD translated into a strong reduction of the life expectancy of TD-MDS cases compared to non-TD counterparts. A significant higher standardized mortality ratio (SMR) had been shown in our series in comparison with the mortality of the general Catalan population, TD vs Non TD patients (Table 1); SMR 5.3 vs 2.6 in men, and 5.7 vs 2.2 in women. Data also seen with the intensity of transfusion >2 PRBC vs ≤2 PRBC monthly 6.7 vs 3.8 and >3 PRBC vs ≤3 PRBC; 9.1 vs 4 and IO as serum ferritin ≥ 1000 ng/ml vs <1000 ng/mL SMR was 6.3 vs 2.9. The negative impact of TD, intensity of TD and IO was also seen in patients>65. We estimated years of life lost (YLL) attributable to TD, intensity of TD and IO. Large differences in YLL were observed by gender (Table 2).
Summary / Conclusion: Our results confirm in our single-center experience the negative impact of TD, intensity of transfusion requirements and IO on life expectancy of patients with MDS and indicates that strategies to reduce this factors may provide a gain of years of life in all MDS population.

Table 1. Transfusion dependency-SMR.

		TRASNFUSION DEPENDENCY		NON-TRANSFUSION DEPENDENCY	
	YEARS	SMR	95% CI	SMR	95% CI
MALES	<65	22,9	15,1-33,3	4,0	2,4-6,4
	65-79	6,2	4,7-8,1	2,5	2,0-3,1
	>80	2,3	1,4-3,5	2,4	1,7-3,4
FEMALES	<65	28,1	12,1-55,3	6,6	2,9-13,1
	64-79	7,4	4,6-11,2	2,5	1,6-3,6
	>80	3,2	1,8-5,4	1,7	1,1-2,4

Table 2. Transfusion dependency-intensity of TD and IO-YLL.

	YEARS OF LIVE LOST					
	ALL PATIENTS		MEN		WOMEN	
	Mean	p-value	Mean	p-value	Mean	p-value
TRANFUSION DEPENDENCE No/Yes	6.11 / 9.03	0.005	6.68 / 9.96	0.006	4.95 / 6.87	0.341
≤ 2 vs > 2 PRBC units/4 weeks	5.64 / 10.80	0.004	4.68 / 12.32	<0.001	7.31 / 6.86	0.902
≤ 3 vs > 3 PRBC units/4 weeks	6.49 / 12.34	< 0.001	6.02 / 13.83	<0.001	7.23 / 6.57	0.839
SERUM FERRITIN (µG/L) < 1000 (µG/L) / ≥ 1000 (µG/L)	7.01 / 9.03	0.238	8.34 / 10.57	0.281	3.98 / 5.50	0.590

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NOVEL 3Q26 DELETION BONE MARROW FAILURE SYNDROME IN A NEWBORN RESULTING IN DOMINANT NEGATIVE INTERFERENCE OF HEMATOPOIESIS BY TRUNCATED EVI1/MECOM

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Background: We report on a patient born with severe congenital abnormalities including macrocephaly, brain MRI abnormalities, bilateral clubfeet, and bone marrow failure with pancytopenia presenting with none but T-lymphocytes in the peripheral blood.
Aims: Characterization of the underlying genetic aberration explaining the severe clinical phenotype of the patient.
Results: A 824 kb to 868 kb heterozygous deletion on chromosome 3q26.2 was identified by array-CGH on peripheral blood using an Agilent 180k oligo array (Amadid 023363). The deletion truncated the 3' part of *EVI1/MECOM* and extended into microRNA 551b (*MIR551B*). The 3' *MECOM* deletion was confirmed by FISH using a *MECOM* specific probe on uncultured peripheral blood cells in 10 investigated metaphases and 200 interphase nuclei. To assess whether the deletion was constitutional, FISH analysis was performed on buccal swaps and cultured skin fibroblasts. Loss of one *MECOM* signal was observed in 200 interphases, and 10 metaphases of cells derived from buccal mucosa and skin fibroblasts, respectively, demonstrating the constitutional nature of the *3' MECOM* deletion. Karyotyping and FISH analysis of both parents did not reveal a structural or numerical abnormality of 3q26.2 *MECOM*, indicating the deletion to be *de novo*. EVI1/ MECOM is a transcriptional regulator essential for maintaining embryonic and adult hematopoietic stem cells by directly regulating transcription of *GATA2*. In mouse models, homozygous disruption of *MECOM* results in embryonic lethality, with hypocellular bone marrow, reduced body size, small or absent limb buds, abnormal development of the nervous system and heart and massive hemorrhaging. Furthermore,

MECOM heterozygosity leads to a marked impairment of the self-renewal capacity of hematopoietic stem cells. In our case the heterozygous deletion results in 3' terminal truncated *MECOM* lacking the C-terminal acidic amino acid cluster domain (AD) encoding sequences. The AD of MECOM is important for activation of *GATA2* transcription *in vitro*. This supports the notion that dominant negative interference of normal EVI1/MECOM expression by truncated amino terminal EVI1/MECOM causes severe bone marrow failure, supposedly by deregulating GATA2 mediated control of hematopoietic stem cell homeostasis.

Summary / Conclusion: We will discuss our data in relation to two cases with a milder phenotype likely to be caused by haploinsufficiency of *EVI1/MECOM*. In conclusion, we report on a novel 3q26 *EVI1/MECOM* deletion syndrome with multiple severe congenital abnormalities and bone marrow failure due to a dominant negative effect on *EVI1/ MECOM* regulated hematopoiesis.

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SPANISH PNH REGISTRY: PNH CLONE SIZE, LDH AND THROMBOSIS EVOLUTION

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Background: Paroxysmal Nocturnal Hemoglobinuria (PNH) is a hematopoietic stem cell disorder caused by a PIG-A gene mutation.
Aims: To analyze epidemiological data obtained from patients enrolled in the National PNH Registry as well as clone size, LDH and thrombotic status evolution at enrollment, 6 and 12 months.
Methods: We analyzed 111 patients (45 female and 66 male) from 26 sites classified according to Parker in three groups: classic/hemolytic PNH (group I; n=54), PNH with another bone marrow disorder (group II; n=32) and subclinical PNH (group III; n=25). We compared in these populations demographic data, clone size, LDH levels and incidence of thrombosis at enrollment, 6 and 12 months.
Results: Median age at disease onset was 35 y with no statistical differences among groups. The median age from disease onset to enrollment was 7.7 yrs (11.6 (0.5-41.2) in group I, 4.1 (0.6-33.8) in group II and 4.7 (0.3-41.8) in group III, P=0.0084). Previous history of thrombosis was present in 19 patients (17.1%), 5 of them in group II, 11 were male (57.9%) and 8 women (42.1%), (P=0.0397). Those patients had 41 thrombotic events in total: 8 (one event), 5 (two events) and 5 (four events). Thrombosis is more common in venous thrombosis, but there were some arterial thrombosis. Median granulocytes clone size at enrollment (n=88) was 60% (0.9-99), 86.5% (20-99.9) in group I, 59% (10-98.9) in group II and 3.2% (0-20) in group III. Median LDH at enrollment (n=105) was 643 U/L (135-6000), 1119 U/L (188-6000) in group I (n=52), 866 U/L (135-2685) in group II (n=28), and 312 U/L (167-752) in group III (n=25), P<0.0001. 10 patients in group II had LDH>1000 U/L and clone size >50% at baseline, so probably, were not well classified at enrollment. A total of 46 patients are treated with eculizumab: 36 being classical and 10 in group II. 14 patients started eculizumab on or after enrollment, 65 have never been on eculizumab, and 5 were treated with eculizumab prior to enrollment but did not continue for different reasons (pregnancy, ending trial, government criteria, etc). No patient on eculizumab has presented a new thrombotic event. One patient not treated (in group I) has presented with thrombosis at 6 months. At 6 months, clone size is 87.3% (24.5-100) in group I (n=21), 45.4% (5.0-98.9) in group II (n=12) and 2% (0.0-15) in group III (n=13), P<0.0001. At 12 months, clone size is 82.4% (18.1-99.2) in group I (n=11), 84.0% (31.6-99.5) in group II (n=7) and 2% (0.0-6.5) in group III (n=7), P<0.0007. Median LDH at 6 months (n=72) is 501 U/L (112-4467), 492 U/L (160-4467) in group I (n=36), 739 U/L (153-2317) in group II (n=22), and 391 U/L (112-778) in group III (n=14), P=0.0013. At 12 months, the median is 396 (205-5509) in group I (n=26), 525 (214-2701) in group II (n=14) and 379 (189-655) in group III (n=9), P= 0.2999. LDH ratio (patient value/ULN) is also significant at enrollment (P<0.0001) at 6 months (P<0.0001) and at 12 months (P=0.0316).
Summary / Conclusion: 17.1 % of patients presented with history of thrombosis at enrollment, but most of them had a history of several thrombotic events. Patients treated with eculizumab have never had a new thrombotic event. One patient present venous thrombosis at 6 months and still is without treatment. LDH levels and LDH ratio are significantly higher in patients with classical PNH, lowering with eculizumab treatment at 6 and 12 months. Granulocyte clone size is statistically significant higher among patients with classical PNH with hemolysis at enrollment, 6 and 12 months and does not change in groups I and II although it appears to increase in group II. The data showed that PNH is an evolving disease with some cases from group II moving to group I over time.

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EUROPEAN PATTERN AND IMPACT OF THE USE ERYTHROPOIETIC AGENTS (ESAS)IN LOW AND INT-1 RISK MDS WITH PROPENSITY SCORING TECHNIQUES IN OBSERVATIONAL LONGITUDINAL STUDIES.

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Background: The EU MDS Registry is a non-interventional, observational longitudinal study enrolling patients with lower-risk MDS <3 months from diagnosis. Between 2008 and 2011 1000 patients were recruited from 14 countries and 118 sites, with a follow-up every 6 months.
Aims: This substudy aims to describe the usage of erythropoietic agents (ESAs), and the impact of treatment on overall survival and transfusion patterns in anemic patients.
Methods: Two cohorts were investigated; all patients for distribution of ESA usage, and patients with hemoglobin levels <10 g/dL at registration for the evaluation of outcome. The effect of ESA treatment on survival and other outcomes was assessed using a proportional hazards model. To account for the non-randomised use of ESA within the cohort, a propensity score for receiving ESA treatment was derived using logistic regression and included in the proportional hazards modeling.

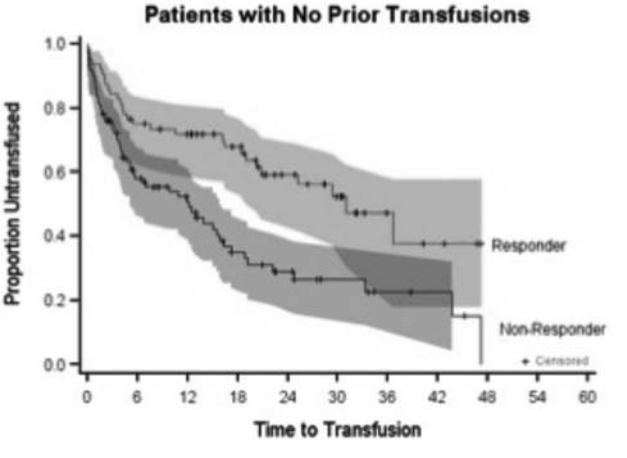

Figure 1. Time to first transfusion in ESA-responders vs ESA nn-responders P=0.0011.

Results: The ESA use varies by country, varying from 16.7% in Poland to 61.1% of the patients in Denmark, as did the pre-treatment Hb levels (range: 4.6-14.9 g/dL). Of the first 1000 patients (median age 74.6 years, range=18.7-95.3), 487 patients (median age 75.8, range: 42-95) were ESA-treated. The median duration of treatment was 8.1 months (0-48 months). The majority of patients started ESA within 6 months after their first visit. In a multivariable

logistic regression model, the strongest predictors of receiving ESA were country, WHO diagnosis subtype, baseline hemoglobin, the percentage of bone marrow blasts and age at diagnosis. The outcome cohort consisted of 419 patients; 260 received ESA and 159 were untreated before their second visit. For a treated patient to be classed as a responder there had to be at least 28 days between the start of ESA treatment and their second visit, with an increased Hb to >10 g/dL or an increase of at least 1.5 g/dL. A patient with transfusions prior to receiving ESA was considered a responder if the transfusion need was abrogated for at least 56 days. Responders appear to have a longer time to first post-ESA transfusion. (P<0.0011) (Figure 1) Median time to transfusion in patients transfused before start of ESA was 5 months (IQR: 2.8-30 months) compared to 15 months (IQR: 3.9-47 months) in patients with no prior transfusions (P=0.0064). The proportional hazards model shows a (non-significant) decreased risk of death (HR=0.74, 95% CI:0.51-1.08, P=0.11) associated with ESA treatment.
Summary / Conclusion: There are marked variations in the patterns of ESA usage across Europe. Treatment with ESA is associated with a tendency to improved survival. Patients starting ESA treatment before receiving transfusions experienced a longer delay in the onset of a regular transfusion need, with ESA–responders having a significant longer time to transfusion after starting ESA compared to non-responders.

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HYPOMETHYLATING AGENTS DOES NOT IMPROVE OVERALL SURVIVAL IN PATIENTS DIAGNOSED WITH CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) COMPARED TO HISTORICAL DATA
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Background: CMML is a clonal hematopoietic disorder that is characterized by a mix of myelodysplastic and myeloproliferative features where its pathologic process is not fully understood and its natural course is variable. Hypomethylating agents (HA) (azacitidine and decitabine) have been approved for the treatment of myelodysplastic syndromes (MDS) and CMML. However, previous studies have included small numbers of patients (pts) who received azacitidine (AZA) and decitabine (DAC) as a treatment for CMML.
Aims: To study the overall response (OR) rates and median overall survival (mOS) in pts who received HA for the treatment of CMML.
Methods: A retrospective chart review of pts diagnosed with CMML at Mayo Clinic Rochester between 1994-2011 was done. All information including hematological labs, bone marrow biopsies results, treatments and responses were obtained from medical records. OR was defined as complete remission (CR), partial remission (PR), marrow remission (MR) and hematological improvement (HI), based on modified International Working group (IWG) criteria in MDS (Cheson B *et al* Blood 2006). IRB approval was obtained in accordance with Helsinki declaration. Comparison between two groups was done using Wilcoxon test, while mOS were calculated using Kaplan-Meier estimates via JMP software V.9.
Results: We found 269 pts diagnosed with CMML, 67% of which were males with a median age of 72 years. Median hemoglobin (Hgb) was 10 mg/dL, white blood cells (WBC) $12x10^9$/L, platelets $88x10^9$/L, and peripheral blood (PB) blasts 0, bone marrow (BM) blasts 4%. Cytogenetics was diploid in 62% (168 pts). Only 25 pts (9%) were considered CMML-2 per WHO criteria. Median overall survival was 515 days. A total of 36 (13%) out of 269 pts received HA (group 1) while 233 (87%) did not receive HA (group 2); of the HA treated patients, 29 (81%) pts received DAC and 7 (19%) pts received AZA. In group2, 69 (30%) pts were treated with other therapies including hydroxyurea, imatinib, prednisone, intensive chemotherapy, or multiple other treatments. No statistical difference was found when comparing both groups where group 1 had median Hgb was 11 mg/dl, WBC $12x10^9$/L, platelets $58x10^9$/L, and PB blasts 0, BM blasts 4%, compared to group 2 of 10, 12, 93, 0,4, respectively. Median OS was not statistically significant between the 2 groups (group 1 of 569 vs group 2 of 513, P=0.39). On multivariate analysis, age, Hgb, platelets, and BM blasts were significant for mOS, but not WBC, PB blasts or HA treatment (P=0.22). Even with subgrouping pts to BM blasts ≥5% or CMML2 (per WHO definition), no statistical significance was found between both groups (P=0.65, P=0.07, respectively). In HA group, 6/32 (19%) pts achieved CR, 5 pts (16%) MR, 3 pts (9%) HI, with a total OR of 44% (in the DAC arm, CR/MR rate was 42%). Median OS for responders (CR/MR) was not statistically significant from non-responders (1083 days vs 409 days, P=0.25). Leukemic transformation into AML was seen in 9/36 (25%) pts in group 1 compared to 11% in group 2 (P=0.028).
Summary / Conclusion: HA are effective therapies in treating pts diagnosed with CMML. HA responders did have better mOS than non-responders but did not reach statistical significance. When compared to historical data of pts who never received HA, HA did not have a improve mOS; both in univariate and multivariate analysis. Our results were limited by small number of pts and being retrospective, therefore more prospective and larger studies are needed to elucidate the effect of HA on CMML pts.

Multiple myeloma: Biology 1

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ANTI-MYELOMA ACTIVITY OF A NOVEL ALKYLATING AGENT MELPHALAN-FLUFENAMIDE
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Background: Despite several recent advances with novel biological agents, multiple myeloma (MM) remains incurable due to the development of relapsed/refractory disease in the majority of patients. The alkylating agent melphalan in combination with prednisone is a standard MM treatment in the non-transplant candidates. High-dose melphalan (HDM) together with autologous stem cell transplantation (ASCT) has enhanced progression-free and overall survival in transplant candidates. Addition of novel therapies such as bortezomib, thalidomide, or lenalidomide into the transplant paradigm as induction, consolidation, and maintenance has further improved patient outcome. Recent research efforts, in Sweden, focused on the development of melphalan prodrug to enhance the therapeutic potential of melphalan. Melphalan-flufenamide (Mel-flufen) is an aminopeptidase N-activated prodrug of melphalan, which enables a more rapid and greater intracellular delivery of melphalan. Mel-flufen has undergone phase-I/IIa clinical trials in solid tumors. Here, we examined the anti-MM activity of mel-flufen using various MM cell lines and patient MM cells, as well as murine models of human MM.
Aims: To study the effects of a mel-flufen on MM cell growth and survival in the bone marrow microenvironment *in vitro* and in xenograft models of human MM. To determine whether the combination of a mel-flufen with other anti-MM agents triggers synergistic or additive anti-MM activity.
Methods: MM cell lines, patient MM cells, and the human MM xenograft animal model were utilized to study the antitumor activity of mel-flufen. *In vitro* assays included measurement of intracellular melphalan, cell viability and apoptosis, Transient transfections, aminopeptidase N activity, and capillary-like tube structure formation assays. Statistical significance was derived using the Student's *t* test. Synergistic anti-MM activity of mel-flufen was obtained with isobologram analysis.
Results: Treatment of MM cells with low doses of mel-flufen induces a more rapid and higher intracellular concentrations of melphalan than is achievable by free melphalan. Analysis of mel-flufen cytotoxicity showed significantly lower IC_{50} of mel-flufen than melphalan; and importantly, mel-flufen triggers apoptosis in melphalan-, and bortezomib-resistant MM cells. Low concentrations of mel-flufen (0.5 micromolar) are able to trigger more potent and greater DNA damage than is observed in cells treated with higher concentrations (3 micromolar) of melphalan.Aminopeptidase N, one of the peptidases mediating melflufen hydrolysis to melphalan, is highly expressed in MM cells; and knockdown of aminopeptidase N with siRNA attenuated mel-flufen induced cytotoxicity. Furthermore, mel-flufen-triggered apoptosis in MM cells was associated with activation of caspases and PARP cleavage, as well as induction of DNA damage, p53, and p21. Using knockdown and knockout cell models, we show that blockade of p21 abrogates mel-flufen-induced cytotoxicity. Moreover, melflu-fen inhibits MM cell migration and tumor-associated angiogenesis. A head-to-head efficacy analysis using our human MM xenograft model showed a more potent inhibition of tumor growth and prolonged survival in mice treated with melflufen than mice receiving equimolar doses of melphalan. Finally, combining mel-flufen with lenalidomide, bortezomib, or dexamethasone triggers synergistic anti-MM activity.
Summary / Conclusion: Our preclinical study supports clinical evaluation of mel-flufen to enhance therapeutic potential of melphalan, overcome drug-resistance, and improve MM patient outcome.

P205

METABOLOMIC PROFILING IDENTIFIES MECHANISMS REGULATING HYPOXIA-INDUCED DRUG RESISTANCE IN MULTIPLE MYELOMA.
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Background: Multiple Myeloma (MM) is the second most prevalent hematological malignancy and remains incurable, with a median survival of 3-7 years. However, despite the success of the new treatments, most patients still succumb to their disease. In about 20-25% of high-risk patients, MM progresses rapidly and does not respond to conventional therapies leading to rapid extramedullary disease and demise of these patients. One such regulator of dissemination and drug resistance is the dynamic process of oxygen depriva-

tion or hypoxia. A number of studies show that hypoxia promotes neo-angiogenesis, cancer progression, epithelial-mesenchymal transition (EMT), acquisition of metastasis potential and stem-cell features, as well as resistance to therapy by activating adaptive transcriptional programs. Targeting hypoxia, and the metabolic pathways regulated by hypoxia in the tumor cells, could lead to novel opportunities for cancer therapy. Rapidly proliferating hypoxic cancer cells undergo a "metabolic switch" to anaerobic glycolysis. This altered energy metabolism has been shown to be associated with activated oncogenes and mutant tumor suppressors, which are more prevalent in patients with high-risk MM.

Aims: Examine the role of HIF1A and HIF2A in regulating drug resistance *in vitro* and *in vivo*. Identify specific hypoxia-regulated genes and regulators of energy metabolism leading drug resistance in MM.

Methods: The effect of hypoxia was analyzed in different MM cell lines (MM1S, RPMI8226, U266 and H929) in basal conditions and after the treatment with bortezomib, dexamethasone or melphalan. The cytotoxicity was analyzed by means of MTT assay. Cell cycle and apoptosis studies were performed by flow cytometry. Proteomic changes induced after treatment were analyzed under normoxic and hypoxic conditions by western-blotting. Gene expression profile of MM1S cells treated with bortezomib was compared in normoxia vs hypoxia using D-chip software. Genes with expression changes greater or lower than 2 fold in either direction were selected. HIF1A and HIF2A knockdowns were performed in MM1S using lentiviral vectors. For metabolite collection, samples were re-suspended using HPLC grade water for mass spectrometry and analyzed using a 5500 QTRAP hybrid triple quadrupole mass spectrometer (AB/SCIEX) coupled to a Prominence UFLC HPLC system (Shimadzu). A total of 254 endogenous water soluble metabolites were analyzed.

Results: We observed that hypoxic conditions (12 hours at 0.7% of oxygen levels) suppressed the effect of melphalan and more significantly the effect of bortezomib. At the transcriptional level and protein level, we observed that cells treated with bortezomib in hypoxic conditions affected a large number of genes/proteins involved in cell cycle, cell death, glucose metabolism and the Wnt signaling pathway. Hypoxia blocked cell cycle progression, which was accompanied by p21, p53 and p57 up-regulation. In addition, apoptosis pathways were inhibited after exposure to hypoxia including inactivation of caspases3, 8 and 9 and PARP cleavage. HIF1A and HIF2A knockdowns restore the effect of bortezomib in MM1S and increased the percentage of apoptosis in cells treated with bortezomib under hypoxic conditions. To further explore the role of hypoxia in the regulation of tumor metabolism, metabolomic studies were performed to characterize metabolic alterations following bortezomib treatment. This analysis revealed that hypoxic tumor cells treated with bortezomib show significant metabolic changes involving multiple pathways, the most significant of which are intermediates in glucose and, sucrose metabolism. Bortezomib treatment under hypoxic conditions was accompanied by a significant decrease in UDP-D-glucose, UDP-D-glucuronate, and glutathione disulfide.

Summary / Conclusion: Hypoxic conditions are essential for drug resistance and glucose utilization. These data provide new therapeutic targets and associated biomarkers for the treatment of Multiple Myeloma.

P206

GENOMIC CHARACTERIZATION OF THE PUTATIVE MULTIPLE MYELOMA STEM CELLS CLONE REVEALS COPY NUMBER ALTERATIONS (CNAS) POSSIBLY CORRELATED WITH THE ORIGIN OF DISEASE

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Background: Although the advances in Multiple Myeloma (MM) therapy and the introduction of novel-agent-based regimens, the disease remains incurable. The existence of Myeloma Propagating Cells (MPCs) is supposed to be one of the major causes of MM drug-resistance, leading to relapse. However, very little is known about the molecular characteristics of MPCs, even if some studies suggested that these cells have phenotypic characteristics resembling the memory B cells that reside in the CD138- compartment.

Aims: To molecularly characterize the CD138+ neoplastic clone and the memory B cells located both in BM and in PBL.

Methods: We collected the CD138+ and CD138-19+27+ cell fractions from bone marrow (BM) and peripheral blood (PBL) of 50 newly diagnosed MM patients (pts), 7 MGUS pts and 15 relapsed pts. For several pts we collected buccal swab sample as a negative control. The complete set of genomic aberrations was evaluated by SNP Array 6.0 (Affymetrix) and the identification of copy number alterations was performed with Genotyping Console and Partek Genomics softwares.

Results: Both BM and PBL CD138+ cell fractions showed exactly the same genomic macro-alterations. In contrast, in the CD138-19+27+ cell fractions from BM and PBL any macro-alteration was detected, whereas several micro-alterations (range: 1-834 Kb) unique of the memory B cells clone, were highlighted. We observed that these micro-alterations are located out of any genomic variants region (Database of Genomic Variants, DGV) and, therefore, presumably associated to the MM pathogenesis. In particular, these micro-alterations involved the following genes: *HMGCLL1*, *DLGAP2* and *RCOR3*, *PRR16*, *TSC1*, *ETS1*, *RBFOX1* , identified in CD138-19+27+ derived from PBL and BM, respectively. These genes are involved in pathways related to the cholesterol metabolism, the embryonic development and the transcriptional regulation. Interestingly, three focal micro-deletions (involving *SKT*, *CES1P1*, *MIR650*) were shared both by the PBL and the BM memory B cells clones. In particular, the *MIR650* gene is located on chr22 in the immunoglobulin lambda gene locus, thus directly controlling its expression and also targeting the expression of several cell cycle genes; indeed, its physiological function allows the inhibition of the cell cycle progression by regulating p16INK4-mediated pathway. By applying a more stringent analysis, the CD138-19+27+ cell fraction obtained from all pts analyzed showed a unique micro-deletion (410 Kb) on chr14, involving *JAG2*, *BRF1*, *PACS2*, *NUDT4* and *BTBD6*. This deletion has been already described as involved in a pediatric *syndrome of chr.14q*, and its presence is correlated to a variety of developmental disorders and mental retardation.

Summary / Conclusion: Reported data suggest that the MM CD138+ clone might resume the end of the complex process of tumorigenesis, proven by the presence of numerous macro-alterations, which might be probably due to an established genomic instability. In contrast, the memory B cells, which lack these macro-alterations, have some intriguing micro-alterations, supporting the idea that these post germinal center cells might be involved in the transforming event that originate the neoplastic clone. Results need to be confirmed in a higher number of pts;additional data will be presented during the meeting.

P207

MULTIDIMENSIONAL FLOW CYTOMETRY FOR SENSITIVE EVALUATION OF PATIENTS WITH IGM MGUS OR WALDENSTROM'S MACROGLOBULINEMIA AND DIFFERENTIAL DIAGNOSIS WITH OTHER LYMPHOMAS

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Background: Although that MYD88 L265P mutation has recently become a genetic hallmark of Waldenström's Macroglobulinemia (WM), approximately 10% of WM patients still lack the mutation, while it can also be present in some cases with B-CLL or Marginal Zone lymphoma (MZL).

Aims: Here, we hypothesized that novel multidimensional flow cytometry (MFC) integrating a total of 16 antigens into a principal component analysis (PCA), after previous merging and calculation of flow cytometric data, would increase the sensitivity to detect the Waldenström's B-cell and plasma cell (PC) clone, as well as contribute towards an automatized-computer-generated differential diagnosis between WM and B-CLL or MZL.

Methods: Bone marrow (BM) samples from newly diagnosed IgM MGUS (n=35), smoldering (n=35) and symptomatic WM (n=21) patients, as well as from MZL (n=7) and B-CLL (n=6) patients were studied by MFC immunophenotyping.

Results: Phenotypically aberrant clonal B-cells were detectable in 77%, 97% and 100% of IgM MGUS, smoldering and symptomatic WM patients, while clonal PCs in 71%, 89% and 100%, respectively. 16-color MFC-derived PCA revealed a complete phenotypic overlap between the Waldenstrom's B-cell and PC clones representative of the three entities, thereby suggesting that the malignant transformation in WM relies mostly in a numeric expansion rather than an evolving phenotype. Clonal B-cells were typically characterized by $CD22^{dim}$, $CD25^{hi}$, $CD79b^{hi}$, $CD81^{hi}$ and $smIgM^{hi}$ expression; bimodal staining for CD27, CD38 and CD200; and absence of CD5, CD10, CD11c and CD103. Clonal PCs were identified by $smIgM^{hi}$ or $CD79b^{hi}$ positive staining. Phenotypically aberrant B-cells and PCs were simultaneously assessed by cytoplasmic kappa and lambda expression to confirm the clonal nature of the selected cell populations. We then investigated if the phenotypic profile of the Waldenström's B-cell clone differed from mutated (n=38) versus wild type (n=4) MYD88, but no significant differences were observed. As expected, in all cases with MYD88 L265P clonal B-cells were detected by MFC; conversely, in 3 out of the 4 cases with wild type MYD88 clonal B-cells were also detectable by MFC. Moreover, ASO-PCR on FACS purified clonal B-cells from these 3 cases confirmed the wild type MYD88, indicating that the absence of MYD88 L265P mutation was not related to a sensitivity limit but a truly non-mutated variant. Finally, automatized-computer-generated PCA between the Waldenström's B-cell clone and clonal B-cells from patients with MZL and B-CLL showed that all cases belonging to the latter group were accurately differentiated within 2 standard deviations (SD), with CD79b, smIgM and CD5 being the most significant markers. Similarly, 5 out of the 7 cases with MZL patients were accurately differentiated from IgM MGUS and/or WM cases within 1SD (with LAIR1, smIgM and CD79b pattern of expression contributing the most). Interestingly, the two cases misclassified were from extranodal MZL of MALT, whereas all 5 cases with nodal

MZL were correctly identified.
Summary / Conclusion: We show here that novel multidimensional flow cytometry is a complementary diagnostic tool for the screening and identification of IgM MGUS and WM patients, as well as to potentially standardize the differential diagnosis between these and other entities potentially concurring with an IgM M-component or MYD88 mutation such as B-CLL and MZL.

P208

IDENTIFICATION OF SERUM MICRORNA PROFILE IN MULTIPLE MYELOMA ASSOCIATED WITH COMPLETE REMISSION AND PROGRESSION AFTER AUTOLOGOUS STEM-CELL TRANSPLANTATION

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Background: MicroRNAs (miRNAs) are small non-coding RNAs that mainly regulate mRNA translation of specific target mRNAs. MiRNAs are deregulated in myeloma cells, and their pattern of expression in MM seems to be associated with specific genetic abnormalities. These molecules can be detected in biological fluids, such as serum, and tumor-associated microRNA profiles can provide potential utility as biomarkers of tumor mass, remission and/or progression.
Aims: To ascertain the expression of miRNA in paired serum samples from patients with multiple myeloma (MM) at diagnosis and at complete remission (CR) after autologous stem-cell transplantation (ASCT), versus sera from healthy donors and those with stable monoclonal gammopathy of undetermined significance (MGUS), and to identify miRNAs related to relapse.

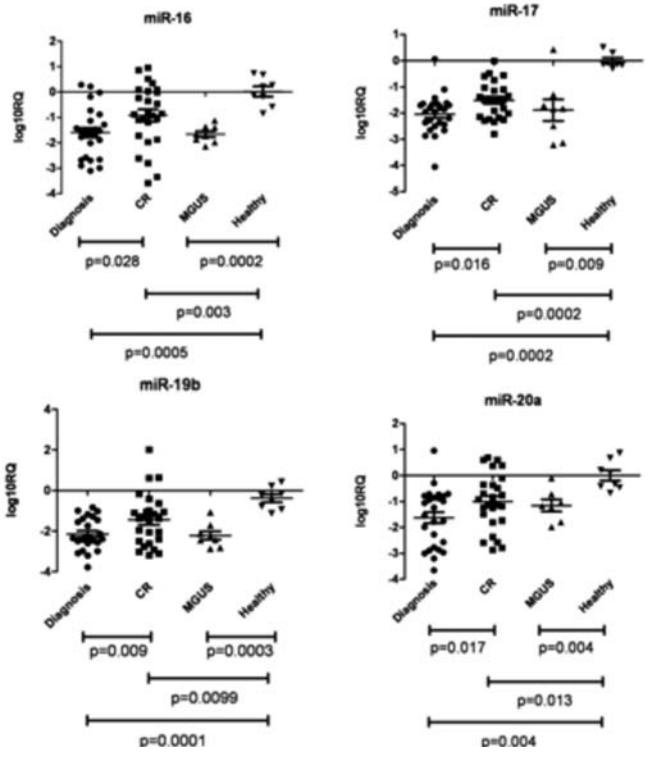

Figure 1. Differential serum levels of four miRNA (miR-16, miR19b, miR-17 and miR-20a in patients with multiple myeloma at diagnosis and complete remission (CR) in comparison with monoclonal gammopathy of undetermined significance (MGUS) and healthy donors.

Methods: First, fifteen MM patients with paired serum samples at diagnostic and at CR after melphalan-based ASCT were studied as screening group. RNA was extracted from frozen serum samples using miRNeasy Mini Kit (Qiagen) and 380 miRNAs and controls were profiled using TaqMan Human MicroRNA Arrays (Array A; Applied Biosystems) using pre-amplification. Expression levels were calculated by 2^{-DDCt} using mir-483-5p as endogenous control, due to the smallest standard deviation among samples, without statistical differences between patients and healthy donors, and the median of 4 serum healthy controls as calibrator. miRNA differentially expressed between diagnosis and CR were selected for validation by single Real Time TaqMan® MicroRNA Assays (without pre-amplification) in a series of 28 patients with paired diagnosis/CR samples after ASCT as first line treatment, plus 8 additional patients with samples in CR, 8 healthy controls and 8 stable MGUS for more than 5 years. Progression-free survival (PFS) after ASCT was recorded in the 36 patients in CR. All PCR reactions were performed using an ABI 7900 HT sequence detection system. Statistical analysis was performed with BRB Array Tools (screening analysis) and GraphPad Prism and PASW Statistics 18 (validation analysis).
Results: Supervised analysis by significance analysis of Microarrays (SAM) and t-test based on multiplex permutations (class comparisons analysis; P<0.001) of the first 15 patients revealed underexpression of 14 miRNAs in sera at diagnosis in comparison with paired samples in CR. The validation in 28 patients with paired samples of this series has shown that this statistical significance remained for miR-16 (P=0.028), miR-19b (P=0.009), miR-17 (P=0.016), miR-20a (P=0.017) (Figure 1), miR-25 (P=0.043) and miR-660 (P=0.048), with a trend for miR-152 (P=0.073). Patients achieving CR showed a partial recovery of the normal serum levels, while MGUS patients had levels very close to patients with MM in CR but lower than normal sera (Figure 1). Trying to explain why all these miRNAs were upregulated in patients in remission, we found that patients with oligoclonal bands had a significantly higher level of miR-25 (P=0.002), suggesting than the cellular origin of at least this miRNA could also be found in non-malignant plasma cells. In the prognostic analysis, patients that had relapsed after ASCT showed a significantly lower levels of miR-19b when in CR (P=0.001). Higher vs. lower levels of serum miR-19b (median PFS 6 vs. 1.8 years; P=0.0001) and miR-331 (median PFS 8.6 vs. 2.9 years; P=0.001) were associated with longer PFS after ASCT.
Summary / Conclusion: In our series of patients with MM after ASCT, measurement of expression levels of several miRNAs in serum showed a underexpression of several miRNA (miR-16, miR-19b, miR-25, miR-17, miR-660 and miR-20a) when compared with normal sera. These results encouraged the potential value of miRNA as serum biomarkers in MM, and even as prognostic factors in patients in CR. Underexpression of some of them in patients with active disease suggested that the origin of these miRNA should be other than malignant plasma cells, such as bone marrow microenvironment. Further studies on this last subject are necessary.

P209

IMPROVED ACCURACY OF DISCRIMINATION BETWEEN IGM MULTIPLE MYELOMA (MM) AND WALDENSTROEM'S MACROGLOBULINAEMIA (WM) BY TESTING FOR MYD88 L265P MUTATIONS

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Background: IgM myelomas account for < 0.5% of all myelomas and are responsible for only a very small proportion of all IgM paraproteinaemias (pp) estimated not to exceed 0.2%. They have been implicated to be frequently associated with an immunophenotype negative for CD20, CD56 and CD117, the occurrence of a translocation t(11;14) and an aggressive clinical course with bad prognosis in the biggest series published of 10 pts., although the universality of these findings have been challenged. WM, which accounts for the waste majority of all IgM pp's , on the other side has recently been shown to be associated with the *MYD88* L265P exon 5 mutation, that triggers IRAK mediated NF-κB signaling**,** in over 90% of cases. The analysis of the frequency of *MYD88* mutations in WM related lymphoid neoplasms showed a positive mutational status in only 16/93 non-WM pts., as well as in only 44/213 pts. in a 2nd published series but included only 3 cases of IgM MM.
Aims: To clarify whether *MYD88* mutational analysis could help to make the clinically critical distinction between IgM MM and WM we analysed 5 further cases of IgM MM with appropriate control samples and extended the analysis to exon 3 and exon 4 of the *MYD88* gene.
Methods: Patients with IgM Myeloma were identified by a search of the Austrian Myeloma Registry (AMR) database (www.myeloma.at), as well as an alignment within the local pathology repository for sample availability. Clinical charts were reviewed for plausibility of the diagnosis (e.g. confirmation of lytic bone disease), as well as an analysis of the cytogenetic and immunophenotypic profil of the neoplasms for myeloma characteristics. Control samples with an established diagnosis of WM were taken from clinical routine diagnostics. DNA from 5 patients (pts.) with IgM MM and 7 pts. with WM was extracted from formalin-fixed paraffin-embedded samples using QIAGEN DNA easy kit (Qiagen, Hilden/Germany). For Sanger sequencing of relevant exons primers were used described elsewhere. Amplified PCR products were isolated by High Pure PCR-Product Purification kit (Roche,Vienna/Austria). For sequencing of the amplified fragments Big Dye Terminator v1.1 ready reaction cycle sequencing kit was used as recommended by the manufacturer (Applied Biosystems, Vienna/Austria). We analysed 5 cases of IgM MM and 6 control cases of active WM, by PCR amplification and Sanger sequencing for MYD88 exon3, 4 and 5 mutations.
Results: We analysed 5 cases of IgM MM and 6 control cases of active WM, by PCR amplification and Sanger sequencing for MYD88 exon3, 4 and 5 muta-

tions. 4/4 IgM MM were found to be unmutated, while 6/7 WM were mutated. No mutations in exons 3 and 4 were found. The clinically important but sometimes difficult discrimination of these two entities can be made with higher precision by MYD88 mutational screening.
Summary / Conclusion: Our results might contradict a role for *MYD88* exon 3 and exon 4 mutations in both WM and IgM MM , but to confirm this findings a higher number of patients needs to be investigated. Furthermore we added another 5 *MYD88* unmutated IgM MM cases to the 3 cases described by (Xu *et al*. 2013). This distinct genetic trait could be exploited to discriminate these entities earlier on and with more precision to allow for a more rationale therapy allocation to the best of our patients.

Reference
Xu L., *et al*.(2013). Blood First Edition Paper, prepublished online January 15, 2013; DOI 10.1182/blood-2012-09-454355.

P210

ABT-199 IS HIGHLY EFFECTIVE AGAINST MULTIPLE MYELOMA AND PLASMA CELL LEUKEMIA HARBORING T(11;14) TRANSLOCATION
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Background: Despite recent advances in the treatment of multiple myeloma (MM) patients invariably relapse and innovative treatment strategies are urgently needed. The development of Bcl-2 inhibitors brings new hope for cancer therapy. We recently demonstrated that the Bcl-2 and Bcl-xL BH3-mimetic ABT-737 induces apoptotic cell death in a sub-group of MM identified by its high Bcl-2/Mcl-1 gene expression ratio. The clinical development of ABT-263 the orally active analog of ABT-737 is impaired due to thrombocytopenia dose-limiting toxicity related to Bcl-x_L inhibition. To overcome this hematologic toxicity, ABT-199, the first-in-class orally bioavailable Bcl-2-selective BH3 mimetic has been developed.
Aims: In the present study, the apoptotic efficiency of ABT-199 was evaluated in both human myeloma cell lines (HMCL) and primary myeloma cells.
Methods: Sensitivity to ABT-199 was assessed by flow cytometry on a panel of HMCLs (n=25) representative of MM heterogeneity and on primary cells from 15 consecutive MM patients. Expression of Bcl-2 and Mcl-1 transcripts by real time quantitative PCR was determined and analyzed in relation to ABT 199 sensitivity.
Results: Among our panel of HMCLs representative of MM heterogeneity, high sensitivity to ABT-199 was restricted to CCND1 subgroup while MMSET HMCLs are always the most resistant subgroup. Indeed, six out eight CCND1 cell lines are efficiently killed by ABT-199 (LD_{50} values ranging from 5 nM to 80 nM). Efficiency of ABT-199 was always superior to the one of ABT-737 as indicated by a decrease of LD_{50} values with ABT-199. Five of sensitive CCND1 cell lines harbors an abnormal p53, indicating that cell death induction is independent of p53 status. Sensitivity to ABT-199 was associated to a higher expression of Bcl-2 (P=.008) and to a lower expression of Mcl-1 (P=.09). The Bcl-2/Mcl-1 ratio defined by Q-PCR was the most powerful biomarker to discriminate ABT-199 sensitive from resistant cell lines (P=.002). Indeed, the median ratio was 6.06 (range 4.47-121) and 1 (range 0.18-3) for sensitive and resistant cell lines respectively. To confirm the role of Mcl-1 in ABT-199 resistance, siRNA against Mcl-1 were transfected in LP1 cells leading to a complete down-regulation of Mcl-1. Mcl-1 silencing highly sensitized these cells to low doses of ABT-199 indicating that Mcl-1 levels are mainly responsible for ABT-199 resistance like it was previously demonstrated for ABT-737. Sensitivity of primary myeloma cell to ABT-199 from 15 consecutive patients was evaluated. A considerable variability in sensitivity to ABT-199 among patient samples was observed. Four patient samples (*de novo* MM, n=2; primary plasma cell leukemia (pPCL), n=1; relapsed MM, n=1) were found highly sensitive to ABT 199 with a LD_{50}<100nM. A FISH analysis of t(11;14), t(4;14) and 17p deletion was analyzed. Of major interest, four ABT-199 sensitive patients bear CCND1 translocation showing that ABT-199 sensitivity is related to genetic subtypes.
Summary / Conclusion: Our data show that ABT-199 lethality is restricted to the specific t(11;14) myeloma subtype. The t(11;14) translocation is found in up to 40% of pPCL which represent a very aggressive form of MM. ABT-199 therapy could represent a very interesting opportunity for targeted therapy for MM and pPLC harboring t(11;14).

P211

BRUTON TYROSINE KINASE EXPRESSION IN MULTIPLE MYELOMA
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Background: Bruton Tyrosine Kinase (BTK) is expressed in normal B and myelo-monocytic lineage cells, where it functions in several signal transduction pathways including the B cell receptor (BCR), the CXCL12 (SDF-1α/β) receptor CXCR4, Toll-like receptors, FcRε, and GpIb. BTK is prominently expressed in normal and malignant B cells and is now an important molecular target for treatment of CLL/SLL, MCL, DLBCL and other malignancies with the oral BTK inhibitor ibrutinib. BTK expression is not detected upon B cell differentiation into plasma cells, however significant BTK expression has been reported in MM patient samples and some MM cells lines. Although over-expression may not necessarily be directly correlated with sensitivity to ibrutinib, tumor types identified as having high levels of BTK expression would merit further investigation.
Aims: Determine the patterns of BTK expression in clinical and molecular subgroupings of MM, characterize the role of BTK in the pathophysiology of MM, and identify subgroups of interest for clinical investigation.
Methods: BTK mRNA expression was analyzed in relation to clinical and molecular variables in gene expression databases, accessed through Compendia Oncomine, which includes 2196 MM clinical accessions from multiple public databases. BTK over-expression was defined as >4-fold average mRNA expression. Results are presented descriptively below.
Results: BTK mRNA was over-expressed in most MM patient samples (74.1%), which was similar to CLL (79.2%), or B cell lymphomas (91-100%), and to a much greater extent than seen in any solid tumor grouping. In comparison with MM, trends towards a higher level of expression were observed in cases of MGUS subtype (92.7%, N= 57) and in plasma cell leukemia (91.7%, N=12). Previously treated patient samples had a slightly greater incidence of over-expression (82.8%, N=842). No differences however were identified between patients with ISS stages I, II or III, patients with or without hyper-diploid cytogenetics, or in patients with light chain myeloma vs IgA/IgG myeloma. BTK was broadly expressed across molecular and cytogenetic subgroupings, however patients with gains of chromosome 11 or CCND1 amplification tended to have higher levels of BTK expression, whereas samples from patients in the MS (Zhan *et al*, Blood 2006;108:2020-2028) or TC 4 (Agnelli *et al*, J Clin Oncol 2005;23:7296-7306) molecular subgroups (generally associated with t(4;14) and related abnormalities) had lower BTK expression, as compared to other subgroups. BTK over-expression was similarly seen to correlate inversely with FGFR3 expression (characteristic of t(4;14)). BTK further showed some degree of co-expression with BCR associated B cell determinants such as CD79a, CD79b, and SYK; a correlation with CXCR4 was also noted.
Summary / Conclusion: BTK is widely expressed in MM, including previously treated patients. Over-expression is frequent however in some molecular and cytogenetic subgroups (e.g. CCND1 amplification, chromosome 11 abnormalities) characterized by high CCND1 expression. This pattern overlaps t(11;14) which has features in common with MCL, a disease known to be sensitive to treatment with ibrutinib. Whether these differences correspond to differences at the protein level and sensitivity to agents such as ibrutinib, or could be the basis of a predictive biomarker, will require further study. BTK co-expression with CXCR4 in MM suggests a prominent functional role of BTK in chemotaxis independently of possible BCR signaling.

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PROGRESSING THROMBOTIC MICROANGIOPATHY IN ATYPICAL HAEMOLYTIC URAEMIC SYNDROME PATIENTS: LONG TERM IMPROVEMENTS IN OUTCOMES WITH ECULIZUMAB
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Background: Thrombotic microangiopathy (TMA) is associated with a number of life-threatening conditions with poor outcomes without appropriate treatment. Establishing its etiology before treatment is therefore important. Atypical hemolytic uremic syndrome (aHUS) arises from chronic uncontrolled complement activation. Thrombotic thrombocytopenic purpura (TTP) is another disease of systemic TMA characterized by severe deficiency of ADAMTS-13 (<5% activity). Eculizumab (ECU) has been approved for the treatment of aHUS. In the absence of rapid testing, presenting platelet levels >30×10^9/L or serum creatinine levels 150-200 mmol/L can be used to identify patients (pts) with a higher likelihood of aHUS compared to TTP (Coppo P *et al*, PLoS ONE 2010;5(4):e10208).
Aims: To evaluate long-term follow-up data from a clinical trial of ECU, a terminal complement inhibitor, in pts with aHUS and active TMA despite plasma exchange or infusion (PE/PI). TTP was excluded by >5% ADAMTS-13 activity levels in all pts.
Methods: This was an open-label, single-arm, phase 2 trial with a long-term extension. Pts aged ≥12 y with aHUS and progressing TMA were treated with ECU 900 mg/wk for 4 wks, 1200 mg at wk 5 and 1200 mg q2 weeks thereafter. The primary endpoint was change in platelet count from baseline.
Results: 17 pts entered the trial; at baseline (not presentation), mean (SD) values were 109 (32)×10^9/L for platelets, 352 (214) mmol/L for creatinine, 89.1 (13) g/L for hemoglobin, 0.47 (0.37) g/L for haptoglobin and 93.5 (15)% for ADAMTS-13 activity. 16/17 pts had been receiving PE/PI prior to inclusion. Four pts (24%) had no identified complement mutations or complement factor

H (CFH) antibodies. Two pts discontinued at wks 1 and 6 due to a protocol violation and an unrelated adverse event (AE), respectively; 13 pts continued ECU treatment in the long-term extension. Platelet normalization (>150×10^9/L) was achieved by 26 wks in 13/15 pts with low platelets at baseline, and was maintained through 2 y in 12 pts. Hemoglobin improved, likely reflecting control of aHUS-related hemolysis, since study pts were maintained on a constant EPO dose and median number of transfused red cell units per pt was 0 (range 0-16). Improvements in renal parameters were also seen and were sustained through 2 y (Table 1). ECU eliminated the need for dialysis in 4/5 pts receiving dialysis at baseline. Rates of treatment-related AEs remained the same or declined with ongoing treatment.
Summary / Conclusion: Baseline platelet and creatinine levels of study pts were consistent with ongoing active aHUS at the time of study entry and confirmed by ADAMTS-13 levels >5%. Identification of a complement mutation is not required for diagnosis of aHUS (24% in cohort had no identified complement mutation). Long-term ECU therapy in these pts, who had progressing TMA, was associated with significant and continuous improvements in outcomes at a median duration of 2 y (Table 1). Although aHUS carries a poor prognosis, all pts receiving chronic ECU therapy remain alive.

Table 1.

Key outcomes with ECU	26 wks	2 y[a]
Platelet count change, mean, ×10^9/L (SD)	96.7 (80.9) p=0.0004	93.9[b,c] (55.5) p=0.001
Hemoglobin g/L (SD)	125 (18)	128 (22)
Hematological normalization,[d] n (%)	13 (76)	15 (88)
TMA-event-free status,[e] n (%)	15 (88)	15 (88)
eGFR increase ≥15 mL/min/1.73m^2, n (%)	9 (53)	10 (59)
eGFR change from baseline,[f] mL/min/1.73m^2, mean (95% CI)	32.0 (14.5-49.4) p=0.001	35.2[b] (17.3-53.1) p=0.0005

For all parameters, improvement was seen in ≥2 consecutive measurements over 4 wks. Baseline mean eGFR 22.9 mL/min/1.73m^2 (SD, 14.5).
[a]At data cutoff (median 100 wks), unless otherwise noted.
[b]104 wks.
[c]Change from baseline based on sample size at each time point.
[d]Normal platelet count (>150 x 10^9/L) and LDH levels for ≥2 consecutive measurements, ≥4wks apart.
[e]≥12 consecutive wks with no platelet count change >25% + no PE/PI + no new dialysis.
[f]Based on ANOVA.

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ACTIVATION OF THE MTOR PATHWAY AND LOCALIZATION OF THE MTOR PROTEIN IN PRIMARY MYELOMA CELLS AND MULTIPLE MYELOMA CELL LINES: ROLE OF POMALIDOMIDE

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Background: mTOR is a protein kinase that plays a central role in regulating critical cellular processes. Consistent with its primary target being the translation machinery, mTOR is predominantly localized in the cytoplasm. However, a nuclear localization of mTOR has been found in rhabdomyosarcomas and HCT8 colon carcinoma cells. Furthermore, mTOR becomes nuclear in HEK293 cells treated with leptomycin B, a specific inhibitor of nuclear export receptor Crm1 suggesting that mTOR may be a shuttling protein.
Aims: to evaluate: activation of the mTOR pathway in multiple myeloma (MM), cellular localization of mTOR protein in MM cell lines and primary myeloma cells, the role of pomalidomide in regulating mTOR.
Methods: immunohistochemistry with antibody against p-mTOR was performed on bone marrow sections of 92 MM patients. Antibody against p-AKT, p-P70S6K and p-4EBP-1 was also used on bone marrow sections of the 57 newly diagnosed MM patients. Proliferation was evaluated by MTT assays in RPMI8226 and OPM2 cells following incubation with pomalidomide. Apoptosis was evaluated by flow cytometry for the detection of annexin V-positive cells in MM cell lines and in plasmacells from 3 MM patients. Cellular localization of mTOR was evaluated with confocal microscopy in RPMI8226 and OPM2 cells and in plasmacells from 4 MM patients in basal condition and after pomalidomide treatment. MM cell lines untreated or treated with the drug were fractionated and both cytoplasmic and nuclear fraction were analysed by western blotting with antibodies against mTOR and p-mTOR.
Results: overall, cytoplasmic p-mTOR stained positive in 46 out 92 (50%) cases by immunohistochemistry. A nuclear p-mTOR staining was also detected in 11 cases (12%). In the 57 newly diagnosed MM patients, p-mTOR expression significantly correlated with p-AKT (r=0.32, P=0.012), p-P70S6K (r=0.48, P=0.0001), and p-4E-BP1 (r=0.47, P=0.0001). In this population, strong mTOR expression (HSCORE >60) significantly correlated with high β2microglobulin-serum levels (>3.5 mg/L). Pomalidomide 1μM at 48h inhibited proliferation of OPM-2 and RPMI-8226 cells with 50% and 40% decrease in cell numbers by MTT assays. Pomalidomide 1μM was also effective in plasmacells from 3 MM patients at 24h with 23%, 33% and 26% annexin-V positive cells (versus 11%,18% and 3% of controls). Immunofluorescence assays demonstrated that mTOR protein is distributed throughout the cytoplasm and the nucleus at baseline in both MM cell lines and in plasmacells of 3 out 4 MM patients. A clearly increase of the nuclear mTOR protein was detected after pomalidomide treatment in RPMI-8226 and OPM-2 cells (10 μM at 48h) and in plasmacells from 3 MM patients (1 μM at 24h) (2 with nuclear mTOR localization at baseline and 1 without it). Cytoplasmic and nuclear distribution of mTOR and p-mTOR was also evidenced by Western blotting in RPMI-8226 and OPM-2 cells. As expected, the mTOR and p-mTOR protein levels were significantly higher in the cytoplasm when compared to the nucleus. Treatment with pomalidomide 10μM at 48h increased nuclear mTOR and p-mTOR expression levels in the nucleus with a concomitant decrease of cytoplasmic p-mTOR protein amount.
Summary / Conclusion: the AKT/mTOR pathway is activated in a subset of MM patients. In MM cell lines and in a fraction of primary MM cells, mTOR is distributed throughout the cell cytoplasm and in some nucleus. The anti-myeloma activity of pomalidomide may be mediated by the downregulation of the mTOR pathway with a nuclear shuttling of mTOR protein and a reduction of the cytoplasmic p-mTOR.

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MULTIDIMENSIONAL FLOW CYTOMETRY IMMUNOPHENOTYPIC QUANTIFICATION AND CHARACTERIZATION OF IN VIVO MESENCHYMAL STEM CELLS IN MGUS AND MULTIPLE MYELOMA

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Background: The interaction between clonal plasma cells (PCs) and bone marrow (BM) stromal cells plays a crucial role in multiple myeloma (MM), accounting for unbalanced bone remodeling and activation of pleiotropic signaling cascades which can promote chemoresistance. Interestingly though, the mesenchymal stem cell (MSC) - precursor of stromal cells - have only been modestly investigated, recurring to *ex vivo* expansion and mostly in active MM.
Aims: Identify, quantify and characterize the minor BM compartment of MSC in patients with MGUS and MM, using sensitive multidimensional flow cytometry (MFC).
Methods: A total of 61 newly diagnosed patients with a PC dyscrasia are the focus of this study, 32 being diagnosed as MGUS, 5 as smoldering and 24 as symptomatic MM. Ten age-matched normal individuals were used as control. MSC were identified in BM samples according to positive staining for CD73, CD90 and CD105, without expression of CD34 and CD45; the mean intensity of fluorescence (MFI) for CD11b, CD13, CD24, CD29, CD49e, CD73, CD106 and NGFR was specifically assessed on the surface membrane of MSC.
Results: Upon normalization by removing clonal PCs from the differential, the mean frequency of BM MSC was found to be similar between normal individuals (0.03%) versus MGUS (0.03%) and SMM (0.02%) patients (P>.05). By contrast, significantly increased numbers of BM MSC (0.14%) were noted in symptomatic MM patients as compared to MGUS patients (P=.03), with a trend also recorded versus SMM cases (P=.05) and normal individuals (P=.08). Among symptomatic MM patients, a progressive increment in the number of MSC was found between ISS I, II and III (0.01%, 0.07% and 0.23%, respectively; P>.05). Moreover, a significantly inverse correlation between the percentage of BM MSC and hemoglobin values was noted (r=0.51; P=.006); conversely, increasing numbers of MSC were followed by higher BM plasmacytosis (r=0.54; P=.008). We then compared the specific phenotypic profile of MSC from patients with the benign (MGUS and SMM) versus malignant (MM) form of the disease. From a total of 8 markers under analysis, significantly differences between symptomatic MM versus MGUS and SMM patients were found exclusively for the adhesion molecule CD49e (P=.01) and CD90 (P=.02), which was significantly increased in symptomatic MM.
Summary / Conclusion: Our results show a significant increment in the frequency of BM MSC from MGUS and SMM to symptomatic patients, thereby suggesting that in addition to support chemoresistance in the active form of the disease, MSC may be potentially implicated in its malignant transformation. The fact that MSC from symptomatic MM patients show increased expression of adhesion molecules deserves further investigations, as it may facilitate its interaction and support clonal PCs. Accordingly, symptomatic MM patients with advanced disease stage showed increased number of BM MSC.

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HUMAN VDELTA1 T CELLS REPRESENT A DOMINANT GAMMA-DELTA T CELL SUBSET IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Human γδ T cells are potent effector lymphocytes of innate immunity involved in anti-tumor immune surveillance. However, the role of Vδ1 γδ subset in patients progressing from monoclonal gammopathy of undetermined significance (MGUS) to Multiple Myeloma (MM) in this process is unknown. We have recently shown that Vδ1 lymphocytes isolated from myeloma patients exert specific cytotoxicity against primary CD38+CD138+ bone marrow derived plasma cells (Knight *et al*., Cytotherapy 2012).
Aims: We aimed to determine the frequencies and phenotypes of Vδ1 and Vδ2 γδ T cell populations in MGUS patients, newly diagnosed myeloma patients, and treated myeloma patients. We hypothesized that the innate immune responses are gradually deregulated in patients progressing from MGUS to MM and this leads to changes in T cell distribution.
Methods: Fresh whole bone marrow samples were used from MGUS (n=25), newly diagnosed myeloma (n=41) and treated myeloma patients (n=7). We used multicolor flow cytometric analysis to determine the naïve/memory/effector phenotypes in patient cohorts and to compare the data with healthy donors.
Results: In MGUS patients, the median levels of Vδ1 and Vδ2 cells were 2.19 (range 0.55-8.05) and 0.63 (0.04-10.2) of CD3+ T cells respectively. Interestingly, largely elevated Vδ1 cells compared to healthy donors were predominantly of naïve phenotype CD27+CD45RA+ in these patients. In contrast, Vδ1 cells in newly diagnosed MM patients and patients after treatment showed effector memory CD27+CD45RA- and terminally differentiated phenotype of CD27-CD45RA+ expression. More importantly, we detected continuously expanded frequencies of Vδ1 cells in both cohorts of MM patients, (range 0.19-17.50 and 1.35-7.50 respectively). The Vδ2 cell subset was also expanded in MM patient cohorts, however the medians were overall reduced compared to the Vδ1 population. The Vδ2 cells were mostly of memory phenotype with CD27+CD45RA- expression in all patient cohorts.
Summary / Conclusion: Our results show significantly elevated frequencies of Vδ1 γδT cell subset in patients progressing from MGUS to MM indicating a possible compensation for defects in specific T-cell immunity. Given that the Vδ1 subset shows a very restricted TCR repertoire compared to the Vδ2 subset, the overall capacity of γδ T cells to recognise and respond to antigens in the bone marrow microenvironment might be significantly enhanced by the interactions with other cell types. The role of large expansions of Vδ1 cells and factors leading to myeloma progression remain to be elucidated. Nevertheless, this is the first report analyzing the Vδ1 cells in patients with monoclonal gammopathy. The project has been funded by the Royal Free Charity, and IGA NT 11145-4, IGA 12130-4, MSMT 0021622434, P304/10/1395 grants.

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AN EGFP IMAGING BASED *IN VIVO* MODEL FOR STUDYING THE BIOLOGY AND TREATMENT OF MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a B-cell malignancy characterized by the accumulation of abnormal plasma cells in the bone marrow (BM) and the development of progressive bone destruction. New treatments targeting the interactions between MM cells and their host BM microenvironment, may improve the survival of MM patients. Therefore, it is necessary to develop an appropriate model presenting the detail interactions and enables real-time monitoring of tumor burden, for assessment of *in vivo* activity of novel anti-MM therapeutics.
Aims: Here we sought to explore such a model in severe combined immunodeficient (SCID) mice, by utilizing whole-body non-invasive fluorescence imaging.
Methods: RPMI8226 and ARH-77 cell lines were stably transduced with an enhanced green fluorescence protein (eGFP) gene. Then these cells were injected into rabbit bone chips previously inoculated into SCID mice (SCID-rab mice), to induce myelomatous tumor growth and bone disease. Tumor burden was then monitored by real-time whole-body fluorescence imaging and serum human immunoglobulin (Ig) detection. Radiographs of mice were taken weekly by X ray. Changes in rabbit bone mineral density (BMD) were recorded. At the end of the experiment, rabbit bone sections were stained with hematoxylin and eosin (H&E) staining. Then SCID-rab-RPMI8226 mice were treated with bortezomib to further clarify the effects of this model for assessment of novel anti-MM therapeutics.
Results: We showed that both cell lines (RPMI8226 and ARH-77) progressed as myeloma-like tumors predominantly in the rabbit bone marrow. Increased tumor burden and decreased survival were detected in SCID-rab mice inoculated with MM cells (P<0.05). In addition, in our model, the sensitivity of fluorescence imaging technique appeared superior to human Ig for tumor growth monitoring. Moreover, rabbit bones harboring myeloma cells were severely resorbed and BMD was significantly decreased when compared with control (P<0.01). Furthermore, mice treated with bortezomib were preserved, exhibited no radiologically identifiable osteolytic lesions and, unlike the controls treated with PBS, lived longer and showed reduced tumor burden (P<0.05).

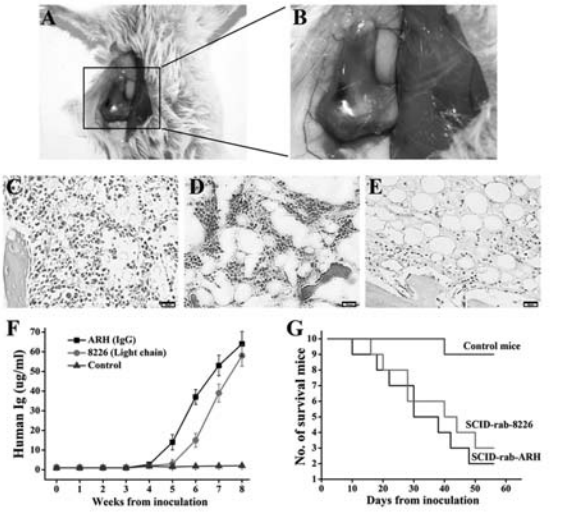

Figure 1.

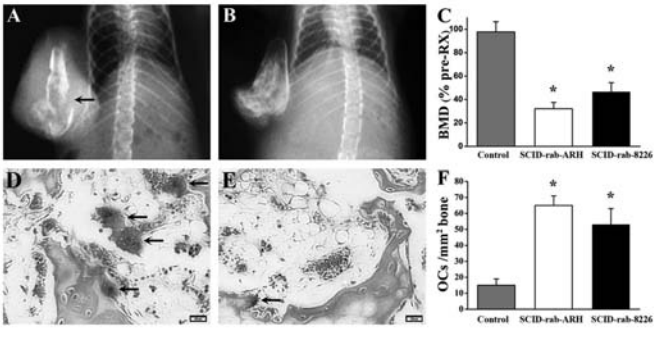

Figure 2.

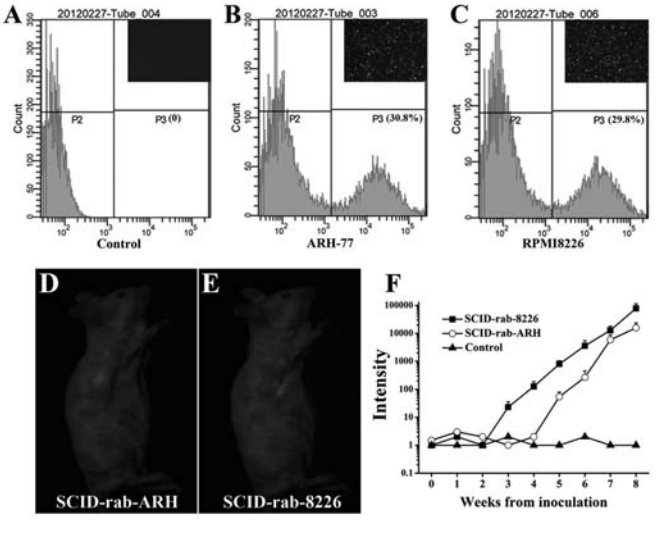

Figure 3.

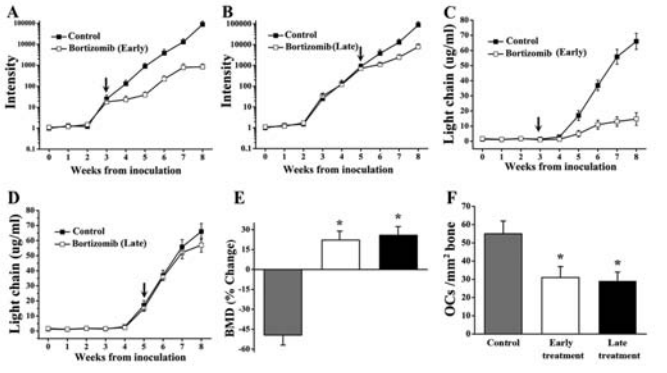

Figure 4.

Summary / Conclusion: This *in vivo* system both exhibits the interaction of MM cells with BM milieu *in vivo* and allows for sensitive real-time monitoring of tumor burden. Therefore, it provides a valuable and sensitive model for the comprehensive preclinical evaluation of anti-MM therapeutics.

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IDENTIFICATION OF POTENTIAL DRUG TARGETS WITHIN THE UBIQUITIN PROTEASOME SYSTEM IN MULTIPLE MYELOMA

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Background: One of the most significant therapeutic advances in Multiple Myeloma (MM) has been the disruption of the ubiquitin proteasome system (UPS) through the use of proteasome inhibitors. Bortezomib, the first-in-class proteasome inhibitor, is now a widely used component of MM therapy. Despite its success a number of patients develop drug resistance and dose-limiting side-effects such as peripheral neuropathy. As knowledge of the UPS has increased, it has become evident that there may be other potentially druggable targets within this system which would confer greater specificity. E3 ligases and de-ubiquitinating enzymes (DUBs) in particular present an ideal therapeutic candidate as they play key roles in substrate selection and may enable direct targeting of an aberrant signalling pathway.
Aims: To identify aberrantly expressed genes within the ubiquitin proteasome system in Multiple Myeloma.
Methods: We carried out comparative gene expression profiling of MM cell lines (U266 and OPM-2) and normal bone marrow (NBM) using microarrays specifically focused on UPS-associated genes (Piqor, Miltenyi). Gene expression levels of select UPS enzymes were validated in MM cell lines (U266, OPM-2, KMS-18) and CD138+ cells from 4 MM patient samples and 3 healthy donors by real-time PCR.
Results: Initial microarray analysis found that 132 genes were significantly differentially expressed in MM cell lines compared to NBM (2 sided 't' test with equal variance using normalised log2-ratio). On the basis of these results, consolidated with publically available data sets comparing healthy donor CD138+ cells with CD138+ cells isolated from MM patients (GSE6691 and GSE6477), 25 E3 ligases/DUBs were found to be consistently differentially expressed in MM and brought forward for further validation. 7 E3 ligases and 2 DUBs demonstrated at least 2-fold higher expression across cell lines and primary MM cells compared to NBM CD138+ cells. One of the UPS enzymes identified though this analysis has recently been demonstrated to be an effective therapeutic target in pre-clinical models of MM, which serves to support the validity of this research strategy.
Summary / Conclusion: The proteasome is already established as a valid therapeutic target in MM and it is becoming increasingly clear that the UPS offers many opportunities for more targeted anti-cancer therapy. The 9 upregulated E3 ligases/DUBs identified in this study will be further investigated to explore their potential as therapeutic targets in MM.

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MULTIPARAMETER FLOW CYTOMETRY DETECTION OF CLONAL PLASMA CELLS IN THE BONE MARROW OF PATIENTS WITH SOLITARY PLASMACYTOMA DETERMINES THE RISK OF PROGRESSION TO ACTIVE MYELOMA

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Background: Although solitary plasmacytoma (SP) represents only 5% of all plasma cell (PC) dyscrasias this is nonetheless a heterogeneous group of patients; half or more develop treatment-requiring myeloma (MM) in 2-3 years, while others remain disease-free at 10 years. Since the diagnosis of SP requires absence of bone marrow (BM) plasmacytosis, no additional lytic lesions (by any imaging modality) or other CRAB, the identification of patients at risk of developing MM is challenging. Albeit patients with SP and an abnormal serum FLC ratio have a shorter time to progression (TTP) to MM, approximately 20% of cases with normal FLCs also develop MM at 5 years.
Aims: Here, we hypothesized that sensitive bone marrow (BM) evaluation of patients with SP through multiparameter 4-color flow cytometry (MFC) would unravel the presence of clonal PCs in otherwise disease-free BM according to conventional techniques, and that BM clonality could identify those patients at higher risk of developing MM.
Methods: Twenty patients with histological confirmation of a solitary plasmacytoma of bone (n=12) or soft tissue (n=8), without BM involvement and absence of PC-related CRAB symptoms are the focus of this study. Clonal PCs were investigated by MFC immunophenotyping based on differential expression of CD38, CD19, CD45, and CD56, and light scatter characteristics, using a single 4-color MoAb combination.
Results: Among the 20 patients with SP, 8 (40%) cases showed phenotypically aberrant PCs in BM (median of 0.04%: 0.004 - 0.9%). In 6 of these 8 patients an MRI was also performed but no BM involvement was noted. After radiotherapy, patients with clonal PCs showed a median TTP to treatment-requiring MM of 36 months versus not reached for those with undetectable clonal PCs (P=.003). Accordingly, 50% of patients with clonal PCs have progressed; by contrast, only 1 of the 12 cases (9%) without BM involvement showed disease transformation (occurring in this case 12.5 years after diagnosis of SP and with multiple skin plasmacytomas but no evidence of BM disease). At the time of diagnosis of SP, 9 out of the 20 patients had an M-protein, with no significant differences in the median TTP to MM between these vs. those cases with undetectable M-protein (P=.97).
Summary / Conclusion: Our results highlight the importance of MFC immunophenotypic studies for a sensitive evaluation of BM samples from patients with SP, and an accurate prediction of their risk of developing treatment-requiring MM.

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CAN IMMUNOPHENOTYPIC CR BE ALSO ACHIEVED IN RELAPSED MULTIPLE MYELOMA PATIENTS?

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Background: Although the introduction of IMiDs and proteasome inhibitors has significantly improved response rates and outcome in relapse multiple myeloma (MM), the management of these patients remains challenging and prognostic biomarkers to identify those at different risk are scarce.
Aims: Here, we hypothesized that in parallel to the front-line setting, novel therapeutic options and autologous or allogeneic stem cell transplantation (SCT) may induce minimal residual disease (MRD) clearance and that this may translate into extended survival in the relapse setting.
Methods: Thirty patients achieving CR after rescue therapy were referred for MRD investigation by multiparameter flow cytometry (MFC) and are the focus of the study. Rescue therapy immediately preceding CR was usually based on novel agent combinations (90%), followed by alloSCT in 37% of cases and autoSCT in 23%. The remaining 40% were not transplanted. Patients were defined to be in immunophenotypic CR when less than one phenotypically aberrant plasma cell was detected among 10^5 cells analyzed.
Results: From the 30 patients in CR, 14 (47%) also achieved immunophenotypic CR whereas the remaining 16 (53%) were MRD+. MRD clearance was most likely achieved in patients submitted to SCT vs. those who were not (61% vs. 25%; P=.05). Only 2 out of the 14 (14%) MRD- cases experienced subsequent relapse as compared to 94% in MRD+ cases (P<.001); median time to progression not reached (NR) vs. 13 months (P=.007), respectively. Median overall survival was NR vs. 38 months (P=.14), respectively. It should be noted that only 2 of the14 MRD- patients have died (both from GVHD without MM progression) in contrast to 10 of the16 MRD+ cases (P=.01). Further sub analysis focusing exclusively on patients submitted to SCT showed that persistent MRD also predicted for significantly inferior TTP (median 11 months vs. NR; P=.018); conversely, no relapses occurred among patients not submitted to

SCT but achieving an immunophenotypic CR (0% vs. 100% of cases MRD+; P=.005).
Summary / Conclusion: We show that achieving immunophenotypic CR is possible in a subset of relapse MM patients particularly after SCT, and identifies a subset of cases with long term relapse free survival.

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MYELOMA PLASMA CELLS ALTER THE BONE MICROENVIRONMENT BY STIMULATING AN INCREASE IN MESENCHYMAL STEM CELLS: A COMPARATIVE STUDY OF MYELOMA PATIENT- AND MURINE MODEL-DERIVED MESENCHYME

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Background: Multiple myeloma (MM) is an incurable haematological cancer characterised by the clonal proliferation of plasma cells within the bone marrow (BM). Numerous studies suggest that the myeloma plasma cells occupy and alter the stromal tissue of the bone marrow as a means of enhancing their survival and growth. However, the nature and magnitude of the changes to the stromal cell tissue remain to be determined.
Aims: To identify changes in stromal tissue in response to myeloma plasma cells.
Methods: In this study, we used mesenchymal stromal cell (MSC) and osteoblast (OB)-related cell surface marker expression and flow cytometry to enumerate MSC and OB numbers in diagnostic BM recovered from myeloma patients and C57BL/KaLwRij mice bearing myeloma disease.
Results: Using this approach, we identified an increase in the number of STRO-1 positive colony forming MSC and a concomitant decrease in alkaline phophatase positive OB. Notably, this increase in MSC numbers correlated closely with plasma cell burden at the time of diagnosis. Additionally, in comparison with the OB population, the STRO-1+ MSC population was found to express higher levels of plasma cell- and osteoclast- activating factors, including RANKL, CXCL12 and IL-6, providing a mechanism by which an increase in MSC may promote and aid the progression of myeloma. Importantly, these findings were faithfully replicated in the C57BL/KaLwRij murine model of myeloma.
Summary / Conclusion: Myeloma plasma cells alter the bone microenvironment by stimulating an increase in mesenchymal stem cells. In addition, the C57BL/KaLwRij murine model presents a clinically relevant system in which to identify and therapeutically modulate the bone microenvironment and in turn, alter the progression of myeloma disease.

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EFFICACY AND SAFETY OF 3 LENALIDOMIDE-BASED COMBINATIONS IN ELDERLY NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: RESULTS FROM THE PHASE 3 COMMUNITY BASED EMN01 TRIAL

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Background: Lenalidomide plus low-dose dexamethasone (Rd) and melphalan-prednisone-lenalidomide (MPR) followed by lenalidomide maintenance showed to be effective and safe in elderly newly diagnosed multiple myeloma(MM) patients (pts). Cyclophosphamide represents a valid alkylant alternative in combination with steroids and novel agents. No formal comparison between these combinations has been performed until now.
Aims: To assess the efficacy and safety of the lenalidomide plus low dose dexamethasone (Rd) vs Melphalan-Prednisone-Lenalidomide (MPR) and Cyclophosphamide-Prednisone-Lenalidomide (CPR) in a community-based setting of MM pts ≥65 years old or not eligible to autologous stem cell transplantation.
Methods: Pts with symptomatic MM were randomized (1:1:1) to receive 9 28-day cycles of Rd, MPR or CPR. Upfront dose reductions of dexamethasone, melphalan and cyclophosphamide were performed, according to pt age (Rd: lenalidomide 25 mg/day for 21 days; dexamethasone 40 mg on days1, 8, 15 and 22 in pts 65-75 years old and 20 mg in those >75 years; MPR: lenalidomide 10 mg/day for 21 days; melphalan orally 0.18 mg/Kg for 4 days in pts 65-75 years old and 0.13 mg/Kg in >75 years pts; prednisone 1.5 mg/Kg for 4 days; CPR: lenalidomide 25 mg/day for 21 days; cyclophosphamide orally 50 mg/day for 21 days in pts 65-75 years old and 50 mg every other day (eod) in >75 years pts; prednisone 25 mg eod). After induction, pts were randomized to receive maintenance with lenalidomide (10 mg/day on day 1-21 every 28) alone or in combination with prednisone (25 mg eod), until disease progression. The primary endpoint was progression-free survival (PFS).

Table 1.

Patients characteristics	CPR		MPR		Rd	
	N=222		N=218		N=223	
SEX						
male	107	48%	108	50%	106	48%
AGE (range-year)	48-88		63-90		63-87	
median	73		73		73	
> 75 y	80	36%	86	40%	83	38%
CLEARANCE CREAT (range-ml/min)	30-150		30-168		30-150	
median	65		70		67	
ISS stage						
I	62	28%	61	28%	59	27%
II	98	44%	97	45%	103	47%
III	60	27%	59	27%	60	27%
Cytogenetic [del17 or t (4;14) or t(14;16)]	54	37%	49	37%	53	36%
Charlson						
0	153	69%	150	69%	135	61%
1	40	18%	39	18%	57	26%
≥2	29	13%	28	13%	28	13%
ECOG PS						
0	73	33%	55	25%	69	31%
1	99	45%	98	45%	106	48%
2-3	40	18%	52	24%	42	19%
Frail						
Yes (ECOG≥2 or Charlson≥2 or age>75)	124	56%	140	65%	143	65%

Results: Between October 2009 and October 2012, 663 pts were enrolled (Rd:222, MPR:218; CPR: 223). Patient characteristics were well balanced in the

three groups. Median age was 73 years in each arm; 37%, 40% and 36% of pts respectively in Rd, MPR and CPR arms were >75 years. Frail pts were 65% in the Rd arm, 65% in the MPR arm and 56% in CPR arm. (Table 1). At data cut-off all pts had completed the 9 induction cycles. Median follow-up was 19 months. Partial response (PR) rate was similar in the 3 arms: 74%, 74% and 75% respectively in Rd, MPR and CPR group, including 35% very good partial response in Rd, 29% in MPR and 26% in CPR. A trend towards a higher complete response (CR) rate was noticed in the MPR group (12%), similar CR rate was reported in Rd (5%) and CPR (7%). At least 1 grade ≥3 hematological adverse event (AE) was reported in 28% Rd pts, 62% MPR pts and 29% CPR pts. The main hematological AE was neutropenia (Rd: 24%; MPR: 59%; CPR: 26%). At least 1 grade ≥3 non-hematological AE was observed in 25% of Rd pts, 29% of MPR pts and 22% of CPR pts. The most common grade ≥3 non-hematological AEs were infections (Rd: 6%, MPR: 9%, CPR:4%) and dermatological toxicities (Rd: 4%, MPR: 4%, CPR: 7%). Treatment discontinuations for toxicity were 7% in Rd, 12% in MPR and 12% in CPR. The main reason for treatment discontinuation was non-hematological toxicity in Rd (6%) and CPR (11%). Only 1% of pts stopped treatment for hematological AEs in Rd and CPR. In the MPR arm 7% of pts stopped treatment for hematological AEs and 5% for non-hematological AEs. Rate of toxic deaths was similar in the 3 arms (4% Rd, 4% MPR and 5% CPR).

Summary / Conclusion: Rd, MPR and CPR produced similar RR in elderly MM patients. The main AEs were hematological, with a higher incidence in MPR in comparison with Rd and CPR. Non-hematological AEs were similar in the 3 groups.

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MELPHALAN/PREDNISONE/LENALIDOMIDE (MPR) VERSUS HIGH-DOSE MELPHALAN AND AUTOLOGOUS TRANSPLANTATION (MEL200) IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS

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Background: The incorporation of new drugs into induction, consolidation and maintenance therapy is changing the treatment paradigm of MM but their role is not well defined.

Aims: To compare in a prospective randomized trial (MM-RV-PI209) conventional chemotherapy plus novel agents [melphalan-prednisone plus lenalidomide (MPR)] with tandem high-dose melphalan (melphalan 200 mg/m2 with stem-cell support; MEL200), both followed by maintenance with lenalidomide or no maintenance.

Methods: A 2x2 factorial randomized trial was designed to assess the role of adding lenalidomide to melphalan/prednisone at induction and as maintenance therapy.The primary end point was PFS on the intent to treat population. An enrolment of 170 pts/arm was required to demonstrate a15% improvement of PFS at 2 years (2-sides a= 0.05, 1- β=80%). At diagnosis, 402 pts (≤ 65 years) were randomly assigned to receive MPR (six cycles of melphalan-prednisone-lenalidomide, N=202) or tandem MEL200 (N=200). After MPR or MEL200, pts were further randomized, within each group, for no maintenance (N=204) or lenalidomide maintenance (10 mg, days 1-21, N=198).

Results: Patients characteristics were well balanced, including median age (58 years in both groups), ISS and FISH abnormalities [presence of t(4;14) or t(14;16) or del17p]. Response rates were similar after consolidation (MPR vs MEL200), with very good partial response (VGPR) or better of 60% vs. 58% (P=0.24) and complete response (CR) rate of 20% vs. 25% (P=0.49). The median duration of maintenance was 26.28 months. Lenalidomide maintenance did not significantly increase response rate: CR rate was 20% after MPR and 25% after maintenance, while it was 25% after MEL200 and 32% after maintenance. After a median follow-up of 45 mos from diagnosis, the median PFS was 25 mos with MPR and 39 mos with MEL200 (corresponding to a PFS of 50% vs 68% at 2 years, HR=1.66; 95%CI 1.27-2.18, P=.0002). Median PFS were 37.5 mos for maintenance and 25.7 mos for no maintenance (HR=0.63, 95%CI 0.48-0.83, P=.0008). The 4-year OS from diagnosis was similar: 71% with MPR and 72% with MEL200 (HR 1.08, 95%CI 0.72-1.63, P=0.71), 76% for maintenance and 68% for no maintenance (HR 0.68, 95%CI 0.45-1.04, P=.08). After a median follow-up of 32 mos from start of maintenance, the median PFS was for 41 mos for maintenance and 18 mos for no maintenance (HR=0.50, 95%CI 0.36-0.68, P<.0001). The 3-year OS from start of maintenance was 81% for maintenance and 72% for no maintenance (HR 0.60, 95%CI 0.37-0.97, P=.04). No meaningful interaction was detected between MPR/MEL200 and maintenance/observation effects.

Summary / Conclusion: MPR at diagnosis was clearly inferior to MEL200 when PFS is used as the main endpoint. Lenalidomide maintenance significantly reduced the risk of progression independently from the previous treatment. At present, OS is similar between MPR and MEL200, with a trend for an improved OS in pts receiving lenalidomide as maintenance therapy.

Table 1.

	First randomization			Second randomization		
	MPR	MEL200	HR (95%CI;p value)	MAINT	No MAINT	HR (95%CI;p value)
From diagnosis						
Median PFS (mos)	25	39	1.66 (1.27-2.18 0002)	37.5	25.7	0.63 (0.48-0.83; 0008)
4-ys OS	71	72	1.08 (0.72-1.63;0.71)	76	68	0.68 (0.45-1.04; 08)
From start of maintenance						
Median PFS (mos)	18	41	2.01(1.45-2.79;<0001)	41	18	0.50(0.36-0.69;<0001)
3-ys OS	77	76	0.98(0.61-1.58; 94)	81	72	0.60 (0.37-0.97; 04)

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BORTEZOMIB-THALIDOMIDE-DEXAMETHASONE VERSUS THALIDOMIDE-DEXAMETHASONE PLUS AUTOTRANSPLANTATION FOR MULTIPLE MYELOMA: UPDATED FOLLOW-UP AND OUTCOMES AFTER RELAPSE IN THE PHASE 3 GIMEMA-MMY-3006 TRIAL

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Background: The phase 3 GIMEMA trial of thalidomide-dexamethasone (TD) versus bortezomib-thalidomide-dexamethasone (VTD) as induction therapy before, and consolidation therapy after double autologous stem-cell transplantation for newly diagnosed multiple myeloma (MM), demonstrated that VTD significantly improves rate of CR/nCR and extends PFS.

Aims: We report an updated follow-up of the GIMEMA trial with a detailed analysis of outcomes after relapse or progression (R/P), an important issue for patients (pts) treated up-front with a triplet or doublet novel agent-based therapy incorporated into ASCT.

Methods: Overall, 474 patients (236 randomized to VTD and 238 to TD) were enrolled in the trial. With a median follow-up of 52 months (mos) 226 pts (48%) relapsed or progressed. 182/226 pts resulted analyzable for outcomes after R/P.

Results: Median PFS for pts randomized to the VTD arm was 56 mos vs 42 mos for those assigned to TD (HR=0.64, P=0.0006). Similarly, median TTP for pts in the VTD group was longer than in the TD group (57 vs 45 mos, HR=0.63, P=0.0006), reflecting a 37% reduction in the risk of R/P with VTD. The probabilities of R/P in the VTD and TD arms were 41% vs 55% (P=0.002). After relapse, in comparison with the VTD-treated group a higher percentage of pts in the TD arm required the immediate start of salvage therapy for symptomatic R/P (67% vs 82.5%, P=0.016). Median time to salvage therapy (TTST) was significantly longer for pts who experienced R/P in the VTD arm than in the TD-treated group (35 vs 29 mos, P=0.018) and the associated salvage therapy-free interval (STFI) was 22.5 mos and 15 mos, respectively (P=0.009). Both bortezomib and lenalidomide were the most frequently used agents at the time of R/P (82% of all cases), while only 18% of pts were treated with conventional cytotoxic drugs. As expected, the majority of pts in the TD arm received a second-line therapy that included bortezomib (68%), while lenalidomide-dexamethasone was received by 12% of pts. By the opposite, in the VTD arm both bortezomib- and lenalidomide-based salvage therapies were equally distributed (40% vs 40%). The probability to achieve at least a partial response after second-line therapy including bortezomib was 60% for pts with prior exposure to VTD vs 63% for those randomized to TD. No difference in post-R/P OS was demonstrated between VTD-treated and TD-treated subgroups of pts who received bortezomib-based salvage therapy after R/P (2-yr estimates: 48% vs 53%, P=0.59). In the overall population, a trend toward longer post-R/P OS was observed for pts who received salvage therapy with novel agents, including bortezomib or lenalidomide (2-yr estimates: 52% and 53%, respectively), in comparison with pts treated with conventional cytotoxic drugs (2-yr estimate: 41%)(P=0.088).

Summary / Conclusion: With an extended follow up of 52 months, VTD incorporated into front-line ASCT was superior in comparison with TD plus ASCT in terms of TTP, PFS, TTST e STFI.VTD-treated pts had a higher probability than TD-treated pts to experience long-lasting asymptomatic R/P not requiring second-line therapy. VTD-treated and TD-treated pts who subsequently received bortezomib-based salvage therapies had similar rates of response and post-R/P OS; this finding suggests that short-term exposure to VTD did not favor the selection of bortezomib-resistant clones at the time of relapse.

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CMP-CARFILZOMIB (CFZ) PLUS MELPHALAN-PREDNISONE (MP)-IN ELDERLY PATIENTS (PTS) WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM): RESULTS OF A PHASE (PH) I/II TRIAL

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Background: MP+thalidomide (MPT) and MP+bortezomib (MPV) have shown significant progression-free survival and overall survival (OS) benefits in NDMM pts > 65 years (y) but are associated with peripheral neuropathy (PN). CFZ, a novel proteasome inhibitor, has shown promising activity and a favorable toxicity profile with low PN rates.
Aims: This PhI/II study in NDMM >65y was designed to determine maximum tolerated dose (MTD) of CMP and assess safety and efficacy.
Methods: In PhI, CFZ was started at 20 mg/m^2, then escalated to 27, 36, and 45 mg/m^2, given IV in 42-day (D) cycles (C) on D1/2/8/9/22/23/29/30 for 9C. Melphalan 9 mg/m² and prednisone 60mg/m² were given PO D1–4 of every 45-day cycle. MTD was based on dose-limiting toxicity (DLT) in C1 defined as any grade (G) 4 hematologic adverse event (AE), any hematologic AE preventing aministration of ≥ 2 C1 CFZ doses except G4 thrombocytopenia without bleeding or G4 neutropenia ≤7D, ≥G3 febrile neutropenia, or any ≥G3 nonhematologic AE.
Results: As of February 26, 2013, 24 pts have been enrolled in PhI: 6 for each dose level. There were 2 DLTs at 45mg/m^2 (fever, hypotension) resulting in a MTD of 36 mg/m². In PhII, 45 additional pts received CMP at 36 mg/m² CFZ for N=69 total PhI/II pts (median age 74y). ORR was 89% with 51% ≥VGPR. With median follow-up of 12 mo, the projected 2y OS was 89.9%. CMP was well tolerated without PN ≥G2. These results compare favorably to those of MPV, MPT, MP+lenalidomide (R), and R+dex in similar pts (ORR 71% San Miguel NEnglJMed2008, 76% Facon Lancet2007, 80% Palumbo JClinOncol2007 and 85% Rajkumar LancetOncol2010, respectively).
Summary / Conclusion: CFZ 36 mg/m^2 +MP is tolerable and effective in elderly NDMM pts. Treatment is ongoing. Final safety and efficacy data will be presented during the meeting.

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RANDOMIZED, OPEN-LABEL, PHASE 2 STUDY OF SILTUXIMAB (AN ANTI-IL-6 MAB) AND BORTEZOMIB-MELPHALAN-PREDNISONE VERSUS BORTEZOMIB-MELPHALAN-PREDNISONE IN PATIENTS WITH PREVIOUSLY UNTREATED MULTIPLE MYELOMA

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Background: For patients newly diagnosed with symptomatic multiple myeloma (MM) and ineligible for autologous stem cell transplantation (ASCT), bortezomib (VELCADE)-melphalan-prednisone (VMP) is a standard treatment regimen. Interleukin (IL)-6 enhances proliferation, differentiation, and survival of malignant plasma cells in MM. Therefore, anti-IL-6-directed treatment could further enhance VMP activity. Siltuximab (S; formerly CNTO 328) is a chimeric, anti-IL-6 monoclonal antibody that has been shown in preclinical experiments to enhance the anti-myeloma activity of VELCADE, melphalan, and corticosteroids.
Aims: To evaluate if the combination of S+VMP demonstrates improved efficacy compared with VMP alone, as assessed by complete response (CR) rate using the European Group for Blood and Marrow Transplantation (EBMT) criteria.
Methods: Newly diagnosed MM patients with measurable secretory disease who were not candidates for high-dose chemotherapy with ASCT received S+VMP for up to 9 cycles in the Part 1 safety run-in of a two-part, phase2, multicenter study. The safety profile of S+VMP was deemed acceptable, and the study proceeded to Part2, in which patients were randomly assigned to receive S+VMP or VMP alone for up to 9 cycles during the treatment period. V 1.3 mg/m^2 was given IV twice weekly at weeks1,2,4, 5 for four 6-week cycles, then weekly for five 6-week cycles with M 9 mg/m^2 once daily and P 60 mg/m^2 once daily on days 1−4 of every cycle. S was given as a 1-hour IV infusion at 11 mg/kg every 3 weeks with VMP. Patients with at least partial response (PR) on the S arm could enter an 18-month maintenance period with S alone. The primary endpoint was safety for Part 1 and CR rate according to EBMT criteria for Part 2.
Results: From June 2009 to May 2011, 12 patients were recruited in Part1, and 106 patients were randomized in Part 2: 52 to S+VMP and 54 to VMP. Data presented here are from the randomized Part 2. Baseline demographics and disease characteristics were well balanced between S+VMP and VMP, except for IgA subtype (40% in S+VMP and 19% in VMP). High-risk cytogenetic abnormalities (ie, t(4;14), t(14;16), and 17p deletion) were present in 17% in S+VMP and 10% in VMP, including 17p deletion in 15% in S+VMP and 4% in VMP. Median treatment duration during Cycles 1−9 was 12.5 months for S+VMP and 12.9 months for VMP alone. Twenty-one patients received maintenance S with a median treatment duration of 6.2 months. The median follow-up was 21.7 months at the current analysis. With S+VMP and VMP, CR was 27% vs. 22%, near CR was 31% vs. 14%, and overall response rate (CR+PR) was 88% vs. 80%, respectively (table). At least very good PR (VGPR) by International Myeloma Working Group (IMWG) assessment was observed in 71% with S+VMP and in 51% with VMP (P=0.0382). Median progression-free survival (PFS) was 17 months with S+VMP and 17 months with VMP. Overall survival (OS) rate with S+VMP and VMP was 88% and 88% at 1 year. During Cycles 1−9, Grade ≥3 adverse events were reported in 92% of patients with S+VMP and in 81% of patients with VMP alone. Common Grade ≥3 adverse events in the S+VMP and VMP arms were neutropenia (62% vs. 43%), thrombocytopenia (44% vs. 25%), and bronchopneumonia/pneumonia (17% vs. 17%). Serious adverse events were reported with S+VMP and VMP in 58% vs. 51% of patients, respectively. The most frequently reported serious adverse events were infections (23% with S+VMP and 17% with VMP). Five patients on S+VMP and 4 patients on VMP died due to an adverse event; 1 death on VMP was considered drug-related.
Summary / Conclusion: The addition of siltuximab to VELCADE-melphalan-prednisone resulted in a numerical increase in CR rates (from 22% to 27%) and at least VGPR rates (from 51% to 71%) and added moderate additional toxicity. No improvements in longer-term outcomes have been observed.

Table 1.

	VMP	S+VMP
Evaluable patients in Part 2	49	49
Overall response (complete response [CR] or partial response [PR]) per EBMT	80%	88%
95% CI	[65.7, 89.8]	[75.2, 95.4]
CR	22%	27%
95% CI	[11.8, 36.6]	[15.0, 41.1]
PR	57%	61%
At least very good PR	51%	71%
Median progression-free survival	17 mos	17 mos
1-Year overall survival rate	88%	88%

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MM-005: A PHASE 1 TRIAL OF POMALIDOMIDE, BORTEZOMIB, AND LOW-DOSE DEXAMETHASONE (PVD) IN RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background: Combinations of lenalidomide (LEN), bortezomib (BORT), and dexamethasone (DEX) have demonstrated preclinical and clinical activity in

patients with multiple myeloma (Kumar S, *et al*. *Blood.* 2012; Richardson PG, *et al*. *Blood*. 2010). POM was recently approved by the US Food and Drug Administration for the treatment of RRMM patients who have received at least 2 prior therapies, including LEN and BORT. POM with low-dose DEX (LoDEX) has demonstrated efficacy in RRMM patients treated with prior LEN and BORT (Leleu X, *et al*. *Blood*. 2013; Lacy MQ, *et al*. *Blood.* 2011). MM-005 was designed to identify the optimal dose of PVD for a phase 3 trial comparing PVD vs. BORT + LoDEX (VD) in patients with RRMM (MM-007).

Aims: Identify the maximum tolerated dose (MTD) of POM in combination with VD.

Methods: Eligible patients had RRMM with 1-4 prior lines of therapy including 2 or more consecutive cycles of LEN and a proteasome inhibitor. Patients must have been refractory to LEN (progressive disease [PD] during or within 60 days of LEN treatment), but not refractory to BORT (at 1.3 mg/m^2 twice weekly). The MTD was determined using a 3 + 3 design in 5 cohorts. Each cohort received 21-day cycles of POM 1-4 mg/day on D1-14; BORT 1-1.3 mg/m^2 on D1,4, 8, and 11; and LoDEX 20 mg/day on D1-2, 4-5, 8-9, and 11-12. All patients received thromboprophylaxis with aspirin or low-molecular-weight heparin. An expansion cohort was enrolled at the MTD. Treatment was continued until PD or unacceptable toxicity. Dose-limiting toxicities (DLTs) were assessed during cycle 1. The primary endpoint was MTD; secondary endpoints included safety, overall response rate (ORR; ≥ partial response), duration of response, and time to response (TTR).

Results: As of December 31, 2012, twenty-one patients were enrolled (3 patients per escalating dose cohort; 6 in the expansion cohort). The median age was 57 years (range, 36-75 years). All patients were LEN refractory and had received prior BORT. Patients had received a median of 2 prior lines of therapy (range 1-4 prior lines). No DLTs have been observed at any dose level. Confirmation of the tolerability of the highest dose level (POM 4 mg, BORT 1.3 mg/m^2, LoDEX 20 mg) is ongoing. The most common grade 3-4 adverse events (AEs) were neutropenia (37%) and thrombocytopenia (21%). With thromboprophylaxis, no deep vein thrombosis was observed and no significant PN (≥ grade 3) has been seen to date. Importantly, none of the patients discontinued treatment due to AEs and 17 patients remain on study. Thus far, the ORR was 73% (n=15 evaluable), including 27% very good partial response. Responses are ongoing and were also observed in patients with adverse cytogenetics. Furthermore, PVD rapidly induced responses with a median TTR of 2 cycles (6 weeks). Updated data will be presented at the meeting.

Summary / Conclusion: PVD was generally well tolerated in RRMM with no DLTs and no discontinuations due to AEs to date. This combination had promising activity in this LEN-refractory population with an ORR of 73%. The maximum planned dose of POM 4 mg/day on D1-14; BORT 1.3 mg/m^2 on D1,4, 8, and 11; and LoDEX 20 mg on D1-2, 4-5, 8-9, and 11-12 of a 21-day cycle has been incorporated into the ongoing MM-007 randomized, prospective phase 3 trial comparing PVD with VD in RRMM patients (n=782).

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TANDEM AUTOLOGOUS/ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATIONS IN FIRST LINE HIGH RISK MULTIPLE MYELOMA PATIENTS: EVOLVING STRATEGIES WITH THE IMMUNOMODULATING DRUGS

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only available potentially curative treatment for patients with hematological malignancies particularly those with high risk factors; its use in multiple myeloma as a first line treatment is still controversial especially not only with discordant results from different studies with different treatment and conditioning modalities but also with different patient/donor characteristics

Aims: We evaluated the efficacy and toxicity of tandem auto-HSCT strategy followed by RIC and allo-HSCT with the post-allo-HSCT use of bortezomib and DLI in high risk (β2 microglobulin level >3 mg/L, del13, t(4;14) or del17p) multiple myeloma patients (Group1). We compared our results to those observed after traditional tandem auto-RIC-allo-HSCT without bortezomib (Group2). Groups 1 and 2 were compared to matched patients not receiving allo-HSCT from the IFM studies.

Methods: Conditioning regimen combined fludarabine 30 mg/m²/d (d-5→d-1), busilvex IV 3.2 mg/kg/d (d-4,d-3) and ATG 2.5 mg/kg/d (d-2,d-1). In group1, by day 90 post-allo-HSCT, patients not in CR received 4 cycles of bortezomib 1.3 mg/kg; if the CR not achieved, increasing doses of DLI were administered. Allo-HSCT groups included 25 patients (12 in group1 and 13 in group2), 18 males and 7 females with a median age of 51 years [28-67]; 14 (56%) patients had del13, 7 (28%) del17 and 17 (68%) had β2 microglobulin level >3 mg/L. The stem cell source was PBSC in 22 (88%) of cases from 16 identical siblings and 9 HLA (10/10) matched unrelated donors. At allo-HSCT, one patient was in CR, 4 in VGPR and 20 in PR. The matched population included 36 controls for group1 and 39 for group2.

Results: At Day 90 after allo-HSCT, all patients engrafted, 10 patients were in CR and 15 patients were in less than CR. Nine patients in group1 received bortezomib, 3 reached a CR while the 6 others were still in PR and received increasing doses of DLI. There were 8 acute GVHD [7 grade II (3 in group1) and 1 grade III in group1] and 11 chronic GVHD [3 lim. (all in group1) and 8 ext. (1 in group 1)]. At the last follow-up, 14 patients are alive (9 in group1 and 5 in group2), 10 were in durable CR1 and 4 in PR after DLI; 11 patients died (3 in group1: all from progression; 8 in group2: 5 from progression and 3 from TRM). After a median follow-up of 55 months [3-142], the median OS was not reached in group1 vs. 65 months (51-NR) in its matched patients (P=0.027); and it was 96 months (49-NR) in group2 vs. 91 months (32-NR) in its matched patients (P=0.77). The median PFS was 49 months (29-NA) in group1 vs. 25 months (21-35) in its matched patients (P=0.004); it was 31 months (22-NR) in group2 vs. 28 months (21-40) in its matched patients (P=0.077).

Summary / Conclusion: The encouraging results in group1 are due to the use of IV busilvex and better ATG administration schedule in addition to the immunomodulating role of bortezomib in the elimination of the residual disease. The use of tandem auto-RIC-allo-HSCT including new agent combinations and immunomodulation after transplantation should be reconsidered in the context of first line treatment for MM especially for patients with poor prognostic factors.

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PHASE II CLINICAL AND CORRELATIVE STUDY OF CARFILZOMIB, LENALIDOMIDE, AND DEXAMETHASONE FOLLOWED BY LENALIDOMIDE EXTENDED DOSING (CRD-R) IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS

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Background: As modern MM treatments induce deeper responses, there is an increasing demand to characterize remissions with sensitive techniques. Flow cytometry and functional imaging techniques are emerging tools that measure minimal residual disease (MRD). Carfilzomib (CFZ) is an irreversible proteasome inhibitor with potent anti-MM effects that induces rapid and deep responses when combined with lenalidomide (LEN) and dexamethasone (DEX).

Aims: In this single arm phase II trial using CRd therapy, we report the results of the first n=28/45 patients. The primary endpoint is the incidence of ≥ grade 3 neuropathy. Secondary endpoints include response rate, profiling CFZ activity to biological endpoints, and impact of MRD studies on clinical outcomes.

Methods: Eligible patients included both transplant and non-transplant newly diagnosed MM patients. Each cycle is 28-days: CFZ IV 20/36 mg/m^2 on days1,2, 8, 9, 15, 16; LEN oral 25 mg days 1-21; and DEX IV/oral 20/10 mg on days1,2, 8, 9, 15, 16, 22, 23. Patients younger than 70-75 years underwent stem cell harvest after 4 cycles of CRd and continue therapy. After 8 cycles of CRd, all patients with SD or better clinical outcome received cycles 9-20 of LEN extended dosing 10 mg days 1-21. MRD assessments with multi-parametric flow cytometry and FDG-PET CT were performed on achievement of CR/end of cycle (during cycles 1-8).

Results: A total of 31 patients meeting eligibility criteria have been enrolled (15 female, 16 male; median age 60; range 40-88). Among enrolled patients, mean baseline M-protein was 3.0 g/dL (range 1.0-7.1) and isotypes included 7 IgA, 19 IgG, 4 kappa, and 1 lambda. 28 patients were evaluable for toxicity and response with a median of 8 cycles of therapy completed (2-17). As no patients reported ≥ grade 3 neuropathy, the primary endpoint of this study was met. The mean M-protein decline after completing one cycle of therapy was 67.2%. Highlighting the rapid rate of response, patients obtaining ≥ VGPR went from 42% after 2 cycles of therapy to 85% after 4 cycles of therapy. Best responses after median of 8 cycles of therapy included 11 – sCR/7-nCR (64.3%), 4 – VGPR (14.3%), 5 – PR (17.9%), and 1 – SD (3.6%). Median time to sCR was 6.9 cycles. One patient had progressive disease (PD) based on biochemical progression and not clinical symptoms. Toxicities were consistent with that previously reported. The most common G3/4 toxicities: non-hematologic - rash, electrolyte disturbance, and LFT elevation; hematologic - lymphopenia and anemia. Among 10 sCR and 4 nCR who underwent MRD assessment, all were negative. Among 1 VGPR, 1 PR and 1 PD patients, there was evidence of immunophenotypically abnormal plasma cells defined by multi-parametric flow cytometry after 8 cycles of CRd. All 11 patients with baseline FDG avid lesions or extramedullary disease showed decrease or resolution of FDG avidity at

MRD assessment time points.
Summary / Conclusion: Combination CRd therapy followed by extended LEN dosing in newly diagnosed MM patients show deep response rates corroborated by MRD assays and tolerable side effects.

Table 1.

	2 cycles n/N (%)	4 cycles n/N (%)	8 cycles n/N (%)
ORR (≥PR)	27/28 (96.4)	20/21 (95.2)	14/15 (93.3)
≥VGPR	12/28 (42.9)	18/21 (85.7)	13/15 (86.7)
sCR	2/28 (7.1)	5/21(23.8)	7/15 (46.7)
nCR	4/28 (14.3)	6/21 (28.6)	4/15(26.7)
VGPR	6/28 (21.4)	7/21(33.3)	2/15 (13.3)
PR	15/28 (53.6)	2/21 (9.5)	1/15 (6.7)
SD	1/28 (3.6)	1/21 (4.8)	1/15 (6.7)

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PROLONGED OVERALL SURVIVAL WITH POMALIDOMIDE AND DEXAMETHASONE IN END STAGE MYELOMA

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Background: MM remains incurable and patients will ultimately acquire resistance to novel agents. Kumar *et al*. have shown that the expected median PFS and OS for patients relapsed or were refractory to bortezomib and an IMiDs (double refractory) was 5 months and 9 months, respectively. Several studies have shown that the combination of pomalidomide plus dexamethasone was active and well tolerated in double refractory MM, with a response rate (PR and greater) of approximately 25% to 35%.
Aims: In this final analysis, we aimed to further demonstrate that treatment with pomalidomide and dexamethasone translated into prolonged survival.
Methods: The IFM2009-02 phase 2 (Leleu *et al*, ASH 2011) randomized study was designed to determine the impact of pomalidomide; given orally either 4 mg daily on days 1–21 of a 28-day cycle (arm 21/28) or continuously on days 1–28 of a 28-day cycle (arm 28/28); in combination with dexamethasone (oral 40 mg weekly). This study included MM patients who did not achieve a response (≤ SD) as per IMWG criteria with the last course of bortezomib and the last course of lenalidomide, or who were refractory to both bortezomib and lenalidomide. The primary objective was ORR, assessed centrally and reviewed by an independent committee. The analysis is performed on the ITT population and combines data from the 2 arms.
Results: Overall, 84 patients (57 male and 27 female)were enrolled, with a median age of 60 years (range 42-83). The median time from diagnosis to enrolment was 70.5 months (range 9-277). The median number of prior lines of therapy was 5 (range 1-13), and 100% of the patients had received prior bortezomib and lenalidomide as per protocol. Additionally, 84.5% were refractory to their last prior line of therapy and 77% were refractory to their last prior lines of both lenalidomide and bortezomib. With a median follow up of 22.8 months, the median (95%CI) PFS and TTP was 4.6 months (4-7) and 5.4 months (CI 4-8), with a median duration of response of 7.3 months (5-15). 28% of patients were free of progression and 44% responded, at 1 year respectively. The most common reason for treatment discontinuation was disease progressions (84%). 95% of deaths wereconsidered to be disease related. Importantly, 10 patients (12%) remain on treatment after 36 months. The median OS was 14.9 months (11-20) with 57% and 44% of patients surviving at 12 and 18 months respectively. We noticed that OS was significantly prolonged in responders when compared to patients with SD. The median OS has not been reached in responders, (18.4;-); and was 13 (8; 20) months in patients with SD, (HR [95% CI) 0.45 [0.2, 0.9], P=0.018). Noteworthy, 83% of responders and 50% of patients with SD were still alive after 12 months of treatment, and 69% and 36% were still alive at 18 months, respectively. Future studies may consider studying response rate and survival improvement on pomalidomide by adding a third agent to Pomalidomide plus dexamethasone regimen.
Summary / Conclusion: The IFM2009-02 study thus pointed out the survival difference in responders as compared to patients with stable disease related to a prolonged duration of response. Pomalidomide and dexamethasone is effective and generally well tolerated in heavily pre-treated MM patients. The combination of pomalidomide and dexamethasone compared very favourably to the expected median OS reported in the historical control study in end stage MM. This study provides further evidence that pomalidomide can provide benefit for patients who have relapsed after other novel therapies.

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A NEW SCORING SYSTEM TO IDENTIFY PATIENTS AT HIGH-RISK OF EARLY DEATH IN THE CONTEXT OF NOVEL AGENT-BASED INTENSIVE THERAPY

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Background: Several biological parameters or indexes including cytogenetics and/or International Staging System (ISS) have been identified to define patients with multiple myeloma with high-risk of progression, but none of them are defined in order to select a subgroup of patients at high risk of early death from progression, especially in the context of frontline therapy using novel-agents based induction and autologous stem cell transplantation.
Aims: We investigated prognostic parameters of patients enrolled in the IFM2005-01 trial comparing bortezomib-dexamethasone versus VAD induction followed by ASCT (Harousseau *et al*, J Clin Oncol 2010;28:4621-4629).
Methods: The risk of death within the first 2 years from the start of therapy from progressive disease (and not toxicity) (42 cases out of 482 patients) was related to 3 independent adverse initial characteristics in a multivariate logistic regression analysis: high LDH > normal value (P=0.0014), ISS 3 (P=0.0097) and cytogenetic abnormalities defined by the presence of either t(4;14) or 17p deletion (P=0.0002). These 3 variables enabled the identification of a simple 0 to 3 scoring system predicting for overall survival (OS). Score 0 was defined by the absence of adverse factor (neither high LDH, nor ISS3, nor t(4;14) and /or 17p); in this group of patients representing 57% of the overall population, the 4-year OS rate was 84%. Score 1 was defined by the presence of only 1 adverse factor (either high LDH or ISS3 or t(4;14) and/or 17p); in this group of patients representing 32% of the overall population, the 4-year OS rate was 73%. Score 2 was defined by the presence of high LDH plus ISS3, without t(4;14) and /or 17p; in this group of patients representing 6% of the overall population, the 4-year OS rate was 68%; Score 3 was defined by the presence of t(4;14) and/or 17p in addition with either ISS3 or high LDH; in this group of patients representing 5% of the overall population, the median OS was only 19 months.
Results: This score was subsequently applied to a larger population of patients enrolled in 4 phase 3 studies independently conducted by HOVON/GMMG (Sonneveld *et al*, JClinOncol 2012), IFM (Harousseau *et al*, J Clin Oncol 2010), PETHEMA (Rosinol *et al*, Blood 2012) and GIMEMA (Cavo *et al*, Lancet 2010) cooperative groups comparing bortezomib-based versus no bortezomib-based inductions prior to single or tandem ASCT. Out of 2169 patients enrolled in these 4 trials, 1601 had data available to use the new scoring system. 903 (56%) had a score of 0, 515 (32%) a score of$_1$, 68 (4%) a score of$_2$, and 115 (7%) a score of$_3$, respectively. 2-year OS was significantly different according to the score: 93% for a score of 0, 85% for a score of$_1$, 67% for a score of$_2$, and 55% for a score of 3 (P<0.0001). This score was also very effective in defining cases with dramatic outcome in the population of 850 patients treated in these 4 trials into the bortezomib-based induction regimens (2-year OS of 52% in patients with score 3).
Summary / Conclusion: We have defined a new and simple scoring system that allows the identification of a small group of patients at very high-risk disease and shortened survival, despite the use of the most recent intensive novel-agent based therapy. The subgroup of patients with a score of 3 associated with a dramatic outcome might be candidate for innovative therapeutic approaches.

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BENDAMUSTINE, BORTEZOMIB AND DEXAMETHASONE (BVD) IN ELDERLY MM PROGRESSIVE AFTER 1ST LINE THERAPY (IFM 2009-01 TRIAL): PREDICTIVE FACTORS OF DEFAVOURABLE OUTCOME

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Background: Prognosis of relapse is severe in elderly multiple myeloma (MM). In recent studies, median survival at progression after 1st line therapy was between 9 and 13 months (T. Facon, Lancet 2007; C. Hulin, J Clin Oncol 2009). Bortezomib (V) plus dexamethasone (D) is a major regimen in the treatment of relapses. Bendamustine (B) demonstrated to be highly active in advanced MM. The IFM 2009-01 trial evaluated the combination of B, V and D in elderly patients with progressive MM on or after 1stline treatment.
Aims: The present analysis aimed to determine predictors of defavourable outcome at 6 months.
Methods: IFM 2009-01 trial was dedicated to pts > 65 years in 1st relapse or refractory to 1st line therapy. Inclusion criteria were measurable disease, PS ECOG 0-2, ANC > 1.5x10^9/L, platelets > 100x10^9/L, serum creatinine level <250 mcmol/l, AST and ALT < 3xULN. Pts with prior exposure to bortezomib were excluded. Treatment regimen was 6 28 days cycles of B 70 mg/m^2 D1-8, V 1.3 mg/m2 D1-8-15-22 and D 20 mg D1-8-15-22. Responders were assigned to receive maintenance treatment with 6 additional cycles given 1 month out of 2. Pts with favourable outcome at 6 months were defined as achieving sustained PR or better and beginning maintenance treatment without major toxicity or unrelated event.
Results: From 03/2010 to 07/2011, 73 pts were included. Median age was 75.8 years (range 66-86). Median time from diagnosis to inclusion was 29 months. All pts received only 1 prior line of therapy: melphalan-prednisone (MP) in 12, MP-Thalidomide in 44, Lenalidomide-Dexamethasone (LD) in 14, other IMiD-based regimen in 3. 49 pts (67.1%) achieved at least partial response [best response CR: 9 pts (12.3%), VGPR: 12 pts (16.5%), PR: 28 pts (38.3%), MR: 6 pts (8.2%), SD: 4 pts (5.5%), progression: 13 pts (17.8%), early discontinuation: 1 pt (1.3%)]. At 6 months, PFS was 67.1% and OS 80.8%. Defavourable outcome was observed in 36 pts (49.3%): failure to achieve sustained PR in 24, treatment toxicity in6, unrelated adverse event in4, patient refusal or lost to follow-up 1 each. Predictive factors of a defavourable outcome were beta 2 microglobulin (B2M) level > 3.5 mg/L (P=0.0029), 17p deletion (P=0.025) and male sex (P=0.04).
Summary / Conclusion: In the IFM 2009-01 trial, elevated serum B2M level, presence of deletion 17p and male sex correlated with defavourable outcome

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CONSOLIDATION WITH VTD SIGNIFICANTLY IMPROVES THE COMPLETE REMISSION RATE IN MULTIPLE MYELOMA

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Background: Several studies have demonstrated the impact of VTd on response rates and PFS either as induction or consolidation regimen upfront in Myeloma (MM). However there are limitations to these studies, especially that no data is available regarding the role of VTd consolidation in the context of bortezomib-triple based VTd induction regimen followed by a single autologous transplantation (auto). The CR rate, relapse rate and median PFS were 29%, 53% and 26 months in the VTd arm of the IFM2007-02 trial with no consolidation. Cavo *et al*. reported 61% CR rate, 39% 3-year progression and 62% estimated 5-year PFS with VTd consolidation after double auto in the GIMEMA study (Cavo *et al*, Lancet 2010).
Aims: We aimed to assess the efficacy and safety of VTd as consolidation therapy in the context of VTd as induction regimen followed by a single auto (VTd-auto-VTd regimen).
Methods: This retrospective multicentre study has included newly diagnosed MM patients eligible for ASCT upfront and aged less than 65 in 2 cohorts across 9 IFM centers. The patients must have completed the procedure. The first group (VTd-auto-VTd) included 121 MM treated with VTd-auto-VTd regimen. The regimen consisted of 4 induction cycles and 2 consolidation cycles of VTd [bortezomib I.V. 1.3 mg/m² on days1,4, 8, and 11, thalidomide 100 mg/day administered orally, and dexamethasone 40mg weekly administered orally]. All patients underwent ASCT with high dose melphalan 200mg/m² as conditioning regimen. The second cohort (VTd-auto) included 96 MM that completed the same VTd-auto regimen but without consolidation. None of the patients had received a maintenance therapy across cohorts.
Results: ORR was identical at completion of therapy across cohorts, nevertheless the CR rate was greater in the cohort VTd-auto-VTd, 52% vs. 30% (P=0.001), while identical across cohorts after induction and autotransplantation. With 30 months median follow-up, the relapse rate was lower in VTd-auto-VTd, 21% vs. 45% (P=0.001) and the median TTP not reached and 25 months in either cohort, respectively (P=0.005). The expected 4-years TTP was 62% and 29% in either cohort respectively. The safety profile of the cohort VTd-auto-VTd was superimposable to that of VTd-auto without consolidation.
Summary / Conclusion: This study further demonstrated the importance of the VTd consolidation to improve clinical outcomes with an acceptable toxicity profile, in the context of VTd as induction and a single ASCT upfront in MM. This study showed an impressive CR rate in relation to the consolidation that translated into a lower relapse rate and a prolonged TTP. This study demonstrated that the VTd regimen, used both as induction and consolidation, in the context of a single ASCT upfront significantly contributed to improve clinical outcomes with an acceptable toxicity profile in MM. VTd-auto-VTd compared very favorably to the other upfront protocols, and may become in the near future a standard of care in newly diagnosed patients with Myeloma.

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CLINICAL PROFILE OF ONCE-DAILY, MODIFIED-RELEASE OPROZOMIB TABLETS IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES: RESULTS OF A PHASE 1B/2 TRIAL

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Background: Oprozomib, a structural analog of carfilzomib, is an orally bioavailable proteasome inhibitor that binds selectively and irreversibly to its target. We have previously shown that oprozomib split-dose powder-in-capsule demonstrated promising clinical activity in patients with hematologic malignancies (Savona MR, *et al*. ASH 2012. Abstract 203). In an effort to improve gastrointestinal tolerability, a once-daily modified-release tablet was introduced into the ongoing phase 1b/2 dose escalation study, the preliminary findings of which were presented at the 14th International Myeloma Workshop (Seigel DS, *et al*. IMW 2013. P-225). More mature data with oprozomib once-daily, modified-release tablets are now available, the results of which are presented herein.
Aims: To determine the maximum tolerated dose (MTD) and the safety and tolerability profile of once-daily, modified-release oprozomib tablets. Secondary endpoints include response according to relevant criteria (eg, International Myeloma Working Group Uniform Response, Third International Workshop on Waldenström's Macroglobulinemia) and pharmacodynamics.
Methods: This is an ongoing, multicenter, phase 1b/2 dose-escalation study (NCT01416428) taking place in 7 centers in the United States in patients with hematologic malignancies. Dose escalation is underway. Two dosing schedules are being investigated: oprozomib once-daily, modified-release tablets have been administered to patients on days1,2, 8, and 9 of a 14-day cycle (QD×2) or on days 1–5 of a 14-day cycle (QD×5). Dose escalation began at 150 mg/d for each schedule and is being increased in 30-mg increments using a 3+3 study design. There is no maximum planned dose for this study.
Results: As of February 2013, 21 patients have enrolled in the study following introduction of oprozomib once-daily modified-release tablets. To date, the patients (16 with multiple myeloma [MM]; 5 with Waldenström's macroglobulinemia) have been enrolled in 6 cohorts (Table 1). No dose-limiting toxicities

(DLT) have been observed for patients who received tablets QD×2. One DLT (acute renal failure) was reported at 180 mg/d from the QD×5 dosing schedule (n=3 patients). This cohort was expanded. For both dosing regimens, no Grade 4 adverse events have been reported, while the most common adverse events have been gastrointestinal disorders that are typically Grade 1/2 in severity. The MTD has not been reached for the QD×2 or the QD×5 dosing regimen. Preliminary response data demonstrated promising activity; updated response data will be presented. Rapid and potent proteasome inhibition in whole blood was observed after administration of oprozomib tablets on Cycle1, Day 1. Proteasome inhibition was >75% at 4 hours postdose for both the 150- and 180-mg cohorts of the QD×2 regimen and for the 150-mg cohort of the QD×5 regimen. This inhibition was sustained and surpassed 95% by Cycle 2.

Summary / Conclusion: Although these data are preliminary, oprozomib once-daily, modified-release tablets have an acceptable safety and tolerability profile with a trend toward lower grades of gastrointestinal adverse events and use of fewer antiemetic medications compared to the previously investigated split-dose powder-in-capsule. Early pharmacodynamic data demonstrate significant proteasome inhibition at lower doses. Dose escalation will continue until the MTD is reached, followed by cohort expansion in patients with MM and Waldenström's macroglobulinemia. Updated safety, tolerability, and efficacy data will be presented at the meeting.

Table 1.

Dosing Schedule	150 mg/d	180 mg/d	210 mg/d	240 mg/d
QD×2 (10)	3	3	3	1
QD×5 (11)	4	7	0	0
Total (21)	7	10	3	1

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AN ASSAY FOR SIMULTANEOUS DIAGNOSIS OF T(4;14), T(11;14), T(14;16)/T(14;20), DEL1P, ADD1Q, DEL13Q, DEL17P, MS/MF EXPRESSION CLUSTERS, AND THE SKY-92 HIGH RISK SIGNATURE IN MULTIPLE MYELOMA PATIENTS

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Background: Multiple Myeloma (MM) is a heterogeneous disease, with several recurring chromosomal aberrations including t(4;14), t(11;14), t(14;16)/t(14;20), del1p, add1q, del13q, and del17p. Gene expression profiling studies have revealed clusters of patients with distinct expression patterns including a signature that identifies high-risk patients (SKY-92) [1]. However, methodologies for assessing these markers have not been standardized yet. Lack of standardization hampers marker interpretation across cohorts, and limits the emerging strategies that combine these markers towards patient stratification and personalized medicine.

Aims: To develop a standardized assay for the detection of SKY-92, t(4;14), t(11;14), t(14;16)/t(14;20), del1p, add1q, del13q, and del17p.

Methods: The MMprofiler assay uses standardized plasma cell purification, RNA and DNA extraction and proprietary sample labeling process for use with Affymetrix HG-U133 Plus2 and a Cytoscan HD chip and proprietary data preprocessing and analysis software. The assay includes ten markers, namely translocations t(4;14), and t(11;14), chromosomal copy number aberrations, del1p, add1q, del13q, and del17p, the MS, and MF gene expression clusters, and the SKY-92 high risk signature. A total of 329 patients from the HOVON-65/GMMG-HD4 trial were used to train nearest mean classifiers for the translocation markers by means of a double loop cross validation protocol.

Results: The SKY-92 signature identifies high risk MM patients, and was previously shown to be a strong independent prognostic risk factor across multiple datasets, that outperforms signatures developed by others for the same goal [1]. The translocations t(4;14), and t(11;14) are associated with strong gene expression profiles, also reflected in their correlation with the clusters. Classifiers with high sensitivity and specificity were developed, as shown in Table 1. Classifiers for the prognostic MS and MF gene expression clusters were also developed. However, as there is no alternative method to compare against (e.g. no FISH etc.) their performance must be assessed by evaluation of their prognostic value in independent cohorts. Gene expression based classifiers performed reasonablefor the chromosomal aberrations add1q, and del13q, and performed poorly for the del17p. Since clinical implementation of these gene expression profiles is suboptimal we decided to detect clinically relevant variants of del1p, add1q, del13q, and del17p using the Cytoscan HD platform, at 99% sensitivity and specificity. An overview of all performances is provided in Table 1.

Summary / Conclusion: We report the development of a research use only assay for evaluation of ten different markers relevant for MM, which can ultimately be applied by qualified laboratories. The assay will be employed for further evaluation along the EMN-02/HOVON-95 clinical trial.

Reference

1. Kuiper R, *et al*. A gene expression signature for high-risk multiple myeloma. Leukemia, 2012, 26:2406–13.

Table 1. Assay performances for the ten markers in percentages.

Marker	Chip	Sensitivity (%)	Specificity (%)
t(4;14)	Expression	86.4	98.0
t(11;14)	Expression	89.7	95.3
t(14;16)/t(14;20)	Expression	82.9	89.2
del1p	Copy number	99.0	99.0
add1q	Copy number	99.0	99.0
del13q	Copy number	99.0	99.0
del17p	Copy number	99.0	99.0
Marker	Chip	HR	p-value
MS cluster	Expression	NA*	NA*
MF cluster	Expression	NA*	NA*
SKY-92	Expression	2.38 to 5.23†	p<0.0001†

* No alternative method available to compare against
† in four independent datasets [1]

P235

A COMPARISON BETWEEN NEXT-GENERATION SEQUENCING AND ASO-QPCR FOR MINIMAL RESIDUAL DISEASE DETECTION IN MULTIPLE MYELOMA

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Background: Although molecular complete remission (mCR) in multiple myeloma (MM) can be assessed by allele-specific oligonucleotide (ASO)-PCR, this technique requires preparation of clonotype-specific primers for each individual which is laborious and time-consuming. We utilized a sequencing method, termed LymphoSIGHT™, which employs consensus primers and high-throughput sequencing to amplify and sequence all rearranged immunoglobulin gene segments present in a myeloma clone. The sequencing method is quantitative at frequencies above 10^{-5} and the lower limit of detection is below 10^{-6}. Usage of the sequencing method for minimal residual disease (MRD) detection in MM may provide increased sensitivity and specificity, while overcoming the challenges associated with ASO-PCR.

Aims: We compared the LymphoSIGHT method with ASO-qPCR for MRD detection in autografts in the autologous peripheral blood stem cell transplantation (ASCT) setting.

Methods: Seventeen Japanese patients with newly diagnosed MM who received various induction regimens prior to ASCT were retrospectively analyzed. All patients had achieved a partial response (PR) or complete response (CR) after ASCT. Bone marrow (BM) slides from 13 MM patients and fresh BM cells from 4 MM patients at diagnosis as well as autografts were obtained for DNA extraction. IGH-based ASO-PCR was performed as described previously (van der Velden *et al*. Methods Mol Biol 2009). In addition, we used the LymphoSIGHT platform, which employs universal primer sets to amplify *IGH* variable (V), diversity (D), and joining (J) gene segments, *IGH-DJ*, and *IGK* from genomic DNA (Faham *et al*. Blood 2012). Amplified products were subjected to deep sequencing using next-generation sequencing (NGS). Reads were analyzed using standardized algorithms for clonotype determination. Myeloma-specific clonotypes were identified for each patient based on their high frequency in BM samples. The presence of the myeloma clonotype was then assessed in follow-up samples.

Results: MRD in autografts was detected in 6 of 17 (35%) by ASO-qPCR and

13 of 17 (76%) by NGS (Figure 1A). When MRD was assessed by NGS, 6 MRD (+) cases received post-ASCT therapy while 4 MRD (-) cases and 7 MRD (+) cases were followed without post-ASCT therapy. The MRD (-) cases tended to show a better PFS than the MRD (+) cases with post-ASCT therapy (P=0.26) and those without post-ASCT therapy (P=0.09) (Figure 1B) although overall survival rates were comparable among the three groups. There was no difference in PFS between MRD (-) and MRD (+) cases when MRD was assessed by ASO-qPCR (P=0.6). These studies will be extended in 30 additional MM patients, and results will be presented.

Summary / Conclusion: MRD-negativity in autografts revealed by NGS may be more closely associated with durable remission of MM than that revealed by ASO-qPCR.

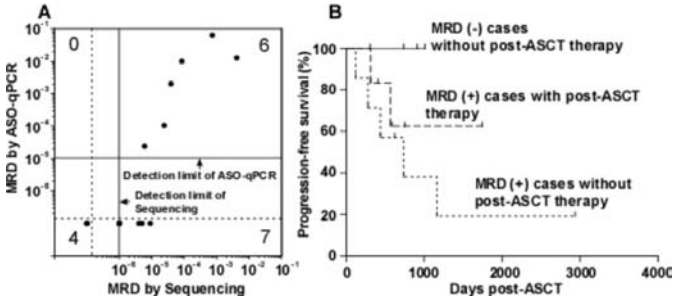

Figure 1. Minimal residual disease detection in autografts by next-generation sequincing.

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WEEKLY ORAL INVESTIGATIONAL PROTEASOME INHIBITOR MLN9708 PLUS LENALIDOMIDE-DEXAMETHASONE IN ELDERLY PATIENTS (PTS) WITH PREVIOUSLY UNTREATED MULTIPLE MYELOMA (MM): SUBSET ANALYSIS OF A PHASE 1/2 STUDY

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Background: Novel and active combination regimens that are well tolerated as long-term therapy are required for elderly MM pts who are ineligible for ASCT. The investigational agent MLN9708 (ixazomib citrate) is the first oral proteasome inhibitor in the boronate peptide class with reported clinical activity in MM. This phase 1/2 study investigated oral MLN9708 plus lenalidomide-Dex in both transplant-eligible and -ineligible pts with previously untreated MM (NCT01217957).

Aims: Phase 1 objectives included evaluation of safety, tolerability, the MTD, and recommended phase 2 dose (RP2D). The phase 2 primary objective was CR+VGPR rate. Secondary objectives included ORR and PFS. The aims of this subset analysis were to evaluate efficacy and safety in elderly vs younger pts. This analysis includes data up to Jan 23, 2013 (median follow-up 8.5 mos).

Methods: Pts received oral MLN9708 on days1, 8, 15, lenalidomide 25 mg on days 1-21, and Dex 40 mg on days1, 8, 15, 22, in 28-day cycles. After 12 cycles, pts received MLN9708, on the same weekly schedule, as maintenance therapy until progression or unacceptable toxicity. Transplant-eligible pts could undergo stem cell collection after 3 and ASCT after 6 cycles. In phase1, MLN9708 dose escalation proceeded from 1.68 to 3.95 mg/m^2 based on cycle 1 DLTs. The MTD was 2.97 mg/m^2; RP2D was 2.23 mg/m^2 (converted to a fixed dose of 4.0 mg based on population PK findings).

Results: 65 pts (15 phase1, 50 phase 2) were enrolled, including 31 aged <65 yrs and 34 aged ≥65 yrs, of whom 12 were aged ≥75 yrs; 25, 28, and 10 pts, respectively, were treated at the RP2D. Disease characteristics were similar among the age groups, including the proportion of pts with ISS stage II/III MM (55%, 56%, and 67% in pts aged <65, ≥65, and ≥75 yrs, respectively). Data on treatment exposure, disposition, and common AEs at the RP2D are shown in the table. Constipation occurred less frequently, while diarrhea occurred more frequently in older pts (≥65, ≥75 yrs) compared to the <65 yrs group. The incidences of grade ≥3 AEs and serious AEs were similar across the age groups. Peripheral neuropathy (PN), which was primarily grade1, was seen in 24%, 29%, and 40% of pts at the RP2D in the <65, ≥65, and ≥75 yrs groups, respectively; grade 3 PN was reported in only 2 pts, both ≥65 yrs (1 ≥75 yrs). There were 2 on-study deaths, 1 due to cardio-respiratory arrest after abdominal surgery in a pt aged 86 yrs (considered unrelated by the investigator) and 1 due to drug-related RSV pneumonia in a pt aged 68 yrs. The acceptable safety profile of MLN9708-lenalidomide-Dex induction enabled non-transplant-eligible pts to proceed to the maintenance phase. MLN9708 maintenance was generally well tolerated, with no evidence of cumulative toxicities. Preliminary response data showed clinical activity across the age groups, with a CR+VGPR rate in pts aged <65, ≥65, and ≥75 yrs of 42%, 70%, and 73%, respectively (including 29%, 24%, 18% CR, and 13%, 45%, 55% VGPR), and an ORR of 94%, 91%, and 82%, respectively. A total of 3 pts had disease progression, including 0,3, and 2 pts aged <65, ≥65, and ≥75 yrs, respectively.

Summary / Conclusion: These data suggest the all-oral combination of MLN9708 plus lenalidomide-Dex is associated with reversible and manageable toxicities, with limited PN, regardless of age. Preliminary data also suggest encouraging antitumor activity across the age groups analyzed, with CR+VGPR rates of 42–73%, including CR rates of 18–29%. Small pt numbers limit the power of this analysis; however, data support further studies, including phase 3 investigations.

Table 1.

	<65 yrs (n=31)	≥65 yrs (n=34)	≥75 yrs (n=12)
Median no. of cycles (range)	7 (1–16)	9.5 (1–24)	5.5 (1–24)
Received ≥12 cycles, %	16	26	17
Remain on treatment, %	35	47	33
Proceeded to ASCT, n	14	7	2
At RP2D:			
Discontinued due to AE, n	2	2	1
Grade ≥3 AEs, all-cause, n	17	19	7
Serious AEs, all-cause, n	9	12	4
Dose reductions, any drug, n	11	15	4
Common (≥30% pts overall) all-grade, all-cause AEs at RP2D, %			
Fatigue	56	64	60
Diarrhea	44	54	60
Nausea	52	32	50
Constipation	44	32	20
Peripheral edema	36	36	20
Upper resp tract infection	32	32	10
Vomiting	32	21	40

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PROGNOSTIC VALUE OF DEEP SEQUENCING METHOD FOR MINIMAL RESIDUAL DISEASE DETECTION IN MULTIPLE MYELOMA

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Background: In multiple myeloma (MM), the prognostic significance of achieving complete remission (CR) using the European Group for Blood and Marrow Transplantation (EBMT) criteria has been widely validated (Ladetto *et al*, JCO 2010). Nevertheless, most MM patients will relapse due to persistence of residual tumor cells, or minimal residual disease (MRD) (Paiva *et al*, JCO 2011). As a result, and also due to the introduction of new, more effective treatments, there is a need to evaluate the clinical relevance of achieving deeper levels of cytoreduction, which may be associated with improved prognosis. We developed the LymphoSIGHT™ platform, a high-throughput sequencing method with a sensitivity of 10^{-6}, which universally amplifies immune receptor gene segments and can identify all myeloma-specific sequences at diagnosis, allowing monitoring of disease progression during therapy (Faham *et al*, Blood 2012).

Aims: We compared the prognostic value of traditional response criteria and MRD measurement by the sequencing-based method and multiparameter flow cytometry (MFC) in a cohort of 56 uniformly-treated MM patients from the Spanish Myeloma Group trials.

Methods: Bone marrow samples were obtained from 56 patients at diagnostic and post-treatment time points on GEM clinical trials (GEM00 and GEM05). All patients were in CR or VGPR at the post-treatment time point. The LymphoSIGHT platform was used to amplify IGH variable (V), diversity (D), and joining (J) gene segments, IGH-DJ, and IGK from genomic DNA. Amplified products were sequenced deeply, and reads were analyzed using standardized algorithms for clonotype determination. Myeloma-specific clonotypes were

identified for each patient based on their high frequency in BM samples. We assessed MRD in follow-up samples, analyzed concordance between sequencing and MFC MRD results, and compared the prognostic value of each method with traditional response criteria.
Results: We observed high correlation between MFC and sequencing MRD results (r^2=0.86). 45 patients were positive by sequencing at MRD levels of 10^{-5} or higher and 11 were MRD negative. There was significantly improved overall survival (OS) in the MRD negative group versus the MRD positive group (median not reached vs. 86 mos, P=0.026). Similar differences were found in progression free survival. When limiting the analysis to the 35 patients in conventional CR, 25 of 35 patients were positive by sequencing at MRD levels at 10^{-5} and higher and 10 were MRD negative. There was significantly improved OS in the MRD negative group versus the MRD positive group (median not reached vs. 80.92 mos, P=0.041).
Summary / Conclusion: Our data shows high correlation between MFC and sequencing MRD levels in MM patients. For patients in CR by traditional response criteria, the presence or absence of MRD by sequencing delineated 2 groups of patients with significantly different OS. MRD negativity by sequencing may be a better prognostic indicator than CR by traditional response criteria.

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PHASE 1B DOSE ESCALATION STUDY OF ORAL QUISINOSTAT, A HISTONE DEACETYLASE INHIBITOR, IN COMBINATION WITH VELCADE (BORTEZOMIB) AND DEXAMETHASONE FOR PATIENTS WITH RELAPSED MULTIPLE MYELOMA

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Background: Aggresome formation is a mechanism of resistance to agents (e.g., bortezomib) which block proteasome activity. Histone deacetylase inhibitor (e.g., quisinostat) prevents aggresome formation by deacetylation of tubulin that allows the transport of unfolded proteins to lysosomes for degradation.
Aims: We therefore investigated the safety and efficacy of the combination of the histone deacetylase inhibitor Quisinostat with the proteosome inhibitor Velcade in relapsed multiple myeloma.
Methods: Patients received quisinostat (Q) at escalated doses (6, 8, 10 and 12 mg) on days1,3, and 5 weekly, subcutaneous VELCADE (V) at 1.3 mg/m^2 on days1,4, 8, and 11 of a 3-week cycle, and oral dexamethasone (D) at 20 mg on the day of and the day after VELCADE dosing. The primary endpoint was the maximum tolerated dose (MTD) of Q in the combination (Q+V+D). The secondary endpoints included safety, overall response rate, and pharmacodynamic biomarkers.
Results: Eighteen patients (3,3, 6, and 6 in increasing Q doses) were enrolled: 56% male; median age 69 (range 50-82) years; multiple myeloma stage: IA = 11% and IIIA = 89%; prior lines of therapy: 1 = 100%, 2 = 55.6%, and 3 = 11.1%; prior VELCADE treatment = 50%. At the highest dose (12 mg) 2 patients had dose-limiting toxicity, 1 with QTc prolongation and 1 with atrial fibrillation. The MTD was established at the 10 mg Q for the Q+V+D regimen. The most common adverse events (≥10% of patients) were diarrhea (39%), asthenia (33%), peripheral oedema (22%), nausea (17%), thrombocytopenia (17%), alopecia (11%), constipation (11%), and vomiting (11%); most were grade 2 or lower in toxicity. To date, 13 patients have discontinued treatment, of which 5 completed 11 cycles of treatment. The overall response rate was 87.5% (14/16, 95% CI: 61.7% to 98.5%), including 1 complete response, 2 very good partial responses, and 11 partial responses. Most patients (9/11) showed a decrease in number of circulating multiple myeloma cells after 1 cycle. Two of 5 patients showed an increase in acetylated histone 3 from baseline as measured in peripheral blood mononuclear cells.
Summary / Conclusion: The maximum tolerated dose is 10 mg quisinostat in combination with VELCADE and dexamethasone. The combination is active in the treatment of relapsed multiple myeloma and has an acceptable safety profile.

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IMPACT OF HISTORY OF AUTOIMMUNE DISEASE ON SURVIVAL IN MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Background: The risk of monoclonal gammopathy of undetermined significance (MGUS) is increased in individuals with a personal and a family history of autoimmune disease (AI), and a personal history of several specific AIs increases the risk of multiple myeloma (MM). These previous findings suggest that immune-related conditions or the treatment of them may play a role in the etiology of MM and MGUS. History of AI is a negative predictor of survival in the general population, however, how AI affects survival in MM and MGUS is unknown.
Aims: The aim of the study was to determine whether a personal history of AI has an impact on survival in MM and MGUS.
Methods: Using national Swedish registries we identified2,765 patients with MM (diagnosed 2000-2006) and4,432 patients with MGUS (diagnosed 1988-2006), as well as 28,119 matched control subjects. The Swedish in- and outpatient registries were used to obtain information on AI in patients and controls, diagnosed prior to MM or MGUS diagnosis. MM and MGUS patients with AI diagnosed less than one year previous to diagnosis of MM or MGUS were excluded to avoid detection bias. We used the Kaplan-Meier method with log-rank test and Cox proportional hazards model to compare outcome among patients and controls with and without AI. We calculated hazard ratios (HR) and 95% confidence intervals (CI).
Results: A history of AI was found in 383 MGUS patients and 1,008 MGUS controls, and in 157 MM patients and 605 MM controls. In analyses restricted to MGUS patients, AI was associated with a 1.4-fold increased risk of death in males (HR=1.4; 95% CI 1.1-1.8) and 2.1-fold in females (95% CI 1.7-2.6). Compared to male controls without prior AI, male MGUS patients with a prior AI had a significantly 2.6-fold increased risk of death (95% CI 2.1-3.2), male controls with prior AI 2.1-fold (95% CI 1.9-2.3), and male MGUS patients without AI 1.9-fold (95% CI 1.8-2.1) risk of death. The corresponding numbers for female patients were HR=4.1 (95 % CI 3.5-5.0), HR=2.1 (95% CI 2.2-2.8), and HR=2.0 (95% CI 1.8-2.1; Figure 1). The interaction between AI and MGUS was significant in males but not in female individuals.

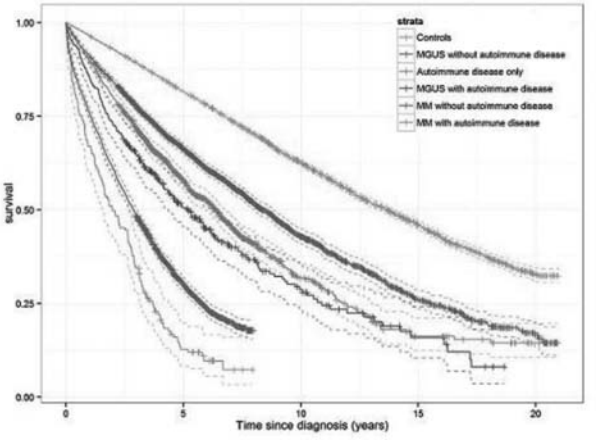

Figure 1.

In patients with MM and a prior history of AI, the risk of death was significantly increased 1.7 fold in females (95% CI 1.2-2.2), but was not significantly increased in males (HR=1.3, 95% CI 0.9-1.7). Compared to male controls without prior AI, male MM patients with prior AI had a significantly 9-fold increased risk of death (95% CI 7.0-11.7), male MM patients without AI 7.1-fold (95% CI 6.5-7.7), and male controls with AI 2.3-fold (95% CI 2.0-2.8) risk of death. The corresponding numbers for females were HR=12.6 (95% CI 9.9-16.0), HR=7.6 (95% CI 6.9-8.3), and HR=2.1 (95% CI 1.7-2.6; Figure 1). The interaction between AI and MM was significant in males and females.
Summary / Conclusion: In this large population-based study that included almost3,000 MM patients, more than4,000 MGUS patients, and their close to 30,000 matched control subjects, we found that a history of AI was linked to

decreased survival in MGUS patients and in female MM patients. AI was a stronger predictor of survival in individuals without MM or MGUS. Our findings suggest that although a history of AI is a poor prognostic factor in the general population, its impact on survival in patients with MGUS and MM is poor but not of similar magnitude. Underlying chronic diseases, such as AI, need to be taken into account when managing patients with MM and MGUS.

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LOW RATE OF SECONDARY PRIMARY MALIGNANCIES (SPMS) IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS TREATED WITH LENALIDOMIDE: FIRST RESULTS FROM THE MRC MM XI TRIAL

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Background: Long term therapy with Lenalidomide (Len) has been associated with an increased risk of developing SPMs.[1-3]
Aims: We are conducting a large phase III study to evaluate Len as induction and/or as maintenance therapy in newly diagnosed MM.
Methods: Patients are treated following an intensive or a non intensive pathway based on their eligibility for stem cell transplantation (ASCT) and are randomised to receive induction therapy with cyclophosphamide and dexamethasone combined with either Len (CRD) or Thalidomide (CTD). Patients failing to achieve an optimal response are randomised to receive additional therapy with cyclophosphamide, dexamethasone and bortezomib (CVD) or no extra therapy. A randomization between Len maintenance and no maintenance is also performed.
Results: We have enrolled 1882 patients, with 1879 having undergone the induction randomisation, and 581 patients having entered the maintenance randomization. The median follow up from initiation of the study is 1.3 years and from maintenance randomization is 1 year. Data on the occurrence of SPMs are being routinely collected as part of safety assessment during all protocol phases and follow up. So far 10 SPMs have been reported (0.5% of the randomised population); three additional patients, reported as having a SPM, were excluded, after central review of the data, either due to a previous history of malignancy or because of the evidence of a neoplasia other that MM at the time of study entry. The median time from trial entry to development of SPMs is 8 months (range 2.1-15.4). Seven out of 10 SPMs developed either during maintenance treatment or follow up. Three cases were diagnosed during induction. The SPMs reported during Len maintenance developed early in the course of treatment, 2.0, 3.3 and 5.9 months after starting maintenance (Figure 1).

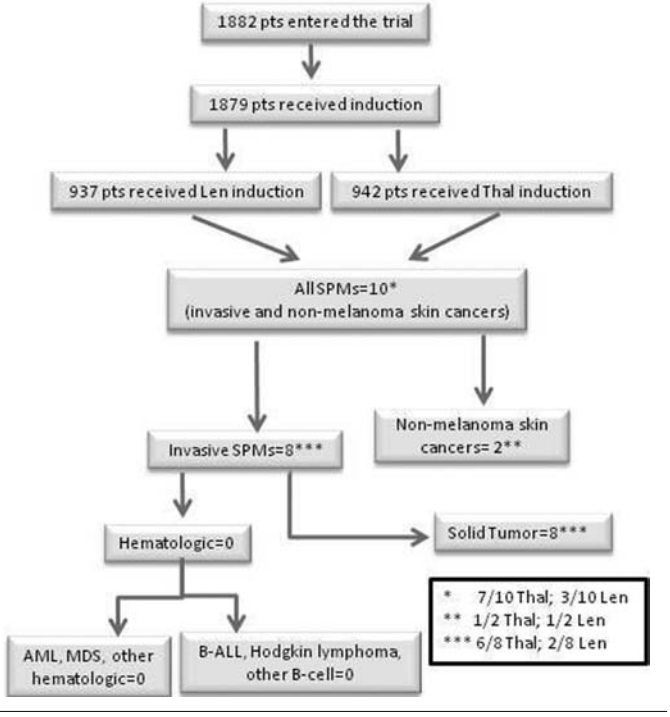

Figure 1.

The median age of patients developing SPM is 72 years (range 61-85), with 8/10 patients having been treated on the non intensive pathway; interestingly 7/10 of the cases received thalidomide induction. One patient, developing a squamous cell carcinoma of the nose received both Len induction and maintenance. Interestingly no hematologic SPM has been reported.
Summary / Conclusion: Our data clearly show a low incidence of SPMs, both overall and in patients treated with ASCT and Len maintenance. With almost 1900 patients enrolled, the cumulative incidence of SPMs reported so far has been 0.5%, with a cumulative rate of 0.1% for non-invasive SPMS and 0.4% for invasive SPMs. The incidence rate of all SPMs (invasive and non invasive) at one year is 0.74% (95% CI 0.39%>1.4%) and at two years is 0.91% (95% CI 0.48%>1.7%).Our data do not confirm previous findings of an excess risk of SPMs in association with the use of Len and melphalan in presenting patients, with only 2/1054 patients enrolled within the intensive pathway developing a SPM (ITT population). Longer follow up together with morphological, cytogenetic and molecular analysis is needed to elucidate the risk of Len associated SPMs.

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P243

THERAPY FOLLOWING RELAPSE ON MELPHALAN-PREDNISONE-LENALIDOMIDE (LEN) FOLLOWED BY LEN MAINTENANCE IN ELDERLY NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: EFFICACY AND SAFETY OF SECOND LINE LEN IN MM-015

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Background: The use of novel agents has dramatically changed the treatment paradigm of multiple myeloma (MM) leading to considerably improved patient (pt) outcomes. However, all pts eventually relapse, requiring subsequent therapy (Tx) to maintain disease remission. The pivotal, randomized, double-blind, placebo-controlled MM-015 phase 3 trial in elderly newly diagnosed MM pts demonstrated unprecedented increase in progression-free survival (PFS) with melphalan-prednisone-LEN followed by LEN maintenance (MPR-R) vs. fixed-cycle MPR or MP (31 vs. 14 and 13 mos, respectively; both P<0.001) (Palumbo A, *et al*. N Engl J Med. 2012;366:1759-69)
Aims: We present the longer term results of a post-hoc analysis assessing the impact of 1st line and 2nd line therapies on post-progression outcomes and safety in pts who went on to receive 2nd line Tx.
Methods: At progression, pts in the MM-015 study could enroll in an open-label extension phase (OLEP) to receive LEN 25 mg (D1–21/28 day cycle)±dexamethasone (DEX; 40 mg on days (D1–4, 9–12, and 17–20), or receive any other antimyeloma treatment outside of the protocol at investigator's discretion. This analysis includes data up to July 31, 2012 (median follow-up: 53 mos). 2nd line time-to-progression (TTP) was defined as time from 2nd to 3rd line Tx. Safety data were assessed only for pts enrolled in the OLEP (MPR-R, n=21; MPR, n=53; MP, n=79).
Results: A total of 459 pts were enrolled in MM-015; MPR-R(152); MPR (153); and MP (154). Fewer pts (53%) received 2nd line Tx in the MPR-R arm vs. the MPR (77%) and MP (82%) arms, consistent with the superior PFS of MPR-R. The median time from 2nd to 3rd line Tx was comparable across the arms (14, 16, and 15 mos for MPR-R, MPR, and MP, respectively; Figure A), suggesting that the addition of LEN did not induce resistant relapses. A total of 185 (57%) pts received LEN-based 2nd line Tx across the arms: 30% (MPR-R), 59% (MPR), and 72% (MP); 49%, 27%, and 21% of pts received bortezomib (BORT); and 21%, 15%, and 7% of pts received other 2nd line Tx, respectively. Pts receiving 2nd line LEN Tx had longer TTP from 2nd to 3rd line Tx (18, 23, and 18 mos for MPR-R, MPR, and MP, respectively) vs pts receiving BORT (14, 16, and 12 mos) or other Tx (6,4, and 6 mos; Figure B). Newly occurred or worsening grade 3–4 adverse events (AEs) reported in ≥ 5% of pts receiving LEN±DEX (n=153) in the OLEP were neutropenia (17%), thrombocytopenia (9%), and anemia and leukopenia (both 5%). Grade 3–4 deep-vein thrombosis and peripheral neuropathy occurred in 3% and 1% of pts, respectively. No cases of febrile neutropenia were reported. Updated data will be presented at the meeting.
Summary / Conclusion: 2nd line Tx is effective in pts who received prior LEN maintenance in the frontline setting indicating that LEN maintenance does not

appear to induce resistant relapses. Results with 2nd line LEN-based Tx compared favorably to outcomes with other Tx and further support the efficacy of LEN as 2nd line Tx for MM consistent with its improved indication. LEN as a 2nd line Tx was generally well tolerated with manageable AEs.

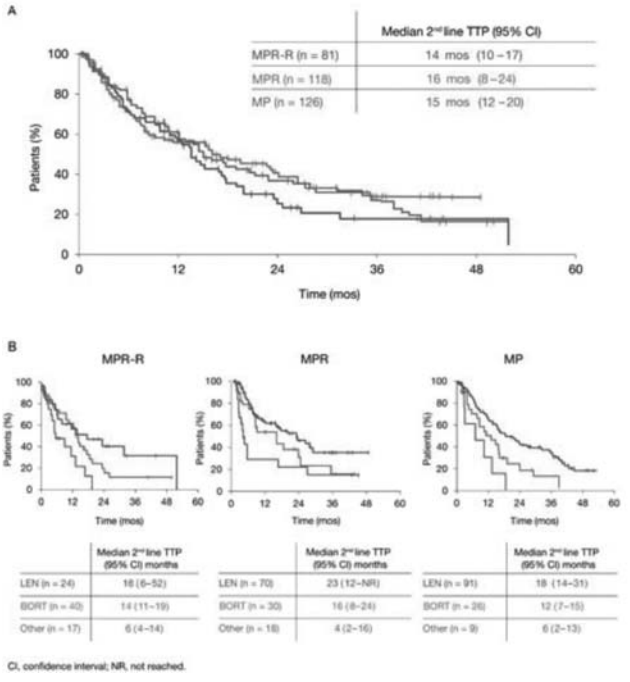

Figure 1. Median time from: (A) 2nd to 3nd line Tx in MM-D15; (B) 2nd to 3nd line Tx according to the type of 2nd line Tx.

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ANALYSIS OF MM-003 PATIENTS WITH MODERATE RENAL IMPAIRMENT USING POMALIDOMIDE + LOW-DOSE DEXAMETHASONE (POM + LODEX) VS. HIGH-DOSE DEXAMETHASONE (HIDEX) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background: Renal failure is a common complication for patients with multiple myeloma (MM) and is the second leading cause of death in this disease (Korbet SM, *et al*. *J Am Soc Nephrol*. 2006). The incidence of renal failure increases during the course of the disease (Eleutherakis-Papaikovou V, *et al*. *Leuk Lymphoma*. 2007) and is particularly relevant in advanced MM. Patients who have exhausted treatment (Tx) options such as bortezomib (BORT) and lenalidomide (LEN) have limited durability of responses and shorter overall survival (OS) (Kumar S, *et al*. *Leukemia*. 2012). POM was recently approved by the US Food and Drug Administration for Tx of RRMM patients who have received at least 2 prior therapies, including BORT and LEN. POM + LoDEX was efficacious with favorable tolerability in RRMM patients with prior BORT and LEN, including those with renal impairment (RI) (Siegel DG, *et al*. ASH. 2012). MM-003 is an open-label, multicenter, phase 3 randomized trial comparing POM + LoDEX vs. HiDEX in RRMM patients who have failed BORT and LEN and progressed on their last therapy.

Aims: This prospective analysis examined RRMM patients with or without moderate RI (creatinine clearance [CrCl] <60 vs. ≥60mL/min).

Methods: Patients must have failed BORT and LEN after ≥ 2 consecutive cycles of each (alone or in combination) and must have been refractory to their last prior therapy (progressive disease [PD] during Tx or within 60 days). Patients with CrCl < 45 mL/min were excluded. Patients were randomized 2:1 to POM 4 mg D1-21 + LoDEX 40 mg (20 mg for patients aged > 75 years) weekly; or HiDEX 40 mg (20 mg for patients aged > 75 years) D1-4, 9-12, and 17-20 (28-D cycles). Tx continued until PD or unacceptable adverse event (AE). Thromboprophylaxis with low-dose aspirin, low molecular weight heparin, or equivalent was required for all patients receiving POM and those at high risk for thromboembolic events. AEs were graded according to the National Cancer Institute Common Terminology Criteria for AEs (v 4.0). Tx was withheld and started at a lower dose in subsequent cycles for any grade 4 hematologic or ≥ grade 3 non-hematologic AE. Supportive care in the form of bisphosphonates, hematopoietic growth factors, erythropoietin, and platelet or red blood cell transfusions was allowed. The primary endpoint of the trial was progression-free survival (PFS), and the secondary endpoints included OS, overall response rate (ORR; ≥ partial response [PR]), duration of response (DOR), and AEs.

Results: A total of 302 patients received POM + LoDEX; 153 patients received HiDEX. Within each group, 94 (31%) and 59 (39%) patients had moderate RI (CrCl < 60 mL/min), respectively. Patients with moderate RI vs. normal renal function were more likely to be older (64% vs. 36% aged > 65 years). Median PFS and OS were significantly longer with POM + LoDEX vs. HiDEX regardless of RI (Table; P<.001) with a median follow-up of 4 months. Similar rates of AEs for both POM + LoDEX and HiDEX Tx were seen in patients with and without moderate RI (Table). Discontinuations due to AE were: 5% vs. 7% (normal renal function) and 11% vs. 5% (moderate RI). Updated data to be presented at the meeting.

Summary / Conclusion: POM has shown activity in patients refractory to LEN and BORT. POM + LoDEX significantly extended PFS and OS compared with HiDEX in RRMM patients with or without moderate RI. The tolerability of POM + LoDEX was acceptable and comparable across subgroups, with few discontinuations due to AE.

Table 1.

CrCl	≥ 60 mL/min			< 60 mL/min		
	POM + LoDEX	HiDEX	HR (*P*)	POM + LoDEX	HiDEX	HR (*P*)
Efficacy						
Median PFS, mo	3.7	1.8	0.47 (< .001)	3.2	1.6	0.44 (< .001)
Median OS, mo	Not reached	9.2	0.57 (.021)	10.3	4.6	0.51 (.008)
Grade 3-4 AEs						
Neutropenia, %	41	15	—	44	15	—
Anemia, %	24	26	—	33	34	—
Infection, %	23	23	—	28	24	—

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SALVAGE TREATMENT WITH POMALIDOMIDE-CYCLOPHOSPHAMIDE-PREDNISONE PRODUCES SIMILAR OUTCOMES AS COMPARED TO PRIOR THERAPIES IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: Multiple myeloma patients who relapse or are refractory after novel agents have a poor prognosis. The median event-free survival is 5 months and overall survival is 9 months (Kumar SK *et al*, Leukemia (2012) 26, 149-157). Pomalidomide is an effective option for patients relapsed or refractory after lenalidomide and bortezomib. In a phase 1/2 study pomalidomide-cyclophosphamide-prednisone (PCP) has demonstrated favorable tolerability and promising activity in myeloma patients relapsed or relapsed/refractory to lenalidomide.
Aims: We evaluated outcomes of patients treated with PCP according to prior treatment exposure.
Methods: Pomalidomide was administered at doses ranging from 1 to 2.5 mg/d, cyclophosphamide at 50 mg every other day, and prednisone at 50 mg every other day, for six 28-day cycles, followed by maintenance therapy with pomalidomide-prednisone. Thromboprophylaxis with aspirin 100 mg/day or low-molecular weight heparin was recommended at physician's discretion.
Results: Fifty-five patients enrolled at the maximum tolerated dose of 2.5 mg were evaluated after completing at least 1 cycle. Patients had previously been exposed to lenalidomide (100%), bortezomib (84%), thalidomide (20%), stem cell transplant (49%). The majority of patients (58%) had received 3 prior therapies (range, 1-3). Fifty-three percent of patients had received lenalidomide, 27% bortezomib, 5% thalidomide, 11% stem cell transplantation, and 4% conventional chemotherapy in their most recent prior line of therapy before enrolment. Median follow-up was 14.8 months. The overall response rate (ORR) was 45% after the last line of previous therapy and 46% after previous lenalidomide. The ORR with PCP was 51%, including 6% of patients attaining complete response, 18% very good partial response and 27% partial response. The ORR with PCP was 61% in patients who had received 1-2 prior therapies and 44% in patients who had received 3 prior therapies. Median progression-free survival (PFS) with PCP (10.4 months) was comparable to that obtained with the last anti-myeloma regimen before study entry (9 months) and with the previous lenalidomide therapy (10 months). Grade 3-4 adverse events included anemia (9%), thrombocytopenia (11%), neutropenia (42%), neurologic (7%), dermatologic (7%), thromboembolism (2%). Grade 3-5 infections occurred in 5 patients (9%). Five patients (9%) discontinued treatment for toxicity.
Summary / Conclusion: Pomalidomide-cyclophosphamide-prednisone is an effective salvage therapy that induced high response rates and prolonged PFS in relapsed/refractory multiple myeloma patients. The outcome achieved with PCP is comparable to that obtained with previous treatments including lenalidomide or bortezomib.

Table 1.

	Previous therapy		PCP		
	Lenalidomide (N=55)	Last line of therapy (N=55)	All Patients (N=55)	1-2 Prior therapies (N=23)	3 Prior therapies (N=32)
≥PR	46%	45%	51%	61%	44%
≥VGPR	11%	11%	24%	26%	12,5%
PFS (median, months)	10	9	10.4	21	9

PCP, pomalidomide-cyclophosphamide-prednisone; CR, complete response, VGPR, very good partial response; PFS, progression-free survival.

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EXPRESSION OF THE TRANSCRIPTION FACTOR NF-E2 IS REGULATED VIA THE NOVEL EPIGENETIC JAK2/H3Y41PH/HP1A PATHWAY

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Background: Despite the recent description of an activating point mutation in the Janus Kinase 2 ($JAK2^{V617F}$) in the majority of MPN patients, the molecular etiology of these diseases remains poorly understood. We have shown that the hematopoietic transcription factor Nuclear Factor Erythroid 2 (NF-E2) is overexpressed in the vast majority of MPN patients and that the JAK2 allele burden is positively correlated with NF-E2 expression. However, we were unable to detect STAT5 binding sites in the NF-E2 promoter. Recently, a novel STAT-independent pathway of JAK2 transcriptional regulation was described. Nuclear JAK2 phosphorylates histone 3 on tyrosine 41 (H3Y41). In the phosphorylated state, H3Y41 no longer binds the repressive heterochromatin protein 1α (HP1α). Therefore, H3Y41 phosphorylation leads to transcriptional activation.
Aims: We tested the hypothesis that NF-E2 is regulated by JAK2 via the novel epigenetic JAK2/H3Y41ph/HP1α pathway.
Methods: To mimic the physiological down-regulation of NF-E2 during myeloid maturation we used HEL cells as we have demonstrated that the demethylating agent 5-aza-2'-deoxycytidine (DAC, decitabine) decreases NF-E2 levels in this model. Untreated HEL cells or cells treated with decitabine were submitted to Western Blot and ChIP analysis using antibodies to $H3K9me^2$, HP1α, H3Y41ph and SHP-1. In addition, granulocytes from healthy controls and PV patients were analyzed for protein expression and chromatin occupancy of $H3K9me^2$, HP1α and H3Y41ph.
Results: In order to assess whether decitabine treatment decreases NF-E2 expression by acting directly on the NF-E2 gene we examined the methylation status of the NF-E2 promoter using pyrosequencing. The NF-E2 promoter is unmethylated in untreated HEL cells as well as in peripheral blood granylocytes of healthy controls and PV patients and remains unchanged by decitabine. Rather, following decitabine treatment we observed an increase in the repressive histone mark $H3K9me^2$ on the NF-E2 promoter. Likewise, in primary mature granulocytes the NF-E2 promoter carries the repressive $H3K9me^2$ mark only in healthy controls, where the gene is physiologically silenced. Conversely, this mark is absent in PV patients which retain unphysiologically high levels of NF-E2. In addition, following decitabine treatment, we observe a decrease in H3Y41ph on the NF-E2 promoter and a concomitant increase in HP1α binding, suggesting that the demethylating agent suppresses JAK2 activity. In support of this model, direct inhibition of JAK2 by TG101348 likewise decreases NF-E2 levels in HEL cells. In order to determine the mechanism by which decitabine treatment reduces NF-E2 levels, we assayed expression of the phosphatase SHP-1, which represses JAK2 activity. Demethylation of the SHP-1 promoter by decitabine has previously been shown. SHP-1 levels were indeed upregulated in HEL cells following decitabine treatment. We therefore propose that the demethylating agent decitabine down-regulates NF-E2 expression via increasing SHP-1 levels, leading to a decrease of $JAK2^{V617F}$ activity, with a subsequent reduction in H3Y41 phosphorylation and increased HP1α binding on the NF-E2 promoter. A separate or interrelated mechanism leads to a concomitant decrease in the $H3K9me^2$ mark on the NF-E2 promoter.
Summary / Conclusion: Our data therefore demonstrate for the first time that NF-E2 expression is regulated by the novel JAK2/H3Y41ph/HP1α pathway and we propose that this epigenetic deregulation contributes to the observed NF-E2 overexpression in MPN patients. These observations strengthen the rationale for using epigenetically active agents, such as decitabine, in the treatment of MPN. Several such trials have recently been initiated.

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CHARACTERIZATION OF JMJD1C AND JMJD2C AS NOVEL NF-E2 TARGET GENES

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Background: We have recently shown that expression of the transcription factor and epigenetic modulator "Nuclear Factor Erythroid-2" (NF-E2) is aberrantly elevated in patients with Myeloproliferative Neoplasms (MPN) and that NF-E2 overexpression in a murine model causes a MPN phenotype. As few NF-E2 target genes are known, the pathways by which this transcription factor exerts its effects remain unknown. We used lentivirally driven NF-E2 overexpression and shRNA mediated NF-E2 silencing in CD34-positive cells as well as *in silico* probing of NF-E2 ChIP Seq data to identify novel NF-E2 targets.
Here, we characterize the histone demethylases JMJD1C and JMJD2C as novel NF-E2 target genes. Both proteins demethylate lysine 9 on histone 3

thereby leading to transcriptional activation of target genes. SNPs in JMJD1C are positively correlated with platelet numbers. JMJD2C is overexpressed in several cancer entities and plays a role in oncogenesis, proliferation, pluripotency and stem cell self renewal. Moreover, it is a known downstream target of JAK2.
Aims: To test the hypothesis that JMJD1C and JMJD2C constitute novel NF-E2 target genes, which contribute to MPN pathophysiology.
Methods: NF-E2 binding to the JMJD1C and JMJD2C loci was analyzed by chromatin immunoprecipitation (ChIP). Functionality of the NF-E2 binding sites in the promoter regions of both demethylases was tested by luciferase reporter gene assays. We used short hairpin RNA (shRNA) mediated knock down of NF-E2 in HEL cells and introduction of the NF-E2 cDNA into CB3 cells to ask whether the transcription factor is required for and/or sufficient to induce target gene expression. Subsequently, we analyzed JMJD1C and 2C expression levels in PV patients and healthy controls as well as in our NF-E2 tg mouse model. Finally, we determined global histone 3 lysine 9 methylation levels in PV patients and healthy controls.
Results: By *in silico* analysis, the JMJD1C locus contains three potential NF-E2 binding sites, whereas the JMJD2C locus contains one. In ChIP assays, we were able to confirm *in vivo* NF-E2 binding to these sites. Luciferase reporter gene assays using NF-E2 binding site containing fragments of the JMJD1C and JMJD2C loci demonstrated NF-E2 driven activation of both promoters. Site directed mutagenesis confirmed the specificity of the NF-E2 effect.
Following shRNA mediated NF-E2 knock down in HEL cells a significant decrease in JMJD1C protein was observed, demonstrating that the transcription factor is absolutely required for JMJD1C expression. Conversely, upon viral reintroduction of NF-E2 into CB3 cells - a cell line that does not express NF-E2 - an upregulation of JMJD1C and 2C mRNA expression was observed. Likewise, NF-E2 transgenic mice demonstrate a significant increase in JMJD1C and 2C mRNA levels compared to wt control littermates, establishing the demethylases as novel NF-E2 targets. JMJD1C and 2C protein levels are statistically significantly elevated in patients with polycythemia vera (PV) compared to healthy controls. As JMJD1C and 2C proteins mediate the demethylation of histone H3K9, increased demethylase activity is expected to result in decreased steady state levels of mono and dimethylated H3K9 in PV patients. Indeed, we observed significantly decreased levels of H3K9me and H3K9me^2 in PV patients compared to healthy controls.
Summary / Conclusion: Here, we describe the histone demethylases JMJD1C and JMJD2C as novel NF-E2 target genes and propose that elevated NF-E2 levels in MPN patients contribute to disease pathophysiology in part by altering histone demethylation. These data provide a rationale for investigating novel JMJD-demethylase inhibitors, currently being developed, in the treatment of MPN patients.

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A MOUSE MODEL FOR HUMAN MYELOFIBROSIS
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Background: Primary Myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN) characterized by bone marrow fibrosis, abnormal megakaryopoiesis and erythropoiesis, extramedullary hematopoiesis and with a high risk of transformation to Acute Myeloid Leukemia (AML). The disease is thought to be a clonal stem cell disorder, however, stem cells that actually drive the disease in xenotransplantation models have not been identified.
Aims: The aim of his study we assess the CD133+CD34+ stem cell compartment of PMF patients for its potential to reproduce the disease phenotype *in vivo*.
Methods: Patient peripheral blood was characterized for stem cell populations expressing CD133+ and/or CD34+ cells. All patients examined carried JAK2V617F mutations. Isolated CD133+ cells were transplanted in immunocompromised mice. Flow cytometry, immuno-histochemistry, *in situ* hybridization and qPCR were used for the detection of human cells and determination of their mutational burden with time.
Results: PMF patient-derived CD133+ stem cells engrafted for more than 6 months in an immuno-compromised mouse model and differentiated into myeloid and endothelial-like JAK2V617F+ progenitors. We observed the constant presence of human, atypical, JAK2V617F+ megakaryocytes in bone marrow and spleen. Anemia, splenomegaly and splenic fibrosis were observed in transplanted mice, mirroring the gradual development of PMF. Strikingly, the transition to AML was observed 9 months post-transplantation of patient cells in mice. Leukemic cells lacked JAK2V617F mutations, which has also been as reported for 20-25% of patients with PMF that transform into a blast crisis.
Summary / Conclusion: We have identified a stem cell population that can drive both chronic and acute phases of PMF. Our mouse model forms the basis to decipher the characteristics of the malignant clones in PMF and to introduce new therapeutic targets for MPN.

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DEREGULATED MIRNAS IN JAK2V617F-NEGATIVE ESSENTIAL THROMBOCYTHEMIA PATIENTS TARGET SOCS FAMILY MEMBERS
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Background: The biological basis for essential thrombocythemia (ET) patients lacking the JAK2V617F mutation is still unknown. MicroRNAs (miRNAs) act as negative regulators of expression of important genes that participate in cellular proliferation, apoptosis and/or carcinogenesis and have been involved in both solid and hematological tumors. The potential role of miRNAs in JAK2V617F-negative ET remains to be elucidated.
Aims: To characterize the expression pattern of miRNA in JAK2V617F-negative ET.
Methods: Total RNA was extracted from platelets from 19 ET patients (10 were JAK2V617F+) and 10 healthy control samples. The expression of 670 mature miRNAs was analyzed using TaqMan Human MicroRNA Arrays v2.0 (Applied Biosystems) in an ABI 7900 HT real time PCR system. miRNA expression data was analyzed by the $2^{-\Delta\Delta Ct}$ method, using RNU48 as endogenous control. Statistical analyses were performed with TiGR MultiExperiment Viewer and R software. To identify molecular pathways potentially altered by the expression of multiple miRNAs we used Diana-mirPath, which performs an enrichment analysis of multiple miRNA target genes, comparing each set of miRNA targets to all known KEGG pathways. After that, mRNA expression of putative selected targets was analyzed by TaqMan gene expression assays (Applied Biosystems). The genes, whose expression was negatively correlated with miRNAs, were selected for further target validation by Renilla Luciferase assay. For Renilla-luciferase assay 100nM pre-miRNAs where transfected in K562 together with 1 μM of modified psicheck2 vector containing the 3'UTR region to be validated and Renilla luciferase levels were measured at 24h.
Results: The unsupervised hierarchical clustering of miRNA expression showed two well-separated clusters between patients and controls indicating that ET platelets had a characteristic miRNA signature (P<0.0001). Supervised analysis by means of significant analysis of microarrays (SAM) analysis showed a distinctive 101-miRNA signature, where most miRNAs (n=99) were downregulated in ET patients compared to controls. Interestingly, SAM analysis identified a 40-microRNA signature differentially expressed between JAK2V617F-positive and JAK2V617F-negative ET patients. Using the 40-miRNA signature we searched for potentially altered targets and pathways using Diana-mirPath. Among the pathways potentially altered, 11 miRNAs had putative targets affecting the JAK-STAT pathway. We quantified the mRNA expression of 8 of these putative targets and correlated it with miRNA levels. An inverse correlation was found between *SOCS1* and miR-221 (r^2=-0.719, P=0.001), *SOCS3* and miR-221 (r^2=-0.644, P=0.005), *SOCS3* and miR-203 (r^2=-0.447, P=0.072) and *PTPN11* and miR-23a (r^2=-0.494, P=0.044). These 3 miRNAs were upregulated in the JAK2V617F-negative ET patients. With these miRNAs we proceeded to target validation by Renilla Luciferase assay at the protein level. Transfection of K562 cells with pre-miRNAs or control pre-miRNA and the modified psicheck2 vector containing the 3'UTR binding sites of the selected miRNAs, resulted in target validation of SOCS1 for miR-221 (28.9% Renilla luciferase protein reduction, P=0.002) and SOCS3 for miR-203 (19.6% Renilla luciferase protein reduction, P=0.04). No significant modifications were observed for PTPN11.
Summary / Conclusion: ET shows a characteristic platelet miRNA-signature with downregulation of most miRNAs in comparison with healthy controls. JAK2V617F-negative ET cases harbor a 40-miRNA signature, differentially expressed from JAK2V617F-positive patients that target regulators of the JAK-STAT pathway, including members of the SOCS family.
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A DIFFERENTIAL CENTROSOME LOCALIZZATION OF WILD TYPE (WT) AND JAK2V617F PROTEIN IN HUMAN LEUKEMIA CELL LINES
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Background: The JAK2^{V617F} mutation is frequently observed in myeloprolif-

erative neoplasm (MPN). Vainchenker's group recently reported that the strong activation of $JAK2^{V617F}$ stimulates homologous recombination, centrosome and ploidy abnormalities. The centrosome ensures symmetry and bipolarity of the cell division process, which is essential for accurate chromosome segregation and cell-cycle progression into mitosis. The centrosome amplification occurs frequently in both solid tumors and hematological malignancies and it is thought to contribute to the development of chromosomal abnormalities in these disorders. The level of centrosome tyrosine phosphorylation is a surrogate marker for activation of centrosomal downstream signaling pathways; constitutive phosphorylation at this site may perturbate centrosome function and cell cycle.

Aims: Main aim of our research is to probe the role of the centrosome in genomic stability, cell polarity and tumor formation. Then, we evaluate if JAK2 status is related to centrosome malfunction and malignant transformation.

Methods: To determine if JAK2 is a centrosomal partner, we performed co-immunoprecipitation and the co-immunofluorescence (CIF) assays between JAK2 and a centrosome marker gamma-tubulin on several cancer cell lines, expressing $JAK2^{WT}$ (K562 and BV173, derived from CML patients; and human bone marrow stromal cell line HS5) or carrying $JAK2^{V617F}$ (SET-2 and HEL cell lines). Furthermore, we also evaluated the JAK2 centrosomal localization in CD34+ cells isolated from MPN patients. Finally we evaluated if centrosome phosphorylation may be modulated by treatment with the JAK tyrosine kinase inhibitor.

Results: CIF assay shows a neat co-localization of JAK2 and gamma-tubulin in K562, BV173 and HS5 cell lines as well as in CD34+ cells isolated from MPN patients with JAK2WT (in more the 90% ±5% of cells) in a cell cycle independent manner. Moreover, we observed that JAK2 centrosomal interaction is strictly dependent on the intact microtubule network, since nocodazole treatment, inducing depolymerization of the microtubule network, was able to reverse JAK2 centrosomal localization. By contrast, in leukemia cell lines carrying $JAK2^{V617F}$ in hemizygosis (SET) or in homozygosis (HEL), we observe colocalization of JAK2 and gamma-tubulin in only 40%±5% and 10±3% of the cells, respectively. In BM CD34+ cells isolated from MPN patients with $JAK2^{V617F}$ mutation, we also observe a partial JAK2 co-localization on centrosome. Notably, in 90% of HEL cells, lacking of JAK2-gamma-Tubulin co-localization, we identified high percentage (61%) structural and/or numeric centrosome abnormalities. Interestingly, we observed that the exogenous expression of $JAK2^{V617F}$ in K562 cell line increased significantly the numbers of centrosome abnormalities. This strongly suggests that JAK2 protein interacts with centrosome structure and that $JAK2^{V617F}$ is associated with centrosome malfunction and transformation.

Summary / Conclusion: Our preliminary data suggest a functional interaction between JAK2 and centrosome in $JAK2^{V617F}$ cell lines and in patients affected by MPN; this interaction may result in a genetic instability of neoplastic cells.

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EVALUATION OF THROMBIN GENERATION POTENTIAL IN POLYCYTHEMIA VERA PATIENTS ENROLLED IN THE CYTO-PV ITALIAN CLINICAL TRIAL

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Background: Polycythemia Vera (PV) is a chronic myeloproliferative neoplasm characterized by a clonal uncontrolled proliferation of bone marrow stem cells. PV typically presents with a hemostatic imbalance featuring an increased risk of both thrombotic and hemorrhagic events. Some studies show that in PV patients an elevated hematocrit (HCT) increments the thrombotic risk. Current guidelines for PV management advise to maintain HCT below 45% in males and 42% in females through phlebotomies and/or the use of cytoreductive drugs. Recently, the results of CYTO-PV clinical trial showed that PV patients with a HCT target <45% have a significantly lower rate of cardiovascular death and major thrombosis than those with a HCT in the range 45-50% (Marchioli *et al*, NEJM 2013).

Aims: In the setting of the CYTO-PV trial, we planned a biological sub-study to assess: 1) thrombin generation (TG) potential according to HCT levels, and 2) TG predictive value for bleeding and/or thrombosis.

Methods: One hundred twenty-four PV patients (HCT<45%: n=66, HCT 45-50%: n=58; age 42-87 years) were enrolled in the biological sub-study. Blood samples were collected at randomization and then after every 6 months for 5 years. TG was determined by the calibrated automated thrombogram assay (CAT assay, Stago), in platelet-poor plasma spiked with 5pM tissue factor (TF). TG results were expressed as endogenous thrombin potential (ETP) and as maximum quantity of thrombin produced (Peak). TG was also performed in the presence of activated Protein C (APC) to evaluate APC resistance, and results expressed as normalized APC sensitivity ratio (nAPCsr). Fifty-one healthy subjects acted as the control group.

Results: At baseline, HCT levels were not different between patients randomized at HCT<45% (46.4%) vs HCT 45-50% (47.9%). A significant correlation was observed between HCT values and TG parameters [ETP (R=0.501) and Peak (R=0.329)]. At baseline we also observed that APC markedly inhibited ETP and Peak of TG in both PV patients and controls; however, patients were more resistant to the anticoagulant action of APC compared to controls, resulting in significantly higher plasma nAPCsr. During follow-up, in patients randomized at HCT <45%, nAPCsr showed an overall decrease over time towards control values, while it remained higher than controls in the HCT 45-50%. The low rate of thromboses (n=9) and major hemorrhages (n=2) in the tested group did not allow us to assess the predictive values of TG for the thrombohemorrhagic events.

Summary / Conclusion: Our data show that in PV patients a correlation exists between HCT levels and TG potential, and a more resistant to APC phenotype occurs compared to healthy controls. The more aggressive cytoreductive regimen, i.e. HCT <45% as target, appeared to be associated to a less APC resistant features.

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A DECREASE IN JAK2 SIGNALLING COULD INDUCE A PARADOXICAL INCREASE IN MEGAKARYOPOIESIS

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Background: Megakaryopoiesis is the hematopoietic process leading from hematopoietic stem cells (HSC) to platelets production. In broad outline, it can be divided into a proliferative stage that generates megakaryocyte progenitors and a maturation stage in which differentiating megakaryocytes no more proliferate. These two stages can be driven by a single cytokine, namely thrombopoietin (TPO), which exerts both proliferative and anti-proliferative effects. Since MPL, the receptor of TPO, is devoid of kinase activity, the receptor associates with intracytoplasmic tyrosine kinases, in particular JAK2, for signal transduction. JAK2 is not only essential for TPO-induced signal transduction, but also for MPL stability and cell surface expression. A preferential amplification of the megakaryocytic lineage is observed in two myeloproliferative neoplasms (MPN), essential thrombocytemia (ET) and primary myelofibrosis (PMF). MPL is down-regulated in megakaryocytes and platelets from MPN patients, although this result is controversial in ET. A JAK2V617F mutation is detected in ~60% of PMF and ET whereas a mutation in MPL is detected in 2-8% of these diseases. A common feature of PMF and ET is the abnormal proliferation of megakaryocytes, suggesting that maturing megakaryocytes could escape the differentiation-associated induction of proliferation arrest by TPO.

Aims: The aim of our study was to demonstrate that a decrease in MPL or JAK2 expression or in JAK2 signalling could induce an increase in megakaryocytes production.

Methods: We derived clones from the human megakaryoblastic UT7-MPL cell line to explore the mechanisms allowing cells to escape the antiproliferative action of TPO. We confirmed our results by an RNA-interference strategy (shRNA anti-JAK2). We studied MPL and JAK2 expression, at the mRNA and protein levels, during the human CD34+ megakaryocytic differentiation process from cells cultured in presence of TPO. We then determined MPL/JAK2 expression in platelets from patients diagnosed with MPN. Finally, we studied the effects of a chemical JAK2 inhibitor on megakaryocytes production.

Results: In the present study, we show that TPO-induced cellular response depends on MPL and JAK2 protein levels. When one of these proteins is expressed at low level, the cytokine induces a weak signal that promotes cell proliferation. At higher MPL and JAK2 expression levels, TPO promotes cell cycle arrest and megakaryocytic differentiation. In our cell line model, a decrease in JAK2 expression can be sufficient to escape the antiproliferative action of TPO. We show that MPL and JAK2 expressions increase in a progressive and regular way during normal megakaryopoiesis and observed a decrease in MPL or JAK2 protein expression in platelets from MPN patients, correlated with the presence of a MPL or JAK2 mutation. We show that in some conditions a JAK2 inhibitor could paradoxically increase the production of megakaryocytes.

Summary / Conclusion: We propose that the modulation of MPL and JAK2 expression levels accounts for the two steps of normal megakaryopoiesis (ie proliferation versus proliferation arrest and differentiation) and that an MPL or JAK2 down-regulation may play a role in the abnormal proliferation of megakaryocytes in various MPN. Our results suggest that in some conditions, the clinical use of JAK2 inhibitors could paradoxically increase megakaryocytes production and result in an increase in the platelets number.

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A TRANSLOCATION T(3 ;13)(Q13 ;Q12) FUSES FLT3 TO A NOVEL PARTNER GENE, GOLGB1, IN A MYELOID/LYMPHOID NEOPLASM WITH EOSINOPHILIA.

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Background: The "Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB or FGFR1." category has been added to the 2008 WHO classification. This group, characterized by constitutive activation of tyrosine kinase, often associated a myeloid neoplasm to a high grade lymphoma (most frequently of T-cell phenotype), and eosinophilia. FLT3 is a class III receptor tyrosine kinase (TK) that can be constitutively activated by mutations in the juxtamembrane or the tyrosine kinase domains. Fusion genes involving this TK have already been described. FLT3 has been found to be fused to ETV6 in two myeloid/lymphoid neoplasms with eosinophilia cases.
We report here the case of a patient with a myeloid/lymphoid neoplasm with eosinophilia harbouring a new t(3;13)(p13;p12) translocation.
Aims: The aim of this study was to determine genes involved in this chromosomal rearrangement and identify the product of this translocation.
Methods: The patient was a 70-year old woman who was referred to our institution for asthenia with elevated leukocytes and neutrophils, presence of myelocytes, hypereosinophilia and increased monocytes, revealed by examination of peripheral blood. LDH level was elevated without tumour syndrome. Bone marrow was hypercellular with granular hyperplasia, no excess of blast and no dysplasia. Lately, the patient developed lymph node enlargement and hepatosplenomegaly with degradation of general status. Histology and phenotypic analysis of lymph node revealed a T-cell lymphoblastic lymphoma. Conventional cytogenetic analysis on bone marrow and lymph node cells revealed a t(3;13)(q13;q12) translocation, highlighting the hypothesis of a lymphoid/myeloid disorder. The patient rapidly died in despite of CHOP chemotherapy initiation. Final diagnosis was a 8p11-like myeloid/lymphoid neoplasm with hypereosiniphilia and with a new translocation t(3;13).
Results: First, FISH analysis and RT-PCR experiments permit to exclude involvement of FGFR1 gene on 8p11. Then, in order to refine the breakpoint in chromosome 3 and 13, a series of BACs was hybridized on 3q13 and 13q12. Finally, the use of fosmid probes permits to identified GOLGB1 and FLT3 as gene candidate in chromosome 3 and 13 respectively. Thus total RNA was extracted from lymph node cells and a reverse transcription- multiplex PCR was used to amplify a putative GOLGB1-FLT3 transcript. Sequence analysis of PCR product revealed a fusion of GOLGB1 exon 14 with a part of FLT3 exon 14. This fusion transcript was in-frame that incorporated 36 pb of intron 14 of GOLGB1. The GOLGB1-FLT3 transcript sequence is predicted to encode a chimeric protein containing the N-terminal portion of Giantin, a large coiled-coil protein encode by GOLGB1, and the C-terminal portion of FLT3 including the two tyrosine kinase domains.
Summary / Conclusion: We report here a novel partner gene for FLT3 identified in a patient with a myeloid/lymphoid neoplasm with eosinophilia harbouring a t(3;13)(p13;p12) translocation. Functional analysis will have to be performed and FLT3 inhibitors tested to determine the consequences and significance of the fusion

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DEFINING THE ONCOGENIC SIGNALLING PATHWAY OF JAK2-V617F BY TRANSCRIPTOME SEQUENCING AND IDENTIFICATION OF SPECIFIC DOWNSTREAM TARGETS

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Background: Myeloproliferative neoplasms (MPN) constitute a heterogeneous group of diseases in which there is an excess of terminally differentiated myeloid cells. In recent years, the *JAK2*-V617F mutation has emerged as a key player in the development of the disease, present in up to 90% of cases of polycythemia vera (PV) and 50-60% of cases of essential thrombocythemia (ET) and primary myelofibrosis (PMF). Major research efforts have been made to delineate the exact mechanism by which *JAK2*-V617F influences the development of the disease and how it results in starkly different phenotypes, however, many questions remain. The murine IL3-dependent Ba/F3 cells have been successfully used in the past to characterize cytokine responses of hematopoietic oncogenes and may serve as a useful model for investigating the action of *JAK2*-V617F in humans.
Aims: In order to gain a deeper insight into the *JAK2*-V617F signalling pathway and identify novel downstream targets, we analysed the transcriptome of the Ba/F3 cell line expressing the human *JAK2*-V617F and *JAK2* wild type cDNA in a tetracycline inducible manner
Methods: Ba/F3 cells were incubated with IL-3 and doxycycline for 72 hours with a 12 hour IL-3 starvation step prior to cell lysis. RNA was extracted and fragment libraries were synthesised. Sequencing was performed on the HiSeq2000 Illumina platform. The reads were analysed with the help of the TopHat/Cufflinks software suite and custom R scripts. Patient samples from *JAK2*-V617F negative PMF (n=9) and *JAK2*-V617F positive PMF (n=9) were also sequenced alongside healthy controls (n=6).
Results: Overall, 216 genes were found to be specifically expressed in *JAK2*-V617F cells upon doxycycline induction. Surprisingly a small number of genes (n=48) were induced upon expression of the wild type *JAK2*, 20 of which were specific to the wild type *JAK2*. We detected known *JAK2*-V617F targets such as *Myc*, *Osm*, *Stat1* and *Socs3*. Analysis of the gene set using the DAVID tool showed a strong enrichment for a variety of pathways including JAK-STAT and interferon response (P=0.0039). We also detected a set of novel dysregulated kinases, microRNAs and secreted proteins which included *Mir715*, *Pim1*, *Aurkb* and *Lif*. These hits represent novel pathways and kinases that may play a key role in the oncogenic signalling of the *JAK2*-V617F mutation.
Summary / Conclusion: Validations of the identified pathways and dysregulated genes are currently in progress, using data from sequenced patient samples. Moreover, functional assays will enable the identification of the genes that play a pivotal role in *JAK2*-V617F oncogenic signalling.

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INCREASED PREVALENCE OF AUTOIMMUNE PHENOMENA IN MYELOFIBROSIS: RELATIONSHIP WITH CLINICAL AND MORPHOLOGICAL CHARACTERISTICS, AND WITH IMMUNOREGULATORY CYTOKINE PATTERNS

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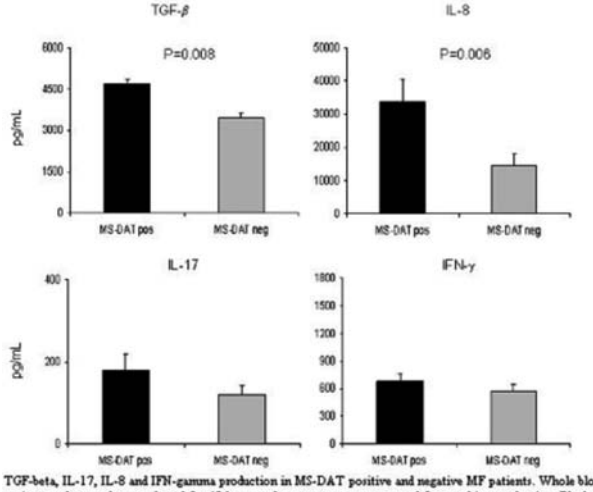
Background: Autoimmune phenomena are frequent in myelofibrosis (MF), both in terms of serologic markers of autoimmunity and of clinical defined diseases; however their prevalence and relationship to the grade of bone marrow fibrosis and clinical risk are not known. The role of the immune system in the pathogenesis of MF is supported by the existence of a non-clonal, non-neoplastic disease which resembles MF, named primary autoimmune myelofibrosis. In addition, MF patients showed an abnormal cytokine expression, which could contribute to bone marrow fibrosis, angiogenesis and constitutional symptoms; on the other hand, it is well established that immunoregulatory cytokines play a critical role in the pathophysiology of autoimmune diseases.
Aims: The aim of this study was to investigate the prevalence of autoimmune phenomena in MF, focusing on anti-erythrocyte antibodies, anti-platelets antibodies and organ- and non organ-specific autoantibodies. Further aim was to relate the presence of autoimmune phenomena with cytokine profiles (IL-17, TGF-β, IFN-γ, and IL-8) and with the grade of bone marrow fibrosis and clinical risk.
Methods: 100 consecutive MF patients were enrolled from May 2010 to April 2011 and followed prospectively for a median of 18 months until September 2012 (range 1-23). Patients were classified according to grade of bone marrow fibrosis and DIPSS-plus scoring system. Anti-erythrocyte antibodies were detected by standard direct antiglobulin test and mitogen-stimulated (MS)-DAT, anti-platelets antibodies and organ- and non organ-specific autoantibodies were detected by standard techniques. Cytokine production was evaluated in cultures supernatants.



TGF-beta, IL-17, IL-8 and IFN-gamma production in MS-DAT positive and negative MF patients. Whole blood from patients and controls was cultured for 48 hours and supernatants were assayed for cytokine production. Black columns represent MS-DAT positive patients and grey columns MS-DAT negative patients Values are the mean+SE.

Figure 1.

Results: Patients (51 males and 49 females; median age 72 years, range 34-86) were classified as follows: 58 primary, 32 post-thrombocythemia or 10 post-polycythemia; 18 MF-0, 65 MF-1, 15 MF-2, and 2 MF-3; 13 LR, 54 IR-1, 22 IR-2, and 11 HR. MS-DAT was positive in 45%, anti-platelets antibodies in 15% and serologic autoantibodies in 57% of cases; considering altogether autoimmune phenomena, positivity for at least one of the tests performed was found in 82 patients. None of the positive cases displayed an overt autoimmune disease. Autoimmunity was more frequent in MF-0/MF-1, and in LR and IR-1 DIPSS-plus groups. MS-DAT positive cases displayed increased TGF-β (P=0.008), and IL-8 production (P=0.006); IL-17 and IFN-γ were higher as well, although not significantly (Figure 1). Likewise, IFN-γ production was increased in patients with positive serological markers of autoimmunity (P=0.03). Finally, TGF-β and IL-17 were found elevated in early clinical and morphological stages, while IL-8 was increased in advanced MF stages.
Summary / Conclusion: Our results suggest that autoimmune phenomena and cytokine disregulation are particularly relevant in early MF stages according to clinical and morphological criteria, although they are not associated with clinically-overt autoimmune diseases. Overexpression of autoimmune phenomena could contribute to establish an unfavorable microenvironment, that may provide an advantage for the emergence of neoplastic and non-neoplastic clones, as already hypothesized in myelodysplastic syndromes and in paroxysmal nocturnal hemoglobinuria. The presence of autoimmune phenomena and cytokine disregulation may offer additional hints for therapeutic strategies (such as thalidomide, lenalidomide, and pomalidomide) aimed also at modulating the immune system.

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TYROSINE KINASE (TK) GENE FUSIONS IN CHRONIC MYELOPROLIFERATIVE DISORDERS (CMPD): A FISH STUDY

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Background: CMPDs are a heterogeneous spectrum of hematopoietic stem cell disorders due to the constitutive activation of specific TK genes because of mutations or exceptionally chromosomal translocations often escaping cytogenetic (CC) identification as most TK genes map within poorly stained regions. Their demonstration is clinically relevant as in 2008 the WHO classification introduced a new entity.
Aims: FISH with probes specific for TK genes was applied in order to assess the incidence of these chromosomal translocations, to identify uncommon TK translocation partners, to establish whether eosinophils are part of the clonal cell population and any correlation with clinical parameters and outcome.
Methods: The 31 consecutive patients (pts) analysed came to our observation in the period January 1998-December 2006 and were 9 females and 22 males with a median age of 45 years (range 22-72). According to WHO classification, 17 pts were classified as atypical chronic myeloid leukemia (aCML), 14 as chronic eosinophilic leukemia (CEL) and one as AML/T lymphoblastic lymphoma (T-LL). Median follow-up was 29 months (range 1-124). At the time of the study one pt died and none experienced disease progression. FISH probes were obtained from Kreatech (Amsterdam, NL), Abbot Molecular Inc. (Chicago, Il, USA) and from BACPAC Resources Center at C.H.O.R.I. (Oakland, USA) after determining their Mb position using UCSC genome browser on Human Feb. 2009 assembly. The commercial probes, applied according to manufacturer's guidelines were: ON FIP1L1-CHIC2-PDGFRA (4q12) Del, Break; ON PDGFRB (5q33) Break; ON FGFR1 (8p12) Break; ON JAK2 (9p24) Break; LSI BCRABL. The BAC probes labelled and applied as previously described were RP11-484L21 and RP11-880I16 covering the PCM1 gene. For i-FISH, cut-off values, obtained from the analysis of 300 nuclei from ten normal samples, were fixed at 10%.
Results: An abnormal FISH pattern was revealed in 9 pts (29%): 3/16 (18.7%) with aCML, 5/14 (35.7%) with CEL and one with AML/T-LL. Two aCML pts presented a trisomy 8 and the last one a t(9;13)(q34;q14) which involved the ABL gene and a partner gene not yet identified. Two CEL pts showed a JAK2 rearrangement: one with a t(8;9)(p22;p24) rearrangement on CC presented the classical PCM1-JAK2 gene fusion, the other with a t(3;8)(?;p24) not revealed by CC presented a fusion between JAK2 and a partner not yet identified. Two additional CEL pts showed a PDGFRB rearrangement which escaped CC detection too. In these pts, one with a t(1;5)(?;q33) and loss of the reciprocal translocation product and the other one with a t(5;8)(q33;?), the PDGFRB translocation partner has been not yet identified. The last chromosomally normal CEL pt showed a PDGFRA deletion. The sole AML/T-LL pt carried a t(8;13)(p11;q12) which produced the classical FGFR1-ZNF198 gene fusion. Thus, in 3 pts FISH with BAC probes is still on-going in order to search the unknown translocation partners of JAK2 and PDGFRB. Noteworthy, despite these TK positive pts presented a relevant eosinophilia (≈65%), FISH performed on peripheral blood smears always provided negative results. From a clinical point of view no aCML/CEL pt showed a lymphoid neoplasms. Four pts were treated with TK inhibitors and presented a haematological improvement.
Summary / Conclusion: Our data suggest that FISH i) effectively reveals cryptic TK translocations in about 36% of chromosomally normal CMPDs; ii) these TK rearrangements are more common in CELs than in aCML; iii) peripheral blood eosinophils may show a normal FISH pattern.

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ALL-TRANS RETINOIC ACID POTENTIATES THE INHIBITORY EFFECTS OF INTERFERON ALPHA (IFN) ON CHRONIC MYELOPROLIFERATIVE NEOPLASMS (MPN) PROGENITORS IN VITRO.

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Background: Although IFN has proven efficacy in Polycythemia Vera (PV) and Essential Thrombocythemia (ET), many patients experience unacceptable side effects. ATRA synergizes with the inhibitory effect of IFN on the growth of malignant and normal hematopoietic cells. Their pathways converge on the promoter of STAT1 that induces IRF-1 transcription. This tumor suppressor exerts pro-apoptotic effect trough TRAIL and caspase activation. ATRA activates TRAIL expression also by IRF1 independent mechanisms. These drugs cooperate in blocking cell cycle by inducing $p21^{WAF1/CIP1}$. This study aimed to *in vitro* investigate the synergistic activity of ATRA and IFN in MPN, in order to reduce IFN doses and its related side effects.
Aims: This study aimed to *in vitro* investigate the synergistic activity of ATRA and IFN in MPN, in order to reduce IFN doses and its related side effects.
Methods: HEL and SET2 cell lines characterized by JAK2V617F mutation were treated with a scalar dose of IFN (1-10000U/mL), ATRA (0,1nM-1x10^6nM) and a combination of the two drugs. Proliferation rate, apoptosis and cell cycle were evaluated after 24-48-72-96-120-168 hrs.
$CD34^+$ cells purified from BM of four MPN patients (PV=1, ET=3) at the time of diagnosis were plated (2x10^3 cells/mL) in semi-solid methylcellulose medium and were incubated with IFN (10000U/mL), ATRA (0,01mM) or their association. Colony forming cell assay (CFC) was performed at day 14. RARα,β,γ and RXRα,β,γ gene expression was evaluated both for cell lines and CD34+ cells from MPN patients.
Results: We observed in SET2 cell line an antiproliferative effect of IFN (10000U/mL) and ATRA (0,01mM) after 120-168 hrs of treatment.
Apoptosis assay showed that, at this concentration, HEL cell line is less sensitive to IFN (P=0,0002), ATRA (P=0,0026) and their combination (P=0,00001) than SET2. RT-qPCR showed a significant down-regulation of RARβ and up-regulation of RARγ in HEL respect to SET2. This differential expression of RAR subunit may explain the different responsiveness to ATRA. IFN and ATRA treatments were effective in inducing SET2 growth inhibition (P=0,0006; P=0,0008) and apoptosis (P=0,001; P=0,04) compared to untreated cell line. Moreover, their combination synergistically increased both growth suppression (IFN vs ATRA+IFN P=0,0001 and ATRA vs ATRA+IFN P=0,0006) and apoptosis (IFN vs ATRA+IFN P=0,036 and ATRA vs ATRA+IFN P=0,00001). Cell cycle analysis revealed a reduction of SET2 cells in S phase after IFN (6,2%) and IFN+ATRA treatment (3,9%), compared to untreated cell line (13,2%). CFC showed that IFN reduced CFU-GM (54±34; P=0,04) without affecting BFU-E and CFU-GEMM formation, while ATRA alone inhibited BFU-E (34±10 P=0,01) growth. Although the addition of ATRA seems to neutralize the IFN inhibitory effect on CFU-GM (127±24 P=0,25), their combination suppresses BFU-E (3±2 P=0,003) and CFU-GEMM (23±7 P=0,004).
Summary / Conclusion: These results indicate that, the *in vitro* combination of IFN and ATRA acts synergistically in inhibiting SET2 cell line and in suppressing BFU-E and CFU-GEMM growth and suggests that it has a potential interest for the treatment of PV and ET.

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SETBP1 OVEREXPRESSION IN CLASSICAL BCR/ABL1-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background: SET binding protein 1 (*SETBP1*) gene encodes a protein containing a ski homology domain, a SET-binding region, and three nuclear localization signals. The encoded protein has been shown to bind the SET nuclear oncogene and to inhibit the activity of protein phosphatase 2A tumor suppressor protein. *SETBP1* represents the first gene identified to be recurrently mutated in atypical Chronic Myeloid Leukemia (aCML), accounting for about 25% of all cases. aCML belongs to the group of myelodisplastic/myeloproliferative (MDS/MPN) syndromes without specific recurrent genomic or chromosomal alterations and characterized by poor prognosis. *SETBP1* mutations were also detected in 10% of "unclassified MDS/MPN" and in 4% of chronic myelomonocytic leukemia (CMML) cases. The occurrence of *SETBP1* point mutations has also been investigated in classical *BCR/ABL1*-negative myeloproliferative neoplasms (MPN) but no mutation was identified.

Aims: To date the possible activation of *SETBP1* gene expression in MPN has never been explored. The aim of this study was to investigate the occurrence of *SETBP1* gene deregulation in classical *BCR/ABL1*-negative MPN.
Methods: A cohort of cases affected by polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) was examined. In detail, 47*BCR/ABL1*-negative MPN patients at diagnosis (including 24 PV, 12 ET, and 11 PMF cases) were analyzed by quantitative real-time PCR (qRT-PCR) experiments using the LightCycler 480II System (Roche) and specific primers for *SETBP1* gene. Statistical analysis of the relative expression results was performed by the Relative expression software (REST) tool.
Results: qRT-PCR analysis revealed a high *SETBP1* expression level in PV and PMF bone marrow samples when compared with healthy matched controls. In fact, *SETBP1* gene expression in PV and MPF was more than 5 (P<0.0001) and 9 (P<0.0001) fold when compared to controls, respectively. Regarding ET cases, *SETBP1* gene resulted to be overexpressed by a mean factor of 1.839, but the data was not statistically significant. Considering a cut-off of ≥ 5 fold-change, expression values ≥ 5 were revealed in 58% (14 out of 24), 33% (4 out of 12), and in 90% (10 out of 11) of PV, ET, and PMF cases, respectively. No association between *SETBP1* overexpression and *JAK2* mutational status (homozigosity/heterozygosity) was detected in PV whereas all analyzed *JAK2V617F*-negative PMF cases (3 out 11) showed high gene expression level.
Summary / Conclusion: Recently, several acquired mutations in genes such as *TET2, ASXL1* and *IDH1/2* were identified suggesting that, in cooperation with *JAK2V617F*, additional molecular alterations are involved in the MPN pathogenesis and in the initiation of a leukemic transformation. Our data revealed that *SETBP1* gene dysregulation is a recurrent event in MPN. It is noteworthy to note that in our study *SETBP1* overexpression was revealed in more than half of the analyzed cases; moreover, *SETBP1* was upregulated in almost all PMF cases included in our study. Further analysis are needed to verify the association between *SETBP1* gene expression and clinical factors in MPN cases.

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CUSTOM AMPLICON CAPTURE AND BENCH-TOP HIGH THROUGHPUT SEQUENCING TO SCREEN FOR MUTATIONS ASSOCIATED WITH MYELOPROLIFERATIVE NEOPLASMS

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Background: Myeloproliferative neoplasms (MPN) are clonal disorders of terminally differentiated hematopoietic stem cells. Polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are the classical form of Philadelphia chromosome-negative MPN. The *JAK2* V617F missense mutation is found in 95% of patients with PV and 40-50% of patients with ET and PMF. Screening for the V617F mutation in query MPN patients has become a routine diagnostic method. Further identification of other activating mutations such as *MPL* (codons 505 and 515) and complex indels affecting *JAK2* exon 12 have been found in mostly V617F negative patients. In addition, a range of mutations in genes involved in intracellular signalling, epigenetic regulation and leukaemic progression have been identified. Molecular genetic screening for these targets at the required depth of sensitivity using conventional techniques is laborious and expensive, but with the advent of benchtop-class high-throughput sequencers, the ability to test for all relevant mutations in one assay becomes a possibility.
Aims: Optimize a custom amplicon high throughput sequencing assay to screen for the most relevant MPN associated mutations and validate candidate hits using pyrosequencing or high resolution melt curve analysis (HRM).
Methods: 24 patient DNA samples (4 PV, 12 ET, 5 PMF, 4 unclassified; 10 *JAK2* V617F positive by pyrosequencing and 14 negative; 2 of which had known *MPL* mutations) were screened (with appropriate ethical consent) for mutations using the Ion Torrent PGM sequencing platform. AmpliSeq was used for the target capture and the panel was designed via the online portal targeting 23 regions of 13 genes associated with MPN. The workflow involved library preparation and multiplex sample pooling following qPCR quantification (8 samples per run), emulsion PCR template preparation, Ion 316 chip loading and sequencing. Alignment and variant calling was via the Torrent Server 3.4.2 plugins, using a custom BED file to define the regions of interest. Variant calls were annotated using IGV and UCSC and candidate hits matching the Catalogue of Somatic Mutations in Cancer (COSMIC) were taken forward for validation using HRM or pyrosequencing.
Results: All 10 *JAK2* V617F and the 2 MPL mutations were correctly called by the Ion Torrent platform, with very similar variant frequencies and no false positives. Additionally, a V617F positive (70.1%) PV sample also showed a heterozygous *TP53* missense mutation (c.832C>A; p.P278T; COSM43697) at a frequency of 10.6%. This was validated using pyrosequencing (11.9%). Also, a 33.8% *MPL* W515L mutation (c.1544G>T; COSM18918) from a V617F negative ET patient was identified, along with a 47.8% *MPL* W515K in a V617F negative PMF sample (c.1543_1544TG>AA; COSM19193). Both *MPL* mutations were validated using HRM.
Summary / Conclusion: Myeloproliferative neoplasms are a heterogeneous group of disorders with specific mutations that can inform the correct diagnosis, prognosis and subsequent treatment choices. The approach used in this study showed that it is possible to combine a high-throughput qualitative and quantitative analysis of multiple genes that individually are laborious and time consuming, with no loss of sensitivity (1-5%) or specificity. Additionally, the ability to multiplex many samples per run increases the labour and cost effectiveness and makes this technique a feasible diagnostic tool for mutation screening in MPN and other somatic variant disorders.

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QUANTITATIVE RT-PCR FOR PDGFR IS USEFUL FOR DIAGNOSIS AND MOLECULAR MONITORING OF PATIENTS WITH PDGFR REARRANGEMENTS TREATED WITH TYROSINE-KINASE INHIBITORS

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Background: Hypereosinophilia (HE) may be the consequence of a *PDGFR* rearrangement. This possibility must be explored because a specific treatment by tyrosine-kinase inhibitors (TKI) will prevent life threatening complications of hypereosinophilias. However, the diversity of *PDGFR* rearrangements, which are often missed by cytogenetics, makes their detection sometimes difficult. All *PDGFR* fusion genes have an increased kinase activity, but also are subject to overexpression of the kinase domain encoded by the 3' end of the fusion. Thus overexpression of *PDGFRA* or *PDGFRB* may indicate the presence of a rearrangement.
Aims: We tested whether a quantitative PCR, modified form Erben *et al*, (Haematologica, 2010;95:738-744) assessing the level of expression of the *PDGFRA* and *PDGFRB* kinases could help detect *PDGFR* rearrangements, but also evaluate their response to TKI.
Methods: The *PDGFRA/PDGFRB* RT-qPCR was performed on the peripheral blood cells of 51 patients with unexplained hypereosiniphilia. Complete diagnosis work-up eventually allowed to classify these patients in 5 groups: 1- *PDGFRA* rearrangement (n=4), 2- *PDGFRB* rearrangement (n=4), 3- reactive hypereosinophilia (including lymphoid HE) (n=27) 4- myeloid HE (n=3), 5- idiopathic HE (n=13). In four patients diagnosed with a t(5;12) (ETV6-PDGFRB) a fusion specific RT-qPCR was performed on blood cells at diagnosis and during follow-up. Generic PDGFRA and PDGFRB RT-qPCR were also performed on follow-up peripheral blood samples for group 1 and group 2 patients.
Results: The RT-qPCRs for *PDGFRA* and *PDGFRB* had efficiencies ranging from 94 to 97% and coefficients of variation from 7 to 12%. Thresholds of 0.5 copies of PDGFRA for 100 copies of ABL1 and 50 copies of PDGFRB/100 copies ABL1 discriminated patients with PDGFR rearrangements (groups 1 and 2) from all other categories in 100% of cases (100% sensitivity and specificity). In patients without PDGFR rearrangement, there was no difference in PDGFRA or PDGFRB expression between groups 3 (reactive HE), 4 (myeloid HE) or 5 (idiopathic HE). The RT-qPCR for ETV6-PDGFRB (efficiency 96-98%) objectivates a decrease of the transcripts in treated patients ranging from 3 to 5 logs. The "generic" PCRs for the PDGFR kinase domain show similar decreases in treated patients, but with only a 3 log range of variations. The same range of decrease is observed in the 4 patients with FIP1L1-PDGFRA and the patient with CCDC6-PDGFRB.
Summary / Conclusion: The quantification of the kinase moiety of *PDGFRA* and *PDGFRB* is a simple, non invasive and unexpensive tool for the screening of *PDGFR* rearrangements. It also allows for a molecular follow-up of treated patients with such rearrangements whenever a fusion specific PCR is not available.

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CHARACTERIZATION OF PLATELET HYPERCOAGULABILITY IN HIGH-HEMATOCRIT POLYCYTHEMIA VERA PATIENTS

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Background: Polycythemia Vera (PV) is a myeloproliferative neoplasm characterized by a hypercoagulable state and an increased rate of thrombotic complications that significantly impact patients' prognosis and quality of life. The current management of these patients is highly dependent on their thrombotic risk. Maintaining a hematocrit below 45% and platelet count below 400,000/μL are

used as guidance in the treatment intensity. The results of a recent randomized controlled trial show that in PV patients with a hematocrit <45% the rate of cardiovascular death and major thrombosis is significantly lower compared to patients with a higher hematocrit. However all blood cell quantitative and qualitative abnormalities have been repeatedly implicated**.** Platelets have an important role in this process.
Aims: To characterize platelet-associated hypercoagulability in PV patients in relation to hematocrit values.
Methods: Seventy consecutive PV patients have been enrolled in this study after giving an informed consent. 94% of patients were positive for JAK2V617F mutation. At enrolment, 57% of patients were treated with cytoreductive therapy and 20% had a history of thrombosis. The whole blood analysis of hematocrit, white blood cells, platelets and immature platelet parameters were determined by Sysmex XE-2100 hematology analyser. Flowcytometric analysis was used to characterize platelet surface P-selectin and TF expression. The platelet-associated thrombin generation potential was evaluated by the calibrated automated thrombogram in fresh platelet rich plasma spiked with 1pM TF.
Results: Our results show that 38 patients had hematocrit <45% (mean±SD: 41.9± 2.7%) and 32 patients had hematocrit >45% (47.1±1.3%). White blood cell count was higher in high- (>45%) compared to low-hematocrit group (9.9±6.4 vs 7.7±3.6x10^9/L, P=0.07; respectively). Platelet surface TF was significantly increased in high- compared to low-hematocrit PV patients (38.6 ±19 vs 25.4±20%; P<0.05). No significant differences were observed for platelet surface P-selectin or platelet count between the two groups. The TG peak and slope were significantly (P<0.05) increased in high-hematocrit compared to low-hematocrit group (peak: 172.4±44.5 vs 142.9±47.9 nM thrombin; slope: 38.9±20.7 vs 28.6±16.4, respectively).
Summary / Conclusion: This study showed that platelets from patients with high hematocrit (>45%) are characterized by an increased procoagulant profile, as assessed by the higher platelet surface TF expression and PRP-associated TG potential, compared to low hematocrit group of patients. New prospective studies are warranted to evaluate the usefulness of these biomarkers in identifying PV patients at the highest risk for thrombosis.

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CHARACTERIZATION OF MUTATIONS IN ASXL1, SRSF2, CBL AND JAK2 GENES IN CHRONIC MYELOMONOCYTIC LEUKEMIA

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Background: Chronic myelomonocytic leukemia (CMML) is a myelodysplastic/ myeloproliferative neoplasm with a median overall survival (OS) of 20 months and 15-30% of progression to acute myeloid leukemia (AML). Molecular biology of CMML is poorly understood. Clonal cytogenetic abnormalities are found in 20-30% of cases. Recently, some recurrent gene mutations have been reported. The most frequent mutated genes are *TET2* (30-50%), *ASXL1* (35-56%), *SRSF2* (28-47%), *RUNX1* (8-37%), *CBL* (10-22%) and *K/NRAS* (2-22%), though less frequent mutations have also been described in other genes.
Aims: We sought to characterize type, frequency and prognostic implication of genetic mutations (*ASXL1*, *CBL*, *JAK2* and *SRSF2*) in a cohort of 55 patients with CMML.
Methods: According to the WHO classification, there were 43 CMML-1 and 12 CMML-2, while based on the FAB classification, 40 cases belonged to the myelodysplastic variant (MD) and 15 to the myeloproliferative one (MP). Median age at diagnosis was 69 years (range: 48-85 years), with a male predominance of 1.5:1 and a 31% frequency of progression to AML. Conventional cytogenetic G-banding was performed at diagnosis.To perform mutational analysis, DNA was extracted from bone marrow samples at diagnosis (n= 55). Sanger sequencing was performed to study mutations in exon 1 of *SRSF2* (n=52), exon 12 of *ASXL1* (n=34) and exons 8 and 9 of *CBL* (n=20), while *JAK2* V617F mutation was analyzed by endpoint genotyping (n=25). The rest of the cases are currently being assessed. Survival analysis was performed using Kaplan-Meier estimate and log-rank tests were used for comparisons. The χ^2 and Fisher's exact tests were used to analyze differences in the distribution of variables among patient subsets.
Results: Aberrant karyotypes were seen in 10/55 (18%) cases at diagnosis. Somatic *SRSF2* mutations were detected in 17/52 (33%) cases. All except from one corresponded to heterozygous, missense mutations located at hotspot P95 (6 P95H, 6 P95R and 4 P95L), while 1 case harboured a 3 nt duplication located at the same hotspot P95. Heterozygous somatic *ASXL1* mutations were detected in 12/34 (35%) cases. A total of 6 different mutations were seen in these cases, being the recurrent mutation G646WfsX12 the only one seen in more than one patient (7/31). *JAK2* V617F mutation was detected in 3/25 (12%) cases and no mutations were seen in *CBL* gene. *ASXL1* and *SRSF2* mutations did not correlate with either CMML-1/CMML-2 subtypes or MD/MP variants. All 3 cases with *JAK2* V617F mutation belonged to the MP variant. Mutations in these three genes were not mutually exclusive. Survival analysis revealed that the only adverse prognostic factors were CMML-2 subtype and MP variant. Patients with CMML-2 has worse OS and progression-free survival (PFS) compared to CMML-1 (P=0.001 and P=0.000), while MP cases also showed inferior OS and PFS compared to MD (P=0.012 and P=0.013). Mutations in *ASXL1* had no prognostic impact, while a higher PFS was seen in cases with *SRSF2* mutations, although it was not statistically significant.
Summary / Conclusion: Mutational study showed that *ASXL1* and *SRSF2* are frequently mutated in CMML. There are multiple *ASXL1* mutations located throughout the exon 12, while *SRSF2* mutations are located at the hotspot P95. Although patients with *SRSF2* mutations have have a trend towards higher PFS, CMML-2 and MP-CMML are still the only prognostic factors associated to worse OS and PFS. Mutational analysis in the whole cohort may show new prognostic factors.

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ADDITIONAL MUTATIONS TARGETING GENES UNRELATED TO RAS PATHWAY ARE FOUND IN SPORADIC JMML BUT NOT IN SYNDROMIC JMML

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Background: Juvenile myelomonocytic leukemia (JMML) is a rare and severe myelodysplastic/myeloproliferative syndrome of early childhood. Up to 90% of patients present with non overlapping mutations of RAS or RAS regulatory intermediates, leading to RAS overactivation. With mutations found in up to 45% of JMML, *PTPN11* is the gene most frequently involved. *PTPN11* mutations can be either somatically acquired (sporadic JMML) or germline in the context of Noonan syndrome (syndromic JMML). Mutations in *NRAS*, *KRAS*, *NF1* or *CBL* can also be found. Intriguingly, the initiating mutation only partially explains clinical and biological heterogeneity among JMML.
Aims: To get further insight into JMML leukemogenesis, we searched for cooperative lesions in either sporadic or syndromic cases due to *PTPN11* mutations.
Methods: In 16 patients with a known *PTPN11* mutation (8 syndromic JMML, 8 sporadic JMML), whole exome sequencing (WES) was performed on paired tumoral and germline samples. Mutations were then searched by Sanger sequencing in the entire French JMML cohort (n =110, with 77 sporadic cases including 30 with *PTPN11* mutation, and 33 syndromic JMML including 18 with *PTPN11* mutations).
Results: WES revealed the presence of 0 to 5 somatic mutations per JMML. No gene was found mutated in addition to *PTPN11* in the 8 syndromic JMML. In contrast, several additional mutations were identified in the 8 sporadic JMML, including somatic *SETBP1* mutations in 2 patients, in line with Muramatsu *et al*. (ASH 2012). Eight other non-silent somatic variants were uncovered in 4 sporadic JMML. None of these variants affected genes known to be related to RAS pathway. Their analysis is still in progress. Targeted resequencing in the whole JMML cohort confirmed the absence of mutations in the 33 syndromic JMML regardless the underlying condition (Noonan syndrome, CBL syndrome). This finding is consistent with the absence of structural abnormalities previously revealed by SNP array analysis in these patients. With 14 additional mutations present in 11/77 (14%) cases, sporadic JMML showed a higher mutation frequency. *ASXL1* was mutated in 7/77 (9%) sporadic JMML, apparently unrelated with the original mutation. *SETBP1* screening showed 7/77 (9%) mutations in sporadic JMML, in addition to a somatic mutation in *PTPN11* (n=4) or *NRAS* (n=3). Six of the SETBP1 mutations affected codons 868 to 870 and are compatible with defective ubiquitinylation leading to *SETBP1* activation. Additional mutations found in sporadic JMML tended to concentrate on the same patients. Indeed, 5/7 *SETBP1* mutations were associated with a mutation of *ASXL1* and/or a clonal cytogenetic abnormalities. The presence of additional mutations at diagnosis may sign an aggressive clinical course since 9 of the 11 patients with at least one additional mutation presented with blast excess (>10%) in bone marrow at diagnosis, experimented subsequent transformation into acute leukemia, and/or early death.
Summary / Conclusion: Although infrequent, additional abnormalities targeting genes unrelated to RAS pathway are found in a subset of sporadic JMML. This clearly distinguishes them from syndromic forms for which no such abnormalities could be identified. This raises the question of a possible role of secondary alterations affecting chromatin conformation in a difference of prognosis between sporadic and syndromic JMML.

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DETECTION OF LEUKEMIA-ASSOCIATED MUTATIONS IN HEMATOLOGICALLY NORMAL ELDERLY INDIVIDUALS

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Background: New sequencing technologies are facilitating the development of large gene panels that can be screened in patients with suspected hematological malignancies. Emerging evidence, however, has indicated that some malignancy-associated mutations might be detectable in the population at large, raising questions about the utility of broad mutation screening as a diagnostic tool.
Aims: To investigate this further we studied i) the Uppsala Longitudinal Study of Adult Men (ULSAM) cohort, an ongoing epidemiologic study of all available men born between 1920-24 in Uppsala County, Sweden and ii) 56 normal elderly women with known X-chromosome inactivation patterns (XCIP) in both T-cells and neutrophils.
Results: In a pilot study that included Illumina 1M-Duo beadchip analysis of 108 ULSAM cases, we previously identified one individual with normal peripheral blood counts and no other indication of a hematological disorder who had acquired a large (103Mb) region of terminal acquired uniparental disomy (aUPD) at chr 4q (Forsberg *et al*., AJHG. 2012;90:217-28). Since 4q aUPD in myeloid malignancies is associated with inactivating mutations of *TET2*, we sequenced this gene and identified a 21bp deletion that removed part of exon 4 and is therefore likely to be deleterious. The mutation was absent from B-cell, T-cell and fibroblast DNA but present in leukocyte and granulocyte DNA extracted at the age of 90, leukocyte DNA at ages 82 and 88 (aUPD detected at all three timepoints) and at the age of 71 when aUPD was not detected. Interestingly, the mutational load and level of aUPD noticeably declined between the ages of 88 and 90 for no apparent reason. To determine if aUPD is more widespread in elderly men, we analysed array data from a further ~1100 ULSAM cases and identified 14 individuals (1.3%) with aUPD (median size = 27Mb; range 13-87) at 1p (n=1), 6p (n=1), 9p (n=1), 9q (n=1), 11p (n=2), 11q (n=1), 14q (n=1), 15q (n=1), 17p (n=1), 19q (n=2), 22q (n=2). Of the 14 individuals, two had been diagnosed with a hematological malignancy: one with *JAK2* V617F positive PV (9p aUPD) and another with CLL (17p aUPD; p53 not tested). We did not detect *MPL* or *CBL* mutations in the cases with 1p or 11q aUPD; candidate somatically mutated genes have not been identified for the other aUPD regions. We then went on to analyze a cohort of 56 hematologically normal elderly women, of whom 10 had skewed XCIP in neutrophils but not T-cells, 21 had skewed neutrophils and skewed T-cells and 25 were either balanced in both fractions (n=21) or skewed in T-cells but not neutrophils (n=4). The skewing may simply be a consequence of age-related stochastic processes but might in some cases be caused by subclinical clonal expansion. We screened neutrophil DNA for *JAK2* V617F and mutations in *DNMT3A* (exons 15-23) and *TET2* (all coding exons). *JAK2* V617F was not seen but 2 variants in *DNMT3A* (G699D and R882C) and 4 in *TET2* (R1261H, G1365R, 91477insA_91478_delAGGT, 176230delA) were identified in 3 cases that were either absent or markedly reduced in T-cells from the same patient. Of note, *DNMT3A* R882C is seen recurrently in patients with leukemia, and two of the *TET2* mutations are frameshifts. A further 3 *TET2* missense variants (M1028I, M1701I and Y867H) were identified that were also seen in T-cells.
Summary / Conclusion: These findings reveal a complex pattern of mutations in elderly individuals and support the notion that somatically acquired driver variants are detectable in some individuals with otherwise apparently normal hemopoiesis.

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THE IDENTIFICATION OF A PSTAT5 GENE SIGNATURE AND ITS MODULATION IN RUXOLITINIB PHARMACODYNAMIC RESPONSE IN HEMATOLOGIC MALIGNANCY

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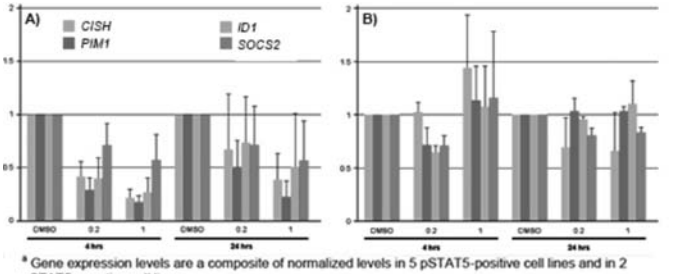
Background: The JAK/STAT pathway is an important signaling pathway downstream of multiple cytokine and growth factor receptors. Receptor-associated JAKs are activated following receptor-ligand binding. Activated JAKs phosphorylate STAT proteins, which then dimerize and translocate to the nucleus where they modulate the expression of target genes. Dysregulated JAK/STAT signaling has been implicated in the pathogenesis of multiple human malignancies. Activating *JAK2* mutations and associated STAT5 activation in myeloproliferative neoplasia is an example of the involvement of this pathway in human cancer. Additionally, overactive JAK/STAT signaling has been suggested to be a survival mechanism in several human cancers. Given the importance of JAK/STAT dysregulation in human diseases, it is important to identify patients with an overactive JAK/STAT pathway for possible treatment with JAK inhibitors. Thus, we developed a gene signature assay to detect overactive JAK/STAT5 signaling.
Results: The cancer cell line encyclopedia (CCLE) and associated gene-expression data were used to correlate the activation status of STAT5 with the induction of a set of *STAT5* target genes. First, we used 27 tumor cell lines of hematologic lineage, with predetermined phosphorylated STAT5 (pSTAT5) status, to derive STAT5 activation gene signatures. Next, the putative gene signatures were validated against a different set of 13 hematologic tumor cell lines. With this approach, a collection of 7 target genes were identified (*PIM1*, *CISH*, *SOCS2*, *ID1*, *LCN2*, *EPOR*, and *EGR1*) whose expression significantly correlated with pSTAT5 status in the 40 hematologic tumor cell lines (P<.0001), either together or in specific subsets of 4 and 6 genes (Table 1). The STAT5 gene signature was then used to examine pharmacodynamic response to ruxolitinib in a preclinical setting. Seven hematologic tumor cell lines (5 positive for pSTAT5 and 2 negative for pSTAT5) were treated with ruxolitinib 0.2 μM or 1 μM, and samples were collected at 4 hours and 24 hours after treatment. Phospho-STAT5 was examined by Western blot analysis, and the expression of the 4 signature genes was determined by qPCR. In the pSTAT5-positive cell lines, ruxolitinib downmodulated pSTAT5, and there was a corresponding reduction of the expression of the signature genes (Figure, A). In the pSTAT5-negative cell lines, there was no clear effect on pSTAT5 modulation or change in signature gene expression (Figure, B).
Summary / Conclusion: These 4-, 6-, and 7-gene signatures provide a transcriptional surrogate for pSTAT5 and, therefore, ruxolitinib pharmacodynamic activity in hematologic cell lines. Additional studies are required to characterize the relevance of these signatures to JAK/STAT pathway activation and inhibition in human malignancies.

Table 1. Correlation of genes with pSTAT5 status in the 40 hematologic tumor cell lines.

4-Gene Signature	6-Gene Signature	7-Gene Signature
PIM 1	*PIM 1*	*PIM 1*
CISH	*CISH*	*CISH*
SOCS2	*SOCS2*	*SOCS2*
ID1	*ID1*	*ID1*
	LCN2	*LCN2*
	EPOR	*EPOR*
		EGR1
P < .0001	*P* < .0001	*P* < .0001



[a] Gene expression levels are a composite of normalized levels in 5 pSTAT5-positive cell lines and in 2 pSTAT5-negative cell lines.

Figure 1. The 4-gene signature in (A) pSTAT5-Positive and (B) pSTAT5-Negative cell lines*.

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DO MYELOPROLIFERATIVE NEOPLASMS (MPNS) HAVE STAGES? ANALYSIS OF RELATIONSHIP OF DISEASE DURATION, PROGNOSTIC SCORES, AND SYMPTOMATIC BURDEN OF 1467 MPN PATIENTS

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Background: The MPNs are comprised not only of three heterogeneous disease types histologically (polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (PMF, post-ET MF, post-PV MF), but of a multitude of variable disease phenotypes within each MPN subtype.

Aims: We sought to assess the relationship between MPN prognosis and symptom burden.

Methods: : Data was prospectively gathered from the MPN-QOL International Study Group. Patients completed the MPN-SAF and EORTC QLQ-C30. Data on diagnosis, clinical features, and clinical course was collected. Prognostic scoring was performed for MF (DIPSS criteria: age (>65), peripheral blasts (≥1%), hemoglobin (<10 g/dL), leukocyte count (>25x10^9/L), constitutional symptoms; Passamonti, 2012), PV (age >70 or 60-69 and leukocyte count (>15x10^9/L); Tefferi, 2012) and ET (IPSET criteria: age (≥60 years) leukocyte count (≥11x10^9/L), hx of thrombosis; Passamonti, 2012). Clusters were based on the nonhierarchical k-means method. Associations between patient/clinical variables and cluster were investigated using ANOVA, Kruskal-Wallis, and chi-squared tests.

Results: Data from 1470 prospectively enrolled MPN patients was collected including MF (n=329; PMF=223, post-ET MF=67, post-PV MF=39; age mean 59 yrs, male 53%), PV (n=519, age mean 61 yrs, male 57%) and ET (n=622, age mean 57 yrs, male 37%). *MF:* Four clusters were identified. Increasing symptom burden was associated with DIPSS risk (P<0.001), leukopenia (P=0.009), thrombocytopenia (P<0.001) and palpable spleen size (P=0.009). Although a correlation exists between clusters and DIPSS risk groups (P<0.001) significant symptom burden occurred in low and intermediate 1 risk disease (Table 1). Within DIPSS groups, MPN-SAF TSS >20 occurred at rates of 37% (low), 56% (int-1), 62% (int-2) and 67% (high). Longer MF duration trended towards higher cluster values (P=0.06). Weight loss, night sweats, fevers and MPN-SAF TSS increased with clusters progression (P<0.001). *PV:* Five clusters were identified. Clusters correlated with leukopenia (P=0.01), splenic size (P=0.002), and anemia (P=0.04). MPN-SAF TSS increased with cluster progression (P<0.001). No correlations were observed between PV risk group (including variables of age [P=0.13] and leukocytosis [P=0.69]) and clusters (P=0.87; Figure 2). There was a trend towards an inverse relationship between PV duration (mean durations of 6.2, 5.8, 6.4, 4.2, 4.4 years) and increasing symptomatic burden (P=0.73). *ET:* Five clusters were identified. Clusters differed by gender (P=0.04), anemia (P=0.01), prior hemorrhage (P=0.047) and median hemoglobin concentration (P=0.002). MPN-SAF TSS increased with cluster progression (P<0.001) while age was inversely associated with cluster progression (P=0.01). No correlations were observed between IPSET risk scores (including variables of leukocyte count [P=0.94] and history of thrombosis [P=0.24]) and clusters (P=0.43; Figure 3).

Summary / Conclusion: Significant heterogeneity exists both between and within each MPN subtypes. This heterogeneity in disease phenotype is not solely a surrogate for disease prognosis as assessed by MPN prognostic scores. Stages (as assessed by symptomatic burden and prognosis) within each of the MPN subtypes may exist but lack a linear chronological progression suggesting biological subsets within MPN subtypes may exist. Further efforts are ongoing on how to incorporate molecular data, prognostic data, and symptomatic phenotype into a useful staging model with MPN subtypes.

Table 1. Total Symptom Scores Comparison Between Risk Scores.

TSS Deciles by Risk Score	Low (N=38)	Int-1 (N=84)	Int-2 (N=37)	High (N=6)	Total (N=165)	p value
MPN-SAF TSS						<0.001[1]
Mean (SD)	14.9 (10.88)	24.1 (14.82)	27.7 (14.75)	43.0 (21.34)	23.5 (15.32)	
MPN-SAF TSS						<0.001[2]
0-10	18 (47.4%)	16 (19%)	4 (10.8%)	0 (0%)	38 (23%)	
11-20	6 (15.8%)	21 (25%)	10 (27%)	2 (33.3%)	39 (23.6%)	
21-30	10 (26.3%)	21 (25%)	8 (21.6%)	0 (0%)	39 (23.6%)	
31-40	4 (10.5%)	15 (17.9%)	9 (24.3%)	0 (0%)	28 (17%)	
41-50	0 (0%)	5 (6%)	4 (10.8%)	1 (16.7%)	10 (6.1%)	
51-60	0 (0%)	4 (4.8%)	1 (2.7%)	1 (16.7%)	6 (3.6%)	
61-70	0 (0%)	2 (2.4%)	1 (2.7%)	2 (33.3%)	5 (3%)	
MPN-SAF TSS						0.11[2]
</=20	24 (63.2%)	37 (44%)	14 (37.8%)	2 (33.3%)	77 (46.7%)	
>20	14 (36.8%)	47 (56%)	23 (62.2%)	4 (66.7%)	88 (53.3%)	

(report generated on 28FEB2013)
[1] ANOVA F-Test [2]Chi-Square

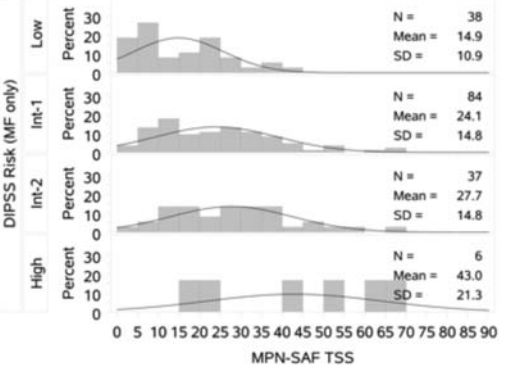

Figure 1. Myelofibrosis risk separated by MPN-SAF TSS

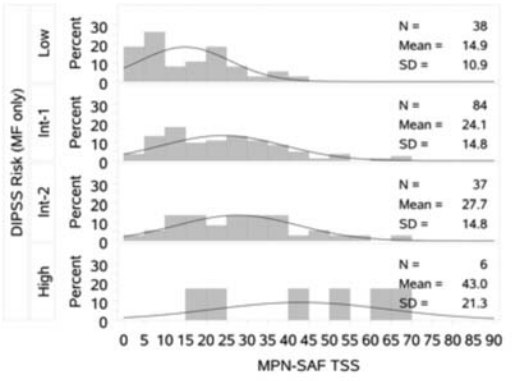

Figure 2. Polycythemia vera risk separated by MPN-SAF TSS.

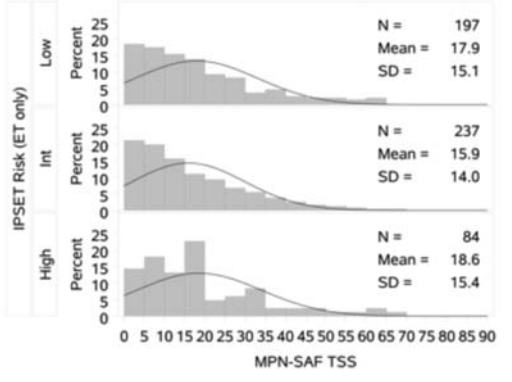

Figure 3. Essential thrombocythemia risk separated MPN-SAF TSS.

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FIRST EUROPEAN JAK2-V617F INTERLABORATORY QUALITY CONTROL STUDY CARRIED OUT BY THE MPN&MPNR-EURONET (COST ACTION BM0902)

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Background: Analysis for the 1849G>T mutation in *JAK2* (encoding JAK2-V617F) is routine in the diagnosis of myeloproliferative neoplasms (MPNs). The quantification of the allelic burden in JAK2-V617F positive patients is increasingly used to monitor treatment response of new targeted therapies as well as in transplanted patients.
Aims: Across Europe the quantitative JAK2-V617F analysis is performed using a number of different assays and analysis platforms and calibration is therefore needed in order to standardise the results.
Methods: Blood samples from 10 JAK2-V617F positive patients were aliquoted (1 ml) and sent out with overnight courier together with 4 reference DNA samples, a JAK2- V617F real time quantitative PCR (qPCR) reference assay and 4 DNA samples (blood samples were collected after informed consent according to the guidelines of the Danish Regional Science Ethics Committee). The reference DNAs were made from a 80 mL pool of normal donor DNA from blood samples by spiking four 10 fold dilutions of a 650 bp PCR product containing the JAK2-V617F mutation into 20 mL aliquots. The copy number of JAK2-V617F and JAK2 wild type in the 4 DNA pools were determined by qPCR and by digital PCR and the allelic ratios of JAK2-V617F were calculated to 75%, 23%, 2.9% and 0.2%, respectively. The qPCR reference assay (Larsen *et al* BJH 2007) that is recommended by the ELN for quantitative PCR (Jovanovic *et al* submitted) was prepared as 10 fold concentrated wild type and V617F mutant primer/probe mixtures. The 4 DNA samples contained either wild type DNA (normal donor DNA), V617F positive DNA (HEL cell line DNA), water (negative control) or normal donor DNA spiked with 1% JAK2-V617F. Two local JAK2-V617F positive DNA samples were also included and these were analysed as both non-diluted and ten fold diluted in order to identify a potential inhibition.
Results: Twenty four laboratories from 13 European countries participated in the study. DNA from the 10 patient blood samples was purified according to local protocols. All samples were run in triplicates (or in duplicates in a few labs) with both the local JAK2-V617F assay and the supplied reference assay (384 qPCR wells per lab). Protocol information, Ct (PCR cycle) values and calculated copy numbers from the local assay were sent to Vejle for analysis (approximately 14,000 data points). Although the reported copy numbers in the 10 patient samples varied between labs the percentage of JAK2-V617F alleles was rather consistent for both the local assay and the reference assay. All labs were able to indentify the 1% JAK2 V617F sample as positive when using the reference assay. However, in several labs the allelic burden of the 1% sample was not significantly different from the normal wild type DNA sample when using the local JAK2 assay indicating limited specificity due to nonspecific amplification
Summary / Conclusion: In 24 labs across Europe the detection and quantification of the JAK2-V617F mutation was relatively consistent in patient samples with an allelic burden above 1%. For values below 1% the specificity and thereby the sensitivity of the analysis varied between labs and this was related to the JAK2-V617F assay used.

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MUTATIONS IN THE RNA SPLICING MACHINERY GENES IN MYELOFIBROTIC TRANSFORMATION OF ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA

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Background: Mutations in genes that codify for several spliceosome subunits, including *SF3B1*, *SRSF2* and *U2AF1* have been recently described in myeloid malignancies including myelodysplastic syndromes, chronic myelomonocytic leukemia, essential thrombocythemia (ET) and primary myelofibrosis (PMF). However, there is limited information about the incidence of alterations in this group of genes in patients with ET or with polycythemia vera (PV) who develop myelofibrotic transformation during the evolution of the disease.
Aims: To analyze the incidence of *SF3B1*, *SRSF2*, and *U2AF1* mutations in a cohort of ET and PV patients who transformed to myelofibrosis.
Methods: A total of 62 patients were included in the study: 22 PV and 14 ET patients who transformed to myelofibrosis, and 26 PMF patients .Screening for mutations of *SF3B1* (exons 14 and 15), *SRSF2* (exon 1) and *U2AF1* (exons2,6 and 7) was performed by pyrosequencing with a next generation sequencing (NGS) 454GSJunior platform (Roche), using DNA extracted from purified granulocytes. Mutations were confirmed by Sanger sequencing.
Results: We identified one out of 22 (4.5%) post-PV MF patient who simultaneously harboured a *SRSF2* mutation (p.P95H) and a *SF3B1* mutation (p.I671T), and two out of 14 (14%) post-ET MF patients with the p.K666N *SF3B1* mutation. A diagnostic sample from one of the *SF3B1* positive ET patients 8 years before transformation, showed the presence of the p.K666N mutation by NGS in 2.4% of sequences in contrast to the 45% observed in the sample at the time of transformation, suggesting an expansion of the clone during the evolution of the disease. On the other hand, a diagnostic sample from the PV patient, 2 years before transformation, showed the presence of both *SF3B1* and *SRSF2* mutations in a similar percentage at both time points. Concerning *U2AF1*, no mutations were detected in any of the patients analyzed. Regarding the group of 26 PMF patients, we detected mutations in the *SRSF2* gene in 6/26 (23%) cases. All mutations affected the P95 hotspot (3 p.P95H, 2 p.P95R and 1 p.P95_R103delinsR). *SF3B1* mutations were detected in 2/26 (8%) patients analyzed (2 p.K666N) and *U2AF1* mutations were observed in 4/26 (15%) PMF patients (2 p.Q157P and 2 p.Q157R).
Summary / Conclusion: Pathogenic mutations in *SRSF2* and *SF3B1* genes are present in a low percentage of post-PV and post-ET MF patients, in contrast to a higher incidence of *SRSF2* mutations in PMF patients. *U2AF1* mutations are found only in PMF patients. These findings suggest a different profile of spliceosome gene mutations in primary and secondary myelofibrosis.
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MULTIVARIATE ANALYSIS OF THE ASSOCIATION OF CYTOKINE LEVELS AND REDUCTIONS IN SPLEEN SIZE IN COMFORT-II, A PHASE 3 STUDY COMPARING RUXOLITINIB TO BEST AVAILABLE THERAPY (BAT)

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Background: Ruxolitinib is a potent JAK1/JAK2 inhibitor that has demonstrated rapid and durable improvements in splenomegaly, disease-related symptoms, and quality of life in the two phase 3 COMFORT studies in patients with myelofibrosis (MF). Prolonged survival was observed in patients receiving ruxolitinib compared with both placebo (COMFORT-I) and BAT (COMFORT-II). Recent findings have suggested that cytokine biomarkers may be prognostic in patients with primary MF, and monitoring cytokine levels may prove valuable in predicting and analyzing responses to therapy.
Aims: To evaluate associations between cytokine levels and spleen size reductions in COMFORT-II to determine whether any cytokines are prognostic for changes in spleen size or predictive of decreases in spleen volume with ruxolitinib treatment.
Methods: COMFORT-II is a randomized (2:1), open-label, phase 3 study comparing ruxolitinib with BAT. Spleen volume was measured by MRI every 12 wk and spleen length by palpation at each study visit. Plasma samples were analyzed using Rules-Based Medicine's HumanMAP v1.6; 89 cytokines were measured at baseline and wk4, 24, and 48.
Cytokines with a high proportion (≥ 30%) of values below the lower level of quantification were excluded. The association between the remaining 59 cytokines at baseline and percent reduction in spleen volume from baseline at wk 48 was estimated via a multivariate linear model. The penalized regression Elastic Net method (Zho and Hastie), which combines the penalty terms of the Ridge and Lasso regression, was used to estimate the linear model. Explanatory variables included in the model were baseline cytokines (standardized after a $\log_2$ transformation), treatment arm, and interaction between the treatment and baseline cytokine levels. The tuning parameters for the Elastic Net were selected via 5-fold cross-validation. 10,000 runs of the Elastic Net were performed with different random splits for cross-validation; the most frequent model was selected as the most likely estimated model.
Results: The model identified 10 markers for which the baseline level had a prognostic effect on spleen volume change: alpha-fetoprotein (AFP), beta-2 microglobulin (B2MICG), CD40 ligand, carcinogenicembryonic antigen (CEA), lymphotactin (LTN), myoglobin (MGB), prostatic acid phosphatase (PAP), RANTES, thyroxine-binding globulin (TBG) and vascular cell adhesion molecule-1 (VCAM1). Lower levels of AFP, CEA, LTN, MGB and TBG and higher levels of B2MICG, CD40 ligand, PAP, RANTES and VCAM1 were associated with enhanced spleen volume changes. Lower baseline levels of AFP, eotaxin, insulin and interleukin-18 were identified as potentially predictive of an enhanced effect on spleen volume reduction with ruxolitinib treatment. The association between spleen volume and change in cytokine levels from baseline to wk 4 was also analyzed using similar methodology and will be presented. Additional validation of the above multivariate model on an independent dataset to determine the utility of these cytokines in predicting response as well as investigation into the potential mechanism of these effects are needed.
Summary / Conclusion: This analysis has demonstrated a set of cytokines that are prognostic for spleen volume changes irrespective of treatment and a different set that are potentially predictive of spleen volume change upon ruxolitinib therapy. The impact of baseline cytokine level, although significant, was small in comparison to the effect of ruxolitinib treatment in reducing spleen volume compared with BAT.

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SPLICEOSOME COMPONENT MUTATIONS AND CYTOGENETIC CORRELATES IN WORLD HEALTH ORGANIZATION (WHO)-DEFINED CHRONIC MYELOMONOCYTIC LEUKEMIA

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Background: Chronic myelomonocytic leukemia (CMML) is a clonal, heterogeneous stem cell disorder characterized by overlapping features between myelodysplastic syndrome (MDS) and myeloproliferative neoplasm (MPN). CMML is characterized by persistent monocytosis, myelodysplasia, and an inherent risk for progression to acute myeloid leukemia. Approximately 20-40% of CMML patients have cytogenetic abnormalities on routine karyotype analysis and approximately 90% have molecular aberrations. The prognostic correlation between cytogenetic and molecular abnormalities is not well established.
Aims: We examined the association of karyotype abnormalities using routine cytogenetics with molecular aberrations involving the spliceosome machinery in World Health Organization (WHO)-defined CMML patients seen at our institution from 1997-2007.

Table 1.

Karyotype	N	*SF3B1* N (%)	*SRSF2* N (%)	*U2AF35* N (%)
Diploid ± -Y	160	6 (4)	69 (46)	15 (10)
Complex/Monosomal	15	1 (7)	2 (13)	1 (7)
Trisomy 8	15	1 (7)	9 (60)	0
Monosomy 7	6	0	4 (67)	0
Other	26	4 (16)	6 (17)	4 (11)
Total	**222***	**12 (5)**	**90 (40)**	**20 (8)**

*: karyotype results were not available in 4 patients

Results: Out of 226 patients with CMML seen during this time interval, 153 (67%) were males, the median age was 71 years (range, 17-90 years), and 192 (85%) had CMML-1. Sixty-two patients (28%) had an abnormal karyotype. According to the Spanish cytogenetic risk stratification model, 163 (72%) patients were low-risk, 31 (14%) were intermediate risk and 32 (14%) were high-risk. The various karyotype abnormalities include the following: diploid with or without loss of Y chromosome (± -Y) in 160 (72%) patients, trisomy 8 in 15 (7%), monosomal karyotype in 10 (4%), monosomy 7 in 6 (3%), complex (≥ 3 abnormalities) in 5 (2%), 20q- in 4 (2%), +21 in 4 (2%), translocations involving chromosome 11 in 4 (2%), idic(X)(q13) in 3 (1%),13q- in 2 (1%), i(17)(q10) in 1 patient and other abnormalities in 8 (4%) patients. Karyotype results were not available in 4 patients. The Spanish cytogenetic risk stratification model was prognostic for overall survival (OS) on univariable analysis (P=0.04), however, on multivariable analysis it lost significance (Patnaik, ASH 2012). Ninety patients (40%) had *SRSF2* mutations, 20 (9%) had *U2AF35* mutations, and 12 (6%) had *SF3B1* mutations, all of which were mutually exclusive. The Table shows the association between various cytogenetic abnormalities and mutations involving the spliceosome machinery. A significantly higher proportion of patients harboring *SF3B1* mutations had >15% ringed sideroblasts in their marrow. None of the molecular aberrations in the spliceosome machinery impacted OS or leukemia-free survival (LFS).
Summary / Conclusion: Approximately 30% newly diagnosed CMML patients have an abnormal karyotype. Fifty-five percent patients have molecular abnormalities in the spliceosome machinery, all of which are mutually exclusive. *SRSF2*, *SF3B1* and *U2AF35* mutations do not predict OS or LFS.

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ACQUIRED COPY-NEUTRAL LOSS OF HETEROZIGOSITY OF CHROMOSOME 1P AS A MOLECULAR EVENT ASSOCIATED WITH MARROW FIBROSIS IN MPL MUTATED MYELOPROLIFERATIVE NEOPLASMS

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Background: The unique *JAK2* (V617F) mutation is found in about 60-70% of patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF). Several studies have been conducted to elucidate the molecular mechanisms underlying the remaining 30-40% of patients with *JAK2* (V617F)-negative ET and PMF. A subset of these patients carry activating somatic mutations in exon 10 of the *MPL* gene, located at chromosome 1p and encoding thrombopoietin receptor. Only minor correlations have been found between *MPL* mutations and clinical phenotype. Acquired copy neutral-loss of heterozigosity (CN-LOH) represents a common molecular mechanism of disease in myeloid malignancies. A paradigmatic example is acquired CN-LOH of chromosome 9p, responsible for the transition from heterozygosity to homozygosity for the *JAK2* (V617F) mutation. The clinical effect of acquired CN-LOH of chromosome 1p and *MPL* mutant allele burden has not been investigated systematically in patients with myeloproliferative neoplasms (MPN).
Aims: To evaluate the clinical phenotype of *MPL*-mutated MPN in comparison with *JAK2*-mutated or *JAK2/MPL*-unmutated MPN and to define whether the

MPL allele burden, rather than the mere presence or absence of *MPL* mutations, might have a clinical effect in *MPL* mutated MPN.
Methods: The study design included 2 parts. First we evaluated a cohort of 892 consecutive patients with Ph-negative MPN other than PV (661 ET, 197 PMF, 44 post-ET MF) diagnosed and followed at the Department of Hematology Oncology, Fondazione IRCCS Policlinico S. Matteo Pavia, between 2002 and 2012. We analyzed *JAK2* (V617F) using a qPCR-based allelic discrimination assay and *MPL* exon 10 mutations using a high resolution melt (HRM) assay. Patients who were found to be *MPL*-mutated at HRM were further characterized using direct sequencing. The 3 genotypic subgroups of MPN patients (*JAK2*-mutated, *MPL*-mutated, *JAK2/MPL* unmutated) were compared in terms of phenotype at diagnosis and outcome. Next, in order to define the effect of *MPL* mutant allele burden on clinical phenotype, we analyzed the Pavia cohort of 43 *MPL*-mutated patients together with a second cohort of 19 *MPL*-mutated patients followed at the University of Florence. *MPL* mutant allele burden was assessed using multiplexed 454 GS-FLX ultramassive sequencing. Analysis of 1pLOH was performed in representative patients with mutant allele burden >75% by SNP genotyping and *MPL* copy number analysis.
Results: Somatic mutations of *MPL*, all but one involving codon W515, were detected in 26/661 (4%) patients with ET, 10/187 (5%) with PMF, and 7/44 (16%) patients with post-ET myelofibrosis. Comparison of *JAK2*-mutated and *MPL*-mutated subjects showed only minor phenotypic differences. In the merged group of 62 *MPL*-mutated patients from Pavia and Florence, the granulocyte mutant allele burden ranged from 1 to 95%, and was significantly higher in patients with PMF or post-ET myelofibrosis compared with those with ET. Patients with higher mutation loads had evidence of acquired CN-LOH of chromosome 1p in granulocytes, consistent with a transition from heterozygosity to homozygosity for the *MPL* mutation in clonal cells. A significant association was found between *MPL* mutant allele burden greater than 50% and marrow fibrosis.
Summary / Conclusion: Acquired CN-LOH of chromosome 1 p, involving the *MPL* location, may represent a molecular mechanism of fibrotic transformation in *MPL*-mutated MPN.

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MODULATION OF PLASMA CYTOKINES AND ITS ASSOCIATION WITH CLINICAL RESPONSE TO TREATMENT WITH THE JAK2-SELECTIVE INHIBITOR SAR302503 IN A PHASE 2 STUDY OF PATIENTS WITH MYELOFIBROSIS (MF)
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Background: Abnormal cytokine expression may represent an inflammatory response that contributes to the clinical phenotype of MF. Some of the constitutional symptoms of MF (e.g. fever, fatigue, pruritus, cachexia) are thought to be caused by elevated cytokine levels. We previously reported that patients in a phase 2 study treated with 3 cycles of the JAK2-selective inhibitor SAR302503 at doses of 300, 400, or 500 mg had clinically meaningful reductions in splenomegaly and disease-related symptoms with acceptable toxicity (*Blood* 2012;120:21 Abs 2837; NCT01420770).
Aims: We analyzed the expression of 97 cytokines in patients enrolled in this study to assess their modulation and the relationship between changes in circulating cytokines and clinical response (change in spleen volume), pharmacokinetic (PK) exposure, and body weight changes in patients with MF.
Methods: Thirty-one patients were randomized to receive 300, 400, or 500 mg of SAR302503 orally, once daily, continuously in 4-week cycles. Plasma cytokines were measured at baseline and at the end of4, 8, and 12 weeks of treatment using a microsphere-based immuno-multiplex assay (Rules-Based Medicine Inc). Spleen volume was measured by MRI/CT at baseline and at the end of 12 weeks of treatment (3 cycles). All patients provided written informed consent.
Results: Complete sample sets were available for 29/31 randomized patients. A total of 28 cytokines predominantly involved in immune/inflammation pathways were regulated ≥1.5-fold (ANOVA P<0.05), of which 19 were regulated at all time points, indicating rapid and sustained modulation by JAK2 inhibition. At 4 weeks, 16 cytokines were down-regulated, including TNFα, IL-1RA, and IL-18, and six were up-regulated, including leptin, EPO, and adiponectin. Hierarchical clustering of the 22 regulated cytokines enriched patients into spleen responder (≥35% reduction in spleen volume) and non-responder groups, suggesting a link between cytokine modulation and clinical response. Moderate correlations (P<0.05) with spleen volume reduction at the end of week 12 were seen for a subset of regulated cytokines, including adiponectin and TNFα. Levels of the majority of the regulated cytokines tended to correlate with steady-state PK exposure at week 4. A positive association with weight gain at week 24 was observed for changes in leptin and adiponectin levels at week 4 (P<0.05).
Summary / Conclusion: This analysis shows that SAR302503 treatment modulated the expression of circulating cytokines in MF patients in association with changes in spleen volume, PK exposure, and weight gain.

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MYELOPROLIFERATIVE NEOPLASM QUALITY OF LIFE (MPN-QOL) STUDY GROUP: DUAL SERIAL ASSESSMENT STUDIES FOR IMPACT OF STANDARD THERAPEUTIC APPROACHES IN MPN POPULATIONS (MEASURE AND SYMPTOMS TRIALS)
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Background: Myeloproliferative neoplasms (MPNs), including polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF) are characterized by burdensome symptom profiles and impaired quality of life. Although patient reported outcomes (PROs) have been assessed in clinical trials with new therapies (JAK-2 inhibitors), little is known about the symptomatic impact of non-experimental therapies including pharmacological therapy, phlebotomy and bone marrow transplant.
Aims: The Myeloproliferative Neoplasm Quality of Life (MPN-QOL) International Study Group seeks to serially assess, in a prospective fashion, the impact of non-clinical trial therapies upon MPN-related symptoms and quality of life.
Methods: Utilizing the validated Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF), we began two prospective trials serially assessing the impact of non-clinical trial therapeutic intervention on MPN patients from baseline and post-baseline time point(s) using paired t-test(s). Additional analyses will include correlation between MPN-SAF total symptom score (TSS) and other PROs at each time point, and correlation between changes in MPN-SAF TSS as well as other PROs at each post-baseline time point. Global Impression of Change items will be used to assess responsiveness of the MPN-SAF TSS in each study. Associations between changes in MPN-SAF TSS and clinical endpoints will also be investigated.
Results: These ongoing, multi-national trials began open enrollment in the summer of 2012 and remain in recruitment phase. Updated data will be presented at the EHA conference.
MEASURE (MPN Experimental Assessment of Symptoms by Utilizing Repetitive Evaluation) trial is a prospective study evaluating the responsiveness of the MPN-SAF in detecting symptomatic changes in target symptoms for ET, PV and MF (including primary MF, post-ET and post PV MF) patients receiving non-experimental medical therapy (aspirin, hydroxyurea, anagrelide, interferon, busulfan, melphalan, cladribine, thalidomide, lenalidomide, prednisone, danazol, commercial ruxolitinib and/or phlebotomy). Patients complete the MPN-SAF (24-hour recall) for seven consecutive days at the time of enrollment and repeat the survey between 90 days and six months. Patients also complete the MDASI and EORTC QLQ-C30 on the first day of the both assessments and Global Impression of Change items on the first day of the second assessment only. Physicians acquire demographic, laboratory, physical examination and radiographic data, along with serial response assessments. SYMPTOMS (Symptoms Yielded in Myelofibrosis Patients after Transplant as Objectified by MPN-SAF) trial is a prospective, randomized study evaluating symptomatic responses in MF patients (including primary MF, post-ET and post PV MF) who are potential candidates for allogeneic stem cell transplant. All participants complete the MPN-SAF (7-day recall) and FACT-BMT at pre-transplant, day 30, 100, and year 1. Global Impression of Change items are completed at follow-up time points only. Physicians will acquire demographic information, along with laboratory, physical examination and radiographic data at each follow-up visit.
Summary / Conclusion: Myeloproliferative neoplasms have been associated with burdensome symptom profiles. To date, no studies have quantified symptomatic improvements to standard-of-care treatments. This abstract introduces two MPN-QOL Study Group prospective studies evaluating improvements in MPN symptoms and quality of life via the use of an MPN-specific, validated PRO instrument.

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LONG-TERM EFFICACY AND SAFETY OF CLADRIBINE (2-CDA) IN ADULT PATIENTS WITH MAST CELL DISEASE: A FRENCH MULTICENTER STUDY OF 68 PATIENTS
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Background: Mast cell disease (MCD) is a myeloproliferative disabling disorder without consensual curative therapy available. 2-CdA, is a synthetic purine analogue that has been used for MCD treatment as a cytoreductive drug for aggressive variants (ASM). Few patients (pts) with indolent (ISM), smouldering systemic (SSM) variants or cutaneous mastocytosis (CM) with mediator related symptoms refractory to symptomatic treatments have been treated so far. In addition, long term follow-up evaluation with this treatment is lacking.
Aims: We conducted a retrospective multicenter study to evaluate the efficacy and safety of 2-CdA in 68 patients with different subtypes of MCD.
Methods: From 2001 to 2010, 68 patients with MCD diagnosis according to WHO criteria were treated by 2-CdA. Patients had ISM (n=28, 41%), SSM (n=2, 3%), ASM (n=14, 21%), mast cell leukemia (MCL=1, 1%), CM (n=6, 9%) SM-AHNMD (n=17, 25%). Mediators release and mast cells infiltration symptoms and biological data (serum tryptase level, complete blood cell count, liver enzymes, serum alkaline phosphatise) were recorded at baseline, after each cycle and two months after the last cycle. A cycle consisted of 2-CdA delivering (0.14 mg/kg/d infusion (Leustatine®) or subcutaneously (Lytak®) from 1 to 5 days. Each cycle was repeated at 4 to 12 weeks intervals from 1 to 15 consecutive cycles which defined a block of treatment (55 pts). 2-CdA cycles with a time interval >9 months were considered as a second block (13 pts). Efficacy was evaluated with the consensus statement for response criteria and notified as complete (CR), major (MR), partial (PR) and no (NR) responses and overall response rate (ORR). Safety of 2-CDA was recorded with acute serious adverse event (SAE) during 2-CdA and late SAE > 3 months after last dose. The severity of neutropenia and lymphopenia was scored (CTCAE V3). Analysis was done by Mc Nemar's chi-squared testing between baseline and end of 2-CdA.
Results: 35 F/33 M with respectively mean age at MCD diagnosis and at 2-CdA treatment of 46 and 54 yrs were included. Clinical mediator release symptoms were improved significantly including fatigue, flush, pruritus, diarrhea, abdominal pain, neuropsychiatric symptoms, headache/pain, and nausea/vomiting (P<0.0001). Mast cell infiltration symptoms improved including urticaria pigmentosa, hepatomegaly, splenomegaly, weight loss/fever/chills/ night sweats and ascitis (P<0.0001). Biological parameters did not improve except for tryptase level (P=0.01). Mean 2-CdA number of cycles was 4.2 and the total dose received averaged 2.34 mg/kg. The ORR was 72% (49 out of 68 pts). The best response rate was observed in indolent subtypes (P<0.0001) (Figure 1). Median time of follow-up was 5.8 years (21d-9 yrs). Disease free survival was 4.5 years. Time to progression averaged 30.5 months (9 to 108). Twenty one patients (31%) died but any case was related to 2-CdA treatment. Causes included MCD progression for 9 pts (43%), other causes for 12 pts (57%). Most SAE were infectious (22%) with 8 recovering opportunistic infections and linked to myelosuppression. Any ISM died of SAE related to 2-CdA. Patients developed neutropenia grade 3-4 (47%) and chronicle lymphopenia (82%). 3 F gave birth after treatment.
Summary / Conclusion: 2-CdA is an effective and safe treatment in symptomatic MCD, which may improve mast cell burden. Its long-term safety and efficacy argue for a possible use in symptomatic CM or ISM with disabilities. Further work is warranted to define optimal regimen and usefulness of maintenance 2-CdA therapy.

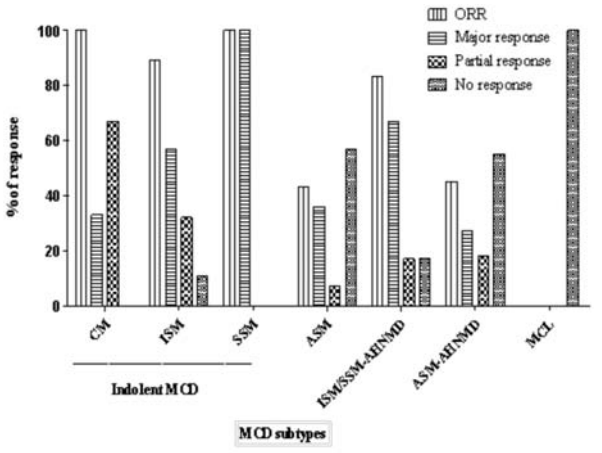

Figure 1.

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CIRCULATING YKL-40 IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET) AND POLYCYTHEMIA VERA (PV) TREATED WITH THE NOVEL HISTONE DEACETYLASE INHIBITOR VORINOSTAT

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Background: Transformations from ET to PV, and from both of these entities, to myelofibrosis are not uncommon. These observations have challenged the conception of the conditions as distinct disease entities. Evidence of a biological continuum has been presented by demonstration of increasing *JAK2*V617F allele burden in blood leukocytes from ET over PV to PMF (Vanucchi *et al*, Leukemia 2006, Larsen *et al*, Eur J Haematol 2007) and evidence has also been presented by the use of gene expression patterns. (Skov *et al*, Exp Hematol 2012). YKL-40 (Chitinase-3-like protein 1) has been implicated in haematological malignancies. Elevated serum levels of YKL-40 have been shown to correlate with disease activity and impaired overall survival in acute myelogenous leukemia (Bergmann *et al*, Clin Can Res 2005) and multiple myeloma (Mylin *et al*, Int J Cancer 2009) The role of YKL-40 in MPNs has never been established.
A multicenter study on the efficacy and safety of vorinostat in ET and PV has most recently been presented, confirming that vorinostat reduces elevated cell counts in a substantial proportion of ET and PV patients in concert with a decrease in spleen size. (Andersen *et al*, ASH 2012)
Aims: Firstly, to investigate YKL-40 in ET and PV as a potential marker of disease burden. Secondly, to investigate circulating YKL-40 in patients during treatment with vorinostat from the recently presented multicenter study.
Methods: From the non-randomized, open-label phase II multicenter study, YKL-40 serum samples were analyzed from 31 PV and 16 ET patients at baseline and again after 3 months of therapy along with quantitative *JAK2* analyses and other routine laboratory parameters. The reference interval for plasma YKL-40 was determined in 3130 healthy subjects (1293 men, 1837 women) aged 21-84 years from the Danish general population.
Results: When adjusting for age YKL-40 baseline values were 1.8 times higher in ET patients than expected from healthy controls (P<0.0001) and PV YKL-40 values were 1.8 times higher than in ET (P=0.02). We observed a significant correlation between YKL-40 at baseline and the level of CRP (rho=0.43, P=0.03), LDH (rho=0.49, P=0.009) and *JAK2* mutant allele burden (rho=0.43, P=0.02) in PV patients. PV patients who experienced a clinicohematological response after 3 months of therapy exhibited a significantly greater reduction of YKL-40 levels compared to non-responders. We did not observe this correlation among ET patients (P=0.5); however, we could only compare 16 patients. Changes in YKL-40 during treatment did not correlate with changes in any of the measured laboratory markers in PV patients, but correlated with platelets (rho=0.76, P=0.003) and leukocytes (rho=0.74, P=0.009) in ET patients.
Summary / Conclusion: We show for the first time that circulating YKL-40 levels are increased in ET and PV patients depicting a continuum between the entities and furthermore correlate significantly with important markers of tumor burden – the leukocyte count, the LDH-value and the *JAK2*V617F-allele burden. The YKL-40 levels also showed a significant correlation with the CRP-concentration, which might reflect YKL-40 to be a novel marker of ongoing inflammation in MPNs. Furthermore; during HDACi-treatment we have shown that YKL-40 to correlate significantly with the clinicohematological response in PV patients. Our observations call for experimental and clinical studies to unravel the cells of origin of YKL-40 in MPN-patients, the importance in disease pathogenesis and progression from early disease stage (ET, PV) to the advanced (MF).

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IMPACT OF HEMATOCRIT ON SYMPTOM BURDEN AMONG POLYCYTHEMIA VERA PATIENTS

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Background: Current guidelines suggest that polycythemia vera patients maintain a strict hematocrit (hct) less than 45%. The recent CYTO-PV trial (N Engl J Med 2013;368:22-33) found that patients undergoing strict hct control had significantly decreased mortality from cardiovascular death and thrombosis, but suffered from overall increased symptom burden. To date, little is known about the relationship between hemocrit control and PV patient symptom burden.
Aims: The purpose of this comparison study is to evaluate the impact hct level on PV associated symptoms and quality of life.
Methods: PV patient data was analyzed from two large prospective investigations including the CYTO PV trial and the MPN-SAF study (JCO 2012; 30(33):4098-4103). The CYTO PV trial was a randomized, clinical trial assessing the impact of traditional (hct maintained at less than 45%) versus experimental (hct maintained at 45% to 50%) hct control. The MPN-SAF study was an international assessment of MPN symptom burden at the time of an office visit. Surrogate hct was calculated from MPN-SAF data as three times hemoglobin. Quality of life assessments included the MPN-SAF and BFI (Cancer 1999;85(5)1186-1196).
Results: Patients and Baseline Symptom Burden: Symptomatic burden was compared between 224 CYTO PV trial patients and 561 MPN-SAF PV patients. Mean age (CYTO-PV 64.7 years, MPN-SAF 63.0), gender (CYTO PV 42% female, MPN-SAF 48% female) and distribution of prognostic scoring were similar between the two studies. Among the MPN-SAF cohort, 299 PV patients had hct up to 45%, 89 had hct greater than 45 to 50%, and 23 with hct greater than 50% (150 no data). Hct Control and Symptom Burden: For both cohorts, end-organ complaints including headache (MPN-SAF 2.1(65%) vs 1.7(54%), CYTO PV 6mo change +0.4 vs +0.0) and cough (MPN-SAF 1.6(48%) vs 1.3(46%), CYTO PV 6mo change +0.3 vs +0.1) were more severe in those with hct greater than 45%. Conversely, strict hct control was associated with increased concentration problems (MPN-SAF 2.4(63%) vs 2.1(59%), CYTO PV 6mo change +0.6 vs +0.4) and insomnia (MPN-SAF 3.0(68%) vs 2.8(66%), CYTO PV 6mo change +0.5 vs +0.2). Strict hct control was also associated with increased weight loss (MPN-SAF 1.1(34%) vs 1.0(32%), CYTO PV +0.2 vs -0.3) and night sweats (MPN-SAF 2.1(53%) vs 1.8(49%), CYTO PV 6mo change +0.4 vs 0.0). Pruritus was most severe and frequent among individuals with stricter hct control for MPN-SAF (2.7(61%) vs 2.3(56%)). HCT Control and Fatigue: When comparing MPN-SAF patients, patients with lower hct had significantly higher rate of fatigue (89% vs 81%, P=0.04). Similarly, analysis of CYTO PV patients undergoing six months of strict hct control had increased worst fatigue (+0.4 vs -0.2, P=0.08) and mean BFI (+0.2 vs -0.3, P=0.04) at 6 months.
Summary / Conclusion: This study assesses the impact of hemodynamic management among two of the largest prospective evaluations of PV symptom burden to date. Iron deficiency likely contributes to cognitive and fatigue complaints seen among patients with strict hct control. Conversely, vascular complications may contribute to the end organ complaints seen in those with lenient hct control. Future studies are needed to further delineate differences in the means of hct control (medicinal vs. phlebotomy) on PV associated symptom burden.

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WHITE BLOOD CELLS COUNT INFLUENCE THE PROGNOSIS OF POLYCYTHEMIA VERA PATIENTS: A SUB-ANALYSIS OF THE CYTO-PV STUDY.
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Background: In Cyto-PV randomized clinical trial (Marchioli *et al*, NEJM 2013), polycythemia vera (PV) patients with a hematocrit (HCT) target of less than 45% had a significantly 4 times lower rate of cardiovascular death and major thrombosis than did those with a hematocrit target of 45 to 50%. Moreover, patients in the high-hematocrit group had significantly higher leukocyte (WBC) counts than did those in the low-hematocrit group, likely due to lower dose of Hydroxyurea prescription.
Aims: To assess whether the persistence of leukocytosis in this trial could have contributed to an excess of fatal and nonfatal cardio vascular events we carried out the present analysis.
Methods: Multivariable time-dependent analysis, adjusted for age, gender, previous thrombosis and hematocrit levels was performed to assess whether the level of exposition to WBC count, recorded in the last clinical visit before the CV event, was associated with the probability of having CV events during follow-up, with censoring at first event, death, or last follow-up visit. WBC count was categorized into four groups and tests for trend were calculated by assigning the median value of each of the 4 WBC categories.
Results: During the study period (median 31 months) of 365 PV patients randomized to different intensity of cytoreductive treatments, the median hematocrit level in the low-hematocrit group was 44.4%, as compared with 47.5% in the high hematocrit group. The WBC count remained significantly higher in the high hematocrit group than in the low-hematocrit group (P<0.001) while no significant difference between groups was noted in the platelet count. Interestingly the risk of thrombosis was clearly increased in patients with a white blood cell count above 6 10^9/L, becoming statistically significant when the white blood cell count was above 12x10^9/L (hazard ratio [HR], 4.89; 95% confidence interval [CI], 1.1-22.7; *P* =0.04).
Summary / Conclusion: Keeping HCT levels >45% and leukocyte count > 12x10^9/L, the risk of fatal and nonfatal cardiovascular events is significantly increased suggesting a role for leukocyte count as well as hematocrit in the pathogenesis of these events. Future clinical studies are needed to evaluate the benefit-risk profile of more aggressive therapy to target the WBC counts.

Table 1. Time-dependent multivariable analysis on the risk of major thrombosis in CYTO-PV study (N=365).

WBC class (x10^9/l)	Events/Pts (%)	Hazard ratio (95% CI), P-value
< 6.0	2/54 (3.7)	1 (Reference)
6.0-10	13/187 (7.0)	2.39 (0.5-10.8), 0.26
10-12	2/50 (4.0)	1.40 (0.2-10.1), 0.74
> 12	11/74 (14.9)	4.89 (1.1-22.7), 0.04

*Model adjusted for: age, gender, previous thrombosis, leukocyte count class, and HCT.

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SAFETY OVERVIEW OF PHASE I-II STUDIES OF PACRITINIB, A NON-MYELOSUPPRESSIVE JAK2/FLT3 INHIBITOR, IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES
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Background: In phase I and II clinical trials, a total of 191 patients (pts) with hematologic malignancies have been treated with pacritinib (SB1518), an oral, once-daily JAK-2/FLT-3 inhibitor. JAK2 inhibition has been associated with anemia and thrombocytopenia, possibly due to inhibition of Epo and TPO signaling, but this was not observed in pacritinib preclinical studies.
Aims: This integrated analysis was performed to quantitate clinical toxicities, including hematologic effects, of pacritinib.
Methods: We reviewed the safety database which included 4 clinical studies: a phase I/II study in advanced myeloid malignancies, a phase I and a phase II study in advanced lymphoid malignancies, and a phase I/II study in myelofibrosis.

Table 1.

Safety population (n=189) (> 1 post-baseline value)	Hb shift from baseline to last value	Platelet shift from baseline to last- value
No shift	101 (53.4%)	108 (57.1%)
Cytopenia Improvement		
1 grade improvement	28 (14.8%)	17 (9%)
2-4 grade improvement	6 (3.2%)	2 (1.1%)
Cytopenia Decline		
1 -2 grade decline	51 (27%)	52 (27.5%)
3-4 grade decline	1 (0.5%)	8 (4.2%)

Results: 191 pts were treated with pacritinib: 129 with advanced myeloid malignancies including 122 myelofibrosis pts [primary 72/122 (59%)] and 7 AML pts; and 62 with advanced lymphoid malignancies including 38 NHL pts and 24 Hodgkin lymphoma pts. The median age was 65 years and the median time from initial diagnosis was 3.8 years. 44% of pts with myeloid disorders had baseline platelet counts <100,000/μL. Pacritinib was dosed from 100 to 600 mg daily during phase I and 400 mg during phase II. The median treatment duration was 306 (range 2- 1210) days for those with myeloid disorders and 910.5 (range 1 – 631) days for lymphoid disorders. 59 pts (31%) stayed on treatment for ≥12 months (including 2 pts for 40 and 36 months, respectively) and another 33 pts (17%) for ≥6 months. The median dose delivered was 98% of intended. The most common adverse events (AEs) were gastrointestinal (GI) (all grades/grade 3-4): diarrhea (73%/8%), nausea (48%/1%), vomiting (30%/1%), constipation (24%/0%) and abdominal pain (21%/4%). Time to onset of diarrhea was ≤30 days in 89% of those affected and was the major cause of dose reduction, interruption, or discontinuation. Anti-motility prophylaxis was not used rou-

tinely in these early studies. Hematological AEs are summarized in the Table 1. Most pts had no decline in hemoglobin or platelet count. Of the 30 myeloid disorder patients with baseline platelet counts <50,000/μL from phase I and II studies, the median decline in platelet count observed at the end of study was3,000/μL. In the 11 patients with myelofibrosis with baseline platelet counts <50,000/μL enrolled in phase II studies, no dose reductions were required for thrombocytopenia. There were no late toxicities noted in the 2 pts treated beyond 3 years. 22 deaths (12%) occurred within 30 days from the last dose of study drug. All but one pt died from a variety of causes not associated with drug related toxicities.

Summary / Conclusion: Pacritinib does not cause suppression of red cell or platelet production in pts with myeloid or lymphoid disorders. Even pts with initial platelet counts <50,000/μL tolerated therapy, maintained stable blood and platelet counts and did not require dose reductions. Grade 1 or 2 GI events, particularly diarrhea, were the most common AEs and were usually controlled with early administration of standard anti-motility agents. Due to the absence of myelosuppression in these studies, ongoing phase III development of pacritinib in myelofibrosis does not restrict study entry due to thrombocytopenia. The lack of bone marrow suppression also suggests that pacritinib could be used in combination with marrow suppressive therapies in pts with JAK2 or FLT3 dependent disorders.

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A PHASE II STUDY OF VORINOSTAT (MK-0683) IN PATIENTS WITH PRIMARY MYELOFIBROSIS (PMF) AND POST-POLYCYTHAEMIA VERA MYELOFIBROSIS (PPV-MF)

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Background: Elevated histone deacetylase (HDAC) levels have been reported in patients (pts.) with PMF and have been shown to correlate to the degree of splenomegaly, suggesting that HDAC may be recruited as ET or PV progress into myelofibrosis or PMF progresses into a more advanced stage. A recent report has documented clinical efficacy of HDAC inhibition (HDACi) in MF pts.

Aims: Primarily to investigate if the HDACi vorinostat as monotherapy in pts. with PMF or PPV-MF could induce a clinical response per the International Working Group response criteria at the end of an intervention (6 months) and observation period (3 months), respectively. Secondly, to investigate whether treatment influenced the *JAK2* mutant allele burden and quality of life as assessed by the Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF) and the EORTC QLQ-C30.

Methods: Fourteen pts. (11 PMF, 3 PPV-MF) from whom informed consent was obtained from Denmark were included and given 400mg of Vorinostat daily.

Results: We report data for 14 pts. (m:f=79%/21%) with a median age of 65 years (52-80). Seventy-one percent were *JAK2*V617F-postive with a median allele burden of 68 % (0-99). Median time from diagnosis to inclusion was 6.4 years (0-22.5). Treatment prior to vorinostat included hydroxyurea (57%), interferon –alpha (21%) and anagrelide (7%). No patient had received experimental therapies or JAK-inhibitors.

Of 9 pts. (64%) evaluable for response assessment after 6 months of therapy, 1 achieved complete remission and 1 achieved clinical improvement. An intention-to-treat response rate of 14% was identified. We were able to compare spleen sizes to baseline values for four pts. In 4/4 we observed a decrease (median =4,5 cm (1-14)). 7/10 *JAK2*V617F-pts. (70%) experienced an increase in *JAK2* tumor allele burden between baseline and 3 months of therapy; (P=0.04). The numerical median increase was 3.2%. We did not observe any significant differences in MPN-SAF total symptom scores, EORTC QLQC30 functional – or symptom scales combined, but could only compare 7 pts. Six pts. (43%) were evaluable after the observation period. Of pts. only having been treated with vorinostat in the intervention period we did not observe any lasting responses after the 3 months without vorinostat. One pt. recommenced vorinostat in the observational period with an ensuing "stable disease"-response.

Seventy-one percent of pts. reported fatigue at least once during the intervention period (grade 2 max) and 50% reported diarrhoea (grade 2 max). Only one pt. was not dose-reduced during the study. Causes of discontinuation included "progressive disease" (3 pts.), missing compliance (1 pt.), transformation to AML (1 pt.) and death caused by pneumonia (1 pt.). One patient discontinued due to a combination of hair loss, fatigue, renal insufficiency and hyperglycemia. One pt. discontinued due to fatigue, dry mouth and progressive splenomegaly.

Summary / Conclusion: Despite that large spleens partially regressed in a proportion of the patients, vorinostat monotherapy 400 mg. daily was associated with significant side effects, which limited long-term treatment. Future studies on the role of HDAC-inhibitor treatment in myelofibrosis should use a lower dosage design, which might allow for longer treatment. Gene expression profiling and epigenome studies are ongoing in an attempt to elucidate particular patterns of deregulation which might contribute to a better understanding of the pathogenetic mechanisms accounting for the observed clinical and biochemical effects of vorinostat in our patient cohort.

P280

CLINICAL FEATURES OF ESSENTIAL THROMBOCYTHEMIA WITH SPLEEN ENLARGEMENT: THE EXPERIENCE OF LAZIAL GROUP FOR THE STUDY OF SMPC, PH-.

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Background: At diagnosis, about 15-20% of patients with Essential Thrombocythemia (ET) have spleen enlargement. The role and the clinical characteristics of these patients are still unknown

Aims: In the present retrospective analysis, we report data from 1,097 patients with ET diagnosed from January 1979 to December 2010 in 11 haematological Centers (5 universitary Institutes and 6 community-based Hospitals) that were collected in the database of our lazial cooperative group.

Methods: The diagnosis was made according to PVSG criteria or WHO 2001 and 2008 criteria, based on the date in which the patients were observed. Spleen enlargement was defined as the presence at diagnosis of a palpable spleen under costal margin or a longitudinal splenic diameter > 12,5 cm at the ultrasound examination. On the whole, 213/1097 patients (19.4%) had a spleen enlargement (sET), while 884/1097 (80.6%) had a normal spleen size (oET). Clinical characteristics of the two groups are reported in the table.

Results: From these data, 2 different types of patients seem to be defined as to gender, WBC, PLTs, rate of positivity and median allelic burden of JAK-2 V617F mutation. Patients without spleen enlargement had features similar to "true-ET" (female prevalence, lower WBC and PLTs count, lower rate of positivity of JAK-2 V617F mutation with lower allelic burden); patients with spleen enlargement were more similar to "early MF" (M/F ratio 1/1, higher WBC and PLTs count, higher rate of positivity of JAK-2 V617F mutation with higher allelic burden). Patients with spleen enlargement had a significantly shorter Thrombosis-Free Survival (P= 0.007) while the Overall Survival was similar in both groups (P= 0.11). At multivariate analysis, spleen enlargement retained its value as independent additional risk factor for thrombosis. In conclusion, the evidence of spleen enlargement at diagnosis in ET patients seems to be associated with a different pattern of disease and should be considered in the evaluation of thrombotic risk.

Summary / Conclusion: The role of spleen enlargement as discriminative feature between "true ET" and "early MF" and its comparison/association with bone marrow biopsy warrant further prospective studies.

Table 1.

Feature	No spleen enlargement (oET)	Spleen enlargement (sET)	P value (95%CI)
N° patients	884	213	--
Median age (yrs) (IQR)	59.7 (47,7 – 72,3)	58.2 (44,4 – 72,4)	0.23
M/F (%)	294/590 (33.3/66.7)	105/108 (49.3/50.7)	0.04
Median Hb (g/dl) (IQR)	13.9 (13,0 – 15,0)	14.0 (12,9 – 15,2)	0.45
Median WBC (x10⁹/l) (Interquartile range)	9.3 (7,1 – 10,8)	9.8 (7,6 – 11,3)	0.0385 (30.3-110.83)
Median PLTs (x10⁹/l) (IQR)	879 (659 – 1000)	933 (699 – 1096)	0.04 (2.28-107.52)
JAK-2 V617F (+/evaluated)	235/566	79/129	0.02
Median allele burden (%) (IQR)	24.3 (8,0 – 37,4)	33.5 (13,8 – 44,2)	0.016 (1.73-16.67)

Non-Hodgkin Lymphoma - Biology

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BMI1, THE POLYCOMB-GROUP GENE, IS RECURRENTLY REARRANGED IN PROGRESSIVE/TRANSFORMED CLL AND MCL

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Background: Chronic lymphocytic leukemia (CLL) has a variable clinical course, ranging from a very indolent to a rapidly progressing disease. In up to 10% of CLL patients, transformation to highly aggressive and usually fatal lymphoma (Richter syndrome) has been reported. Although several factors predisposing CLL to a high grade transformation and/or chemoresistance have been recently identified (e.g. mutations of *NOTCH1, SF3B1* and *BIRC3*), molecular events associated with Richter transformation remain largely unknown. However, accumulating evidence indicate that this process may be conducted by several oncogenes, including *MYC, BCL3* and *NOTCH1*, activated by *IG*-related translocations acquired during clinical course of disease.
Aims: Our study aimed at molecular characterization of the novel t(10;14)(p12;q32)/t(10;22)(p12;q11) identified in six cases of progressive/ transformed CLL, and various 10p11p13 rearrangements recurrently observed in mantle cell lymphoma (MCL).
Methods: The work was performed using FISH, qRT-PCR, IHC, high-resolution array CGH (aCGH), Sanger sequencing and RNA-sequencing.
Results: (i) Six cases of progressive/transformed CLL with the *IGH*- or *IGK*-involving translocations targeting 10p12 were collected. Molecular profiles of these leukemias were heterogeneous (mutated or unmutated VH, presence of either del(11q) or del(13q14), or trisomy 12) and the leukemia cells were negative for common mutations of *NOTCH1* and *TP53*. All patients died within 1-37 months after detection of t(10;14)/t(10;22). The extensive BAC-walking FISH analysis eventually mapped the 10p12 breakpoint in the region harbouring *BMI1* gene. Upregulation of *BMI1* mRNA was demonstrated by QRT-PCR analysis in one case documented by paired diagnostic/follow-up samples. (ii) 16 MCL cases with various 10p11-13 aberrations were initially subjected to FISH and aCGH analysis. All the rearrangements affected *BMI1* which was either duplicated/amplified (4 cases), or involved in non-reciprocal translocations (9 cases), or affected by balanced translocations/insertions (3 cases). RNA-sequencing performed in three available cases did not identify *BMI1*-related fusions/mutations, but demonstrated a significant upregulation of *BMI1*, in addition to overexpressed cyclin D1 and *SOX11*.
Summary / Conclusion: We show for the first time that *BMI1*, the Polycomb group gene and postulated lymphoma-related oncogene, is recurrently targeted by chromosomal aberrations in two human B-cell malignancies, CLL and MCL. In the former neoplasm, *BMI1* was constantly affected by *IG*-mediated translocations exclusively detected at time of CLL progression or Richter transformation. MCL displayed a much broader spectrum of *BMI1* rearrangements of which the most frequent were unbalanced (non-*IG*) translocations. These aberrations were associated with upregulation of *BMI1* by mechanisms which remain elusive. Of note, the previously identified amplification of *BMI1* occurred in only 40% of MCL cases with the 10p12/*BMI1* rearrangements.
Collectively, our work identified *BMI1* as a new player implicated in progression and high grade transformation of CLL and confirmed its involvement in the pathogenesis of MCL. Although many solid tumors and haematological malignancies display an aberrant expression of BMI1, the underlying 10p12 chromosomal rearrangements (except of gain/amplification) have never been reported in human cancers.

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MASSIVE IMMUNOGLOBULIN REPERTOIRE BIAS IN PRIMARY INTRAOCULAR LYMPHOMAS SUGGESTS ANTIGENIC SELECTION OF THE NEOPLASTIC CELLS DURING LYMPHOMAGENESIS.

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Background: Primary intraocular lymphoma (PIOL) is a high grade lymphoma, which affects the retina,vitreous and/or the optic nerve. The vast majority of cases are classified as diffuse large B cell lymphoma and considered as a subtype of primary central nervous system lymphoma.
Aims: To gain insight of the unusual localisation of these lymphoma, we analysed the immunoglobulin (IG) repertoire of 55 cases of PIOL, the largest series to date.
Methods: Monoclonal IG heavy chain rearrangements, and for a fraction of cases also light chain rearrangements, were sequenced from PCR products obtained by amplification using either framework 1 or peptide leader primers. In a few cases sequencing was performed after subcloning of PCR products generated with high fidelity Taq polymerase. Comparison with germline sequences was done using IMGT data and tools.
Results: We observed a highly restricted IGHV usage, with a single gene, namely IGHV4-34, present in 69.1% (38/55) of cases. The second most frequent gene was IGHV3-7, utilized in 7.3% (4/55) of cases. Thus, only two IGHV genes accounted for more than three quarters of the PIOL IGHV repertoire. Bias in IGHD and IGHJ genes were also observed, although to a lesser extent, with an underrepresentation of IGHD6 subgroup genes and a high IGHJ5 / IGHJ6 ratio as compared to other B-cell malignancies. Heavy complementarity-determining region 3 (VH CDR3) were short (median length 14 amino acids) and in half of cases (28/55) carried electropositive residues with predicted isoelectric values of 7.0 or greater (up to 13.0). Remarkably, 3 of the 4 cases expressing the IGHV3-7 gene had 11 aminoacid-long, electropositive VH CDR3s with shared motifs, which could be considered as "stereotyped" antigen-binding sites. Except for two unmutated cases, all sequences had a high number of somatic hypermutations (SHM) as the mean % of identity from their germline counterpart was 86.7%. Analysis of the distribution of SHM in the subgroup of PIOL cases utilizing the IGHV4-34 gene revealed high replacement (R) to silent (S) mutation ratios in CDRs (R/S>3.0) along with low R/S ratios in FRs. Identical replacement mutations ("stereotyped" amino acid changes) at certain codon positions were identified amongst rearrangements utilizing the IGHV4-34 gene, some of which were distinct from those previously reported for IGHV4-34 rearrangements in other B cell malignancies. In addition, the IGVH4-34 specific motif responsible for binding in superantigenic fashion the N-acetyllactosamine antigenic determinant was altered in a minority of sequences (6/38,15.8%); however, the most critical residue of this motif (TRP at position 7 in FR1) was intact in all 38 cases. Sequencing of multiple (at least 20) clones in 9 cases of PIOL (including 6 IGHV4-34 cases) showed intraclonal diversity in all of them. Finally the repertoire of IG light chains was also biased for IGHV4-34 PIOL as 7/23 cases (30.4%) had a IGKV3-20 / IGKJ1 rearrangement. Identical amino-acid replacements resulting from SHM were also observed among these sequences.
Summary / Conclusion: PIOL display a highly biased IG gene repertoire with very precise targeting and distinctive features of SHM, suggestive of selection by specific (super)antigen(s) in lymphomagenesis.

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JUNCTIONAL ADHESION MOLECULE C CONTROLS PROLIFERATION, HOMING AND ENGRAFTMENT OF NORMAL AND MALIGNANT HUMAN B CELLS

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Background: The junctional adhesion molecules (JAMs) are a subgroup of the Immunoglobulin superfamily. JAM members localize at endothelial tight junctions and have been involved in the formation and maintenance of inter-endothelial junctions and in leukocyte transmigration. Earlier studies have also demonstrated that tight junction molecules can regulate cell polarity and vascular permeability, as well as cell proliferation and differentiation. JAM-C is also expressed in human B cells and its expression is tightly controlled during differentiation. Expression on malignant B cells was found to be disease-specific, allowing the classification into JAM-Cpos and JAM-Cneg B-cell lymphomas.
Aims: In the current study, we investigated the role of JAM-C in the proliferation, homing and engraftment of normal and malignant B cells.
Methods: Human B cells were isolated from peripheral blood of healthy donors and lymphoma patients. To analyze the role of JAM-C in proliferation, cells were activated and cultured in the presence of anti-JAM-C antibodies, and proliferation was measured by flow cytometry. The signaling pathways induced by binding of JAM-C antibodies were monitored at a single cell level. Using phospho-specific antibodies for p38, Erk 1/2, JNK (Mitogen-activated protein kinases, MAPK), Stat3 (Signal transducer and activator of transcription), and for Akt (PI3K/AKT/mTOR cell survival pathway), phosphorylation profiles for each protein were analyzed by phospho-specific flow cytometry. To investigate the role of JAM-C in B cell migration, B cells were incubated with six different anti-JAM-C antibodies and injected i.v. into NOD/SCID mice. Homing of cells to lymphoid organs (bone marrow, spleen, lymph nodes) was analyzed one hour later by flow cytometry. To investigate the role of JAM-C in lymphoma dissemination, the JAM-C positive mantle cell lymphoma B-cell line Jeko-1 was used for long-term engraftment assays. Jeko-1 cells were injected into NOD/SCID mice and animals were treated for three weeks with anti-JAM-C antibodies. Tumor burden was evaluated in lymphoid organs on day 26.
Results: Incubation of normal and malignant JAM-Cpos B cells with anti-JAM-

C antibodies significantly reduced proliferation by 30-35%. Moreover, the binding of anti-JAM-C antibodies inhibited the phosphorylation of ERK1/2 by 35%, without affecting other signaling pathways. Treatment with 2 out of 6 monoclonal anti-JAM-C antibodies reduced the homing of normal and JAM-Cpos lymphoma B cells to lymph nodes (50%), bone marrow (30%) and spleen (65%). These two antibodies recognize different epitopes on the JAM-C molecule, as demonstrated by competitive binding and Plasmon resonance assays. Long-term administration of the most efficient anti-JAM-C antibody reduced drastically the engraftment of JAM-Cpos Jeko-1 cells in the bone marrow (94%), spleen (100%) and lymph nodes (99%) of NOD/SCID mice.
Summary / Conclusion: Despite considerable progress in the treatment of mature B-cell lymphomas, aggressive forms still remain incurable and new treatment strategies are needed. Our results demonstrate a functional role of JAM-C in B cell homing, proliferation and engraftment into lymphoid organs. We also identified for the first time the intracellular MAPK cascade as the JAM-C driven signaling pathway in JAM-Cpos B cells. Anti-JAM-C antibodies could thus represent an efficient therapeutic approach reducing cell proliferation and preventing lymphoma B cells from reaching supportive lymphoid microenvironments in bone marrow, lymph nodes and spleen.

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DETECTION OF PERIPHERAL BLOOD MONOCLONAL T LYMPHOCYTOSIS AND RISK OF T LYMPHOPROLIFERATIVE DISEASE DEVELOPMENT
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Background: Occasional evidence of monoclonal gammopathy of undetermined significance (MGUS) or monoclonal B lymphocytosis (MBL) are considered predisposing to the development of multiple myeloma and chronic lymphocytic leukemia (CLL) respectively and it is recommended to monitor them for possible early diagnosis. In cutaneous T lymphoma (CTL), such as mycosis fungoides and Sezary syndrome, has been demonstrated the presence of clonal T lymphocytes even in the peripheral blood.
Aims: It seems possible that the detection of asymptomatic clonal proliferations lymphocytosis attributable to CTL may represent a predisposing condition to the development of cutaneous lymphomas.
Methods: Every year we analyze by flow cytometry about 1700 samples. Patients with peripheral lymphocytosis undergo a standard panel for T, B and NK cells. If clonal B is excluded, we proceed to phenotypic T CD4 subsets (CD7, CD26), analysis of Vβ chains and TCR when necessary.
Results: From January 2010 to September 2012 we observed 28 cases of CD3 T> 80% of total lymphocytes (CD3 ≥ 3.000/mm^3). Lymphocytosis was variable between3,000 and 10.000/mm^3, with an average of5,350. 14 subjects with CD4+CD8- > 75% of T cells showed CD7 low/neg, CD26 low/neg- and TCR Vβ suggestive of monoclonality, then confirmed by molecular biology. These subjects were followed up with blood count and clinical / dermatological evaluation twice a year in the event of increased peripheral lymphocytosis or appearance of symptoms suggestive of lymphoma. Two patients developed erythematous skin lesions and skin biopsy diagnosed the presence of Sezary syndrome, also confirmed on lymph node and bone marrow biopsies. Both these patients belonged to the subgroup with CD3 + CD4 + CD7-/ + CD8-CD26- phenotype, typical of Sezary syndrome.
Summary / Conclusion: 7.1% (2/28) of patients with monoclonal T lymphocytosis have developed a Sezary syndrome in a median follow-up of 20 months. Based on these preliminary data the occasional finding of T lymphocytosis with "atypical" phenotype CD3 + CD4 + CD7-/ + CD26-, may be a useful tool in order to allow early diagnosis of CTL in analogy with the strategy currently used for patients with MBL. If the hypothesis will be confirmed in larger cohort this approach could represent an advantage in terms of early diagnosis and timely treatment in this subset of patients.

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WHOLE EXOME ANALYSIS REVEALS MUTATIONS OF TET2 IN ADULT T-CELL LEUKEMIA/LYMPHOMA
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Background: Adult T-cell leukemia/lymphoma (ATL) is an aggressive form of peripheral T-cell lymphoma (PTCL), which is etiologically associated with human T-lymphotropic virus type I (HTLV-1) infection during early infancy. Although HTLV-1 can effectively immortalize T-cells, there is a long latency period of ~50 years prior to the onset of ATL, suggesting that HTLV-1 infection alone may not be sufficient for the development of ATL, but additional acquired genetic hits that occur in immortalized T-cells during the later life are essential for its pathogenesis. However, little has been known about those genetic hits that are involved in the pathogenesis of ATL.
Aims: The purpose of this study is to understand the genetic basis of ATL, we performed whole exome sequencing and follow-up mutation analysis in a large series to confirm the findings in the exome analysis.
Methods: We performed whole exome sequencing of paired-tumor/normal DNA from a single case with ATL lymphoma type. In addition, mutations of *TET2*, *IDH1/2,* and *DNMT3A* were screened in an extended cohort of 145 ATL cases using targeted deep sequencing.
Results: A total of 77 non-silent somatic mutations were detected by whole exome sequencing. Among them, we identified a *TET2* mutation (R1261C). *TET2* mutations have been found in a wide variety of myeloid malignancies at high frequencies. The TET family of proteins is thought to be involved in the epigenetic regulation of gene expression through catalyzing conversion of 5'-methyl cytosine to 5'-hydroxymethyl cytosine, which are supposed to be further converted to unmethylated cytosine. One of the recent interests is frequent mutation of *TET2*, *IDH2* and *DNMT3A* in other PTCLs, such as angioimmunoblastic T-cell lymphomas (AITL) and PTCL not otherwise specified (PTCL-NOS). So we investigated mutations of these genes in a cohort of 145 ATL.
In total, 17 *TET2* mutations were found in 14 (9.6%) out of the 145 ATL samples. Less frequent mutations of *IDH2* and *DNMT3A* were also identified. Different subtypes of ATL were affected with 6 out of 47 acute, 3 out of 36 chronic and 5 out of 46 lymphoma types having *TET2* mutations. Biallelic involvement was suggested in 4 out of the 14 cases. *TET2* mutations seemed to have no significant impacts on overall survival. Less common mutations were found in *IDH2* (p.R172K and p.R172T) and *DNMT3A* (p.G543fs and p.R882H), of which 2 *IDH2* mutations coexisted with *TET2* mutations. In deep sequencing, it revealed that the *TET2* mutations harbored a major tumor population in most evaluable cases, while the *TET2* mutations in two cases only involved a minor population. In some cases, *TET2* mutations seemed to be among early genetic event in ATL, but nevertheless, it should not before HTLV-1 infection, and in other cases, *TET2* mutations could be relatively late events, found in a subpopulation. So our finding of *TET2* mutations in ATL suggested that mutated *TET2* have unique roles in T-cells, contributing to the development of peripheral T-cell neoplasms, most likely through epigenetic deregulation (Figure).
Summary / Conclusion: *TET2* was mutated in ~10% of ATL patients, indicating common pathogenesis between AITL and other PTCLs. Our finding suggested a common role of deregulated epigenetic machinery in the development of mature T-cell neoplasms including ATL.

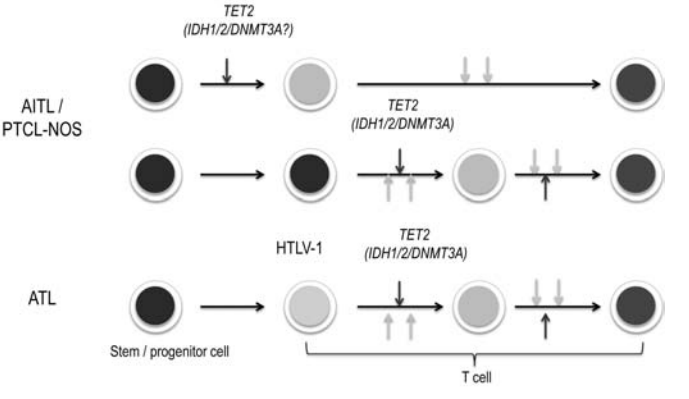

Figure 1.

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VITAMIN D3 AND LENALIDOMIDE SYNERGIZE TO INDUCE APOPTOSIS IN MANTLE CELL LYMPHOMA VIA THE INDUCTION OF THE BH3-ONLY BIK PROTEIN
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Background: Targeted therapies are being tested in the MCL. Among all new treatment options, lenalidomide appears as one of the most efficient molecule. Lenalidomide has multiple modes of action targeting the tumor cell and its environment including immune system. It is widely reported that cancer patients are deficient in vitamin D3 (1,25-dihydroxyvitamin D3, VD3) and recent studies in lymphomas have shown that VD3 rate is significantly associated with survival. While relations between VD3 and incidence of cancer remain unresolved, it was

shown that VD3 contributes as an anticancer agent through its anti-proliferative, pro-differentiation, anti-inflammatory and anti-angiogenic properties.
Aims: We assessed the efficacy of VD3 to potentiate cell death induced by lenalidomide in MCL cell lines and patients' samples and unraveled the mechanism of cell death.
Methods: Experiments were conducted in a panel of 6 MCL cell lines (JEKO-1, MINO, GRANTA-519, UPN-1, REC-1 and Z138) and 8 primary samples.
Results: After 6 days of treatment, MCL cells were weakly sensitive to low doses of lenalidomide (1 μM and 10μM for cell lines and samples, respectively). Addition of physiological doses of VD3 (100nM) significantly and synergistically increased cell death in 67% of cell lines and in 63% of primary samples. Apoptosis, characterized by Annexin V staining, appearance of a subG1 peak and caspase 9 activation, was accompanied by cell cycle arrest in G1 phase. VD3 plus lenalidomide combination dramatically increased expression of the BH3-only Bik without affecting expression of other Bcl2 molecules. By immunoprecipitation assays, we showed that induced-Bik was not bind to anti-apoptotic molecules Bcl2, BclxL or Mcl1 in treated cells but free to activate effectors molecules such as Bax. Moreover, silencing of *BIK* by siRNA prevented apoptosis induced by VD3/lenalidomide, confirming the direct involvement of Bik in cell death. Bik accumulation induced by lenalidomide and VD3 was not related to an increase in transcription factor TEF expression but to an increase of the ratio unmethylated over methylated of *BIK* CpG island. Similar epigenetic regulation of *BIK* expression was obtained with the inhibitor of methylation, 5-azacytidine.
Summary / Conclusion: We show that lenalidomide and VD3, similarly to 5-azacytidine, induce the expression of Bik via the unmethylation of *BIK* GpG island, which is responsible for cell death. Indeed, our results show for the first time an original and non-toxic approach to significantly potentiate apoptosis induced by lenalidomide in MCL cells by simply adding physiological doses of VD3. These data underline the interest 1) to measure the level of VD3 in MCL patients especially those receiving lenalidomide, 2) to define if this level is correlated with the response, 3) to define whether supplementation with VD3 could increase response rate of patients receiving lenalidomide.

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CXCR4 IS CRITICAL FOR NON HODGKIN LYMPHOMA CELL SURVIVAL, INTERACTION WITH BONE MARROW MICROENVIRONMENT AND DRUG RESISTANCE *IN VITRO* AND *IN VIVO* IN ANIMAL MODEL
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Background: CXCR4/CXCL12 chemokine axis has been implicated in the progression of hematological malignancies. CXCR4 is highly expressed in a variety of B cell neoplasms, including NHL. CXCR4 inhibition in combination with rituximab has been shown to be an effective strategy in targeting lymphoma cells in BM environment in disseminated NHL xenograft model.
Aims: To provide a mechanistic insight into CXCR4-regulated lymphomagenesis *in vitro* and *in vivo*.
Results: High mRNA and surface CXCR4 levels were detected in most of NHL cell lines (n=7) and primary samples from NHL patients with BM involvement (n=7). Cell lines expressing high CXCR4 (BL-2 and Raji) demonstrated higher migration rate in response to CXCL12 and firmer adhesion to fibronectin and BMSC monolayer than low CXCR4-expressing BJAB cells. Interaction with BMSC protected the cells with high CXCR4 from rituximab-induced apoptosis. In contrast, BJAB cells with low CXCR4 were not protected by stroma. To further investigate the role of CXCR4 in NHL progression, *in vivo* xenograft model was established. Immuno-compromised mice inoculated subcutaneously with BL-2 cells produced highly invasive local tumors with BM dissemination and succumbed to lymphoma. In contrast, injection of low CXCR4 expressing BJAB cells resulted in local slow-growing tumor development without BM involvement and long-term animal survival. Notably, BL-2 cells that arrived to the BM demonstrated higher levels of mRNA and surface CXCR4, compared to the bulk tumor, therefore suggesting the role of CXCR4 in clonal selection and homing of lymphoma cells to the BM. To further establish the role of CXCR4 in lymphoma progression, we blocked endogenous CXCR4 expression in BL-2 and Raji cells, using anti-CXCR4 shRNA construct. CXCR4 silencing resulted in significant decrease in cell viability *in vitro*, from 92% to 2% in BL-2 cells, and from 85% to 23% in Raji cells (P<0.002). Importantly, we found that anti-apoptotic proto-oncogene BCL-6 was down-regulated in the lymphoma cells following CXCR4 silencing. In accordance, CXCR4 silencing in BL-2 cells significantly (P<0.001) inhibited local tumor growth and prevented NHL spread to BM. As complementary research strategy, we ectopically expressed CXCR4 in low-CXCR4 expressing BJAB cells. Exogenous expression of CXCR4 significantly increased the *in vitro* growth rate and promoted cell survival in the presence of BMSCs (P<0.01). Furthermore, interaction with BMSCs resulted in strong and prolonged pErk1/2 activation in exogenously expressing BJAB-CXCR4 cells, comparing to the native line expressing low CXCR4. Finally, CXCR4 overexpression significantly promoted the growth of xenograft subcutaneous tumors and their spread to the BM.
Summary / Conclusion: Taken together, our findings clearly demonstrate an important pathophysiological role of CXCR4 in NHL. Our model may further serve to elucidate CXCR4-regulated molecular events potentially involved in the pathogenesis of NHL, and strongly support targeting CXCR4 as therapeutic strategy.

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THE HOST GENETIC BACKGROUND MODULATES TREATMENT ACTIVITY AND TOXICITY IN FOLLICULAR LYMPHOMA: FIL-FOLL05 TRIAL
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Background: Follicular lymphoma (FL) is the most frequent indolent lymphoma subtype. Though most FL biomarkers rely on features of the tumor, the genetic background of the host may also be relevant for outcome.
Aims: Here we aimed at verifying the contribution of single nucleotide polymorphisms (SNPs) to the prognostic stratification of FL patients treated with immunochemotherapy.
Methods: The study was based on 428/504 (85%) FL patients enrolled in the FOLL05 phase-III prospective trial comparing R-CVP vs R-CHOP vs R-FM as initial treatment. Seventy-six patients were not assessable due to lack of biological material. Candidate SNPs were selected because known to be relevant for: i) immunochemotherapy outcome or toxicity (MLH1 rs1799977, GSTA1 rs3957357, CYBA rs4673, NCF4 rs1883112, FCGR2A rs18011274, FCGR3A rs396991); ii) FL course (6p21.33 rs6457327). SNPs were genotyped by Sanger sequencing or real time PCR on peripheral blood DNA samples. The primary endpoint was time to treatment failure (TTF). Median follow up of alive patients was 34 months.
Results: Patients (n=428) were representative of the entire FOLL05 study cohort and SNP genotypes distributed in Hardy-Weinberg equilibrium. Though FCGR2A and FCGR3A SNPs have been suggested to influence rituximab single agent activity in FL, our data document that they do not affect treatment results when rituximab is combined with chemotherapy. Indeed, by pooled analysis of the treatment arms, the 3-year TTF did not differ according to FCGR2A (AA: 59% vs AG: 57% vs GG: 62%; P=.742) or FCGR3A (TT: 61% vs GT: 55% vs GG: 61%: P=.252) genotypes. These results were consistent also after compensating for treatment received and FLIPI by multivariate analysis (FCGR2A: P=.793; FCGR3A: P=.490). MLH1 is a component of the DNA mismatch repair that regulates the genotoxic effects of doxorubicin and impairs R-CHOP performance in diffuse large B-cell lymphoma. Consistently, the MLH1 genotype affected the 3-year TTF in the R-CHOP arm (AA: 66% vs AG: 68% vs GG: 30%; P=.011), but not in arms lacking doxorubicin (p for R-CVP=.298; p for R-FM=.601). The impact of MLH1 on TTF was independent (P=.004) after adjusting for FLIPI in the multivariate analysis. The remaining SNPs (GSTA1, CYBA, NCF4, 6p21.33) had no significant effect on TTF. Concerning toxicity, the FCGR2A genotype, which modulates rituximab binding to effector cells, correlated with G3-4 neutropenia (AA: 56% vs AG: 43% vs GG: 40%; P=.026). Also the genotype of GSTA1, which is involved in cyclophosphamide detoxification, affected G3-4 neutropenia risk (CC: 58% vs CT: 43% vs TT: 42%; P=.018).
Summary / Conclusion: Taken together, these data indicate that: i) MLH1 genotype is consistently associated with outcome in FL treated with R-CHOP, thus providing a more general and prospective validation of the usefulness of this host-related biomarker in R-CHOP treated lymphomas; ii) FCGR2A and FCGR3A genotypes have no impact when FL is treated with rituximab combined to chemotherapy; iii) GSTA1 and FCGR2A SNPs may represent biomarkers for the identification of FL patients at risk of severe neutropenia.

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GENERATION AND CHARACTERIZATION OF A CD30 POSITIVE T-CELL LYMPHOMA MOUSE MODEL RESEMBLING HUMAN ANAPLASTIC LARGE CELL LYMPHOMA (ALCL)

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Background: Anaplastic large cell lymphomas (ALCL) are a subgroup of aggressive Non-Hodgkin-Lymphomas mainly affecting children and young adults. In 60% of systemic ALCLs, a translocation t(2;5) (p23;q35) resulting in NPM-ALK fusion gene expression is found. The constitutively activation of ALK tyrosine kinase expressed from the NPM-promoter causes increased proliferation and inhibition of apoptosis thereby promoting cell survival and tumorigenesis.
Aims: Immunphenotypic characterization of human ALCLs revealed highly CD30-positive cells of T- or Null-Cell-origin and resulted in promising clinical trials with CD30-coupled antibodies. However, the impact of CD30 on diseases development as well as NPM-ALK signal transduction in course of disease remain unclear and appropriate mouse models to answer these questions are missing.
Results: In this regard, we established a retroviral murine bone marrow transplantation model resembling a human ALCL-like T-cell neoplasia. Therefore we use an inducible Cre/loxP system where NPM-ALK expression is controlled and expressed in a special type of early T-cells. For generation of this vector, we inserted a floxed translational 'stop-cassette' between the retroviral promoter MSCV-LTR and the NPM-ALK cDNA, which guaranties specific expression of NPM-ALK only in cells, where the enzyme Cre-recombinase is expressed. Recognition of loxP-sites by Cre-recombinase leads in our system to deletion of the stop-cassette and consequently NPM-ALK expression. Using different Cre-expressing cell types allowed us to study pathogenesis of ALCL in more detail. In our recent study, we infected bone marrow of transgenic mice expressing Cre-recombinase under the control of the Lck-promotor with our MSCV-Stop-NPM-ALK-IRES-EGFP (MSNAIE) vector and transplanted it into lethally irradiated C57/Bl6 recipient mice. With a latency of 4-5 months, these mice developed Thy1.2-positive lymphomas and died from neoplastic infiltration of bone marrow and lymphatic organs with T-cells. Immunphenotypic analyses confirmed T-Cell origin of the lymphomas and showed importantly highly CD30-expression. Staining of the different T-cell-subpopulations demonstrated highest NMP-ALK expression in immature CD4/CD8 double negative T-cells and not fully differentiated CD4/CD8 double positive T-cells. Interestingly, FACS-staining of the proliferation marker Ki-67 revealed highest expression in CD4/CD8 double negative T-Cell, in contrast to the other subpopulations where Ki-67 is less detected. Therefore we hypothesized, that the lymphoma initiating cell (LIC) must be within this early T-cell population. Most interestingly we found highest CD30-expression just in the same CD4/CD8 negative T-cell population, pointing to a crucial role of CD30 in lymphoma initiation. To further substantiate our hypothesis we performed secondary and tertiary transplantations with different sorted T-Cell subpopulation and indeed, the immature CD4/CD8 double negative population was able to initiate lymphoma growth in recipient mice. Future analyses of these mice will help to identify the leukemia initiating cell in ALCL.
Summary / Conclusion: Taken together, our murine LckCre-NPM-ALK bone marrow transplantation model represents a precise and versatile tool to study disease initiation and development resembling human ALCL. Moreover, the impact of specific proteins (e.g. CD30) in the course of disease can be addressed by combining Knockout (e.g. CD30)/LckCre transgenic mice with our model.

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LYMPHOMAS AS ESTROGEN RELATED DISEASES - POSSIBLE TARGET FOR ESTROGEN RECEPTOR BETA AGONIST TREATMENT.

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Background: Lymphomas are generally not considered as endocrine related cancers. However, most lymphoid malignancies show a gender differences in incidence and prognosis with males being more affected. Furthermore, epidemiological data in females show an association between reproductive hormonal factors and oral contraceptives with a significantly reduced risk for Non-Hodgkin lymphomas, indicating a protective role of estrogens. More recent studies have demonstrated estrogen receptor beta (ERb) to be the major ER expressed in normal and malignant cells of lymphoid origin.
Aims: The aim of the study was to investigate whether estradiol and selective ERa and ERβ agonists may affect lymphoma growth in culture and *in vivo.*
Methods: We have analyzed the effects of estradiol and selective ERa and ERβ agonists on lymphoma growth by grafting mice with lymphoma cells. RT-qPCR, microarray analysis and immunohistochemistry/immunofluorescence were used in our study.
Results: Treating lymphoma cells with estradiol or ERa selective agonist had minor or no effect on cell growth, while selective ERβ agonist treatment showed an anti-proliferative effect. When grafting mice with murine T lymphoma cells, male mice developed larger tumors compared to female mice, a difference that was abolished following ovariectomy, demonstrating estrogen dependent growth *in vivo*. To investigate whether lymphoma growth may be inhibited *in vivo* by ERβ agonist treatment, mice were grafted with murine T and human B lymphoma cells and treated with ERb selective agonists. Results showed that treatment with ERb selective agonists strongly inhibited lymphoma growth of several lymphoma types due to reduced proliferation and in some cases increased apoptosis. In addition, ERb selective agonists reduced lymphoma dissemination. Gene expression studies have identified target genes and mechanism that could explain the above effects of ERb agonists. Preliminary results from immunohistochemical staining of primary mantle cell lymphoma material showed that ERb is expressed in the tumor cells.
Summary / Conclusion: In summary, our results demonstrate an ERβ ligand-dependent antiproliferative effect on lymphoma cells expressing endogenous ERβ and that lymphoma cell growth and dissemination *in vivo* can efficiently be reduced by ERβ agonists. The results suggest that ERβ agonists may be useful in the treatment of lymphomas. The results may also explain the gender difference in incidence and prognosis of lymphomas.

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A COMPARATIVE ANALYSIS OF NEXT-GENERATION SEQUENCING AND REAL-TIME QUANTITATIVE PCR FOR MINIMAL RESIDUAL DISEASE DETECTION IN FOLLICULAR LYMPHOMA

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Background: The detection of minimal residual disease (MRD) by t(14;18) Real-Time Quantitative (RQ) PCR has become an important tool for treatment monitoring in follicular lymphoma (FL). However, only 50% to 65% of patients can be assessed by t(14;18) RQ-PCR and alternative targets as the immunoglobulin heavy chain variable region (IGH) can be used only with limitations. IGH-based next-generation sequencing (NGS) might provide an alternative approach with further increase in sensitivity, specificity and accuracy. Therefore we comparably analysed both approaches on diagnostic and post-treatment follow-up samples in 29 FL patients.
Aims: To verify the suitability of NGS for MRD detection we comparably analysed diagnostic and post-treatment follow-up samples in 29 FL patients.
Methods: Overall, 206 samples (85 bone marrow, 114 peripheral blood, 6 stem cell aliquots and one lymph node sample) were investigated from 29 FLenrolled in clinical trials. Overall, 33 diagnostic and 173 follow-up (FU) samples were analysed. 23/29 patients had a PCR detectable t(14;18) rearrangement, 5 patients had a clonal IGH rearrangement only and one patient had no marker by consensus PCR. RQ-PCR was carried out as previously published by our group and results were analysed according to ESHLO criteria. NGS was performed independently at the Sequenta facilities in San Francisco and data remained blinded until comparison. Using universal primer sets IGH variable, diversity, and joining gene segments were amplified and sequenced with a coverage of 14 reads per each IGH molecule and analyzed using standardized algorithms for clonotype determination. Tumor-specific clonotypes were identified based on their high-frequency in diagnostic sample and then quantitated in FU samples. A quantitative and standardized measure of clone level among all leukocytes was determined using internal reference DNA. Discordances of MRD results by both methods were classified as follows: a positive/negative discordance between two results was defined as major when the positive result was >1 E-05 and minor when ≤1 E-05; a quantitative discordance was defined as two positive results with a quantitative discrepancy >1 log.
Results: 29 patients were evaluable with at least one method. 15 patients were evaluable for MRD by RQ-PCR and NGS. Here, in 97 FU samples a significant concordance between both MRD methods could be demonstrated (r^2=0.80) (P<0.0001). Of these samples, 44 were MRD positive and 45 were MRD negative with both tools .Quantitative discordances occurred in 12/44 MRD+ samples where in 7 samples MRD was higher and in 5 was lower by NGS. A major discordance occurred in 4 samples where RQ-PCR was positive and NGS was negative. A minor discordance was detectable in 4 samples where in 2 samples RQ-PCR was positive and NGS was negative, and in the other 2 samples the opposite was correct. Seven patients were only quantifiable by RQ-PCR while NGS did not identify an index clone for sequencing. In all but one diagnostic samples demonstrated low level lymphoma infiltration with MRD below 10^{-3}. In one of these cases, IG kappa could be successfully sequenced for MRD indicating that not only low level MRD but also somatic mutation of IGH is a potential pitfall for MRD detection by NGS. In 5 t(14;18)

negative cases with a clonal IGH rearrangement IGH-RQ-PCR resulted in non quantifiable assays. Here, NGS detected MRD successfully, as well as in one further case where an unusual large t(14;18) rearrangement did not allow MRD quantification by RQ-PCR.
Summary / Conclusion: NGS represents a feasible tool for MRD monitoring that allows analysis of a larger group of FL patients. Our results show that lymphoma infiltration of diagnostic samples is critical for identification of the tumor-specific clonotypes by NGS and that different MRD methods may complement each other to allow MRD assessment for the majority of FL patients.

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MYD88 L265P MUTATION IN WALDENSTROM MACROGLOBULINEMIA: INCIDENCE AND FUNCTIONAL STUDY

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Background: Mutation of *MYD88* gene has recently been identified in activated B-cell like diffuse B-cell lymphoma, and enhanced JAK STAT and NF-kB signalling pathways. Whole exome sequencing study in Waldenstrom macroglobulinemia (WM) suggested a high frequency of MYD88 L265P mutation in WM. Although the genetic background is not fully deciphered in WM, the role of NF-kB and JAK STAT pathways has been demonstrated in WM.
Aims: We aimed to analyze MYD88 mutation in exon5, to characterize the clinical significance of this genetic alteration in 67 WM and to study the effects of MYD88 inhibition in WM cell lines
Methods: 67 patients (42 males, 25 females) diagnosed with WM were included in this study. Patients were untreated at time of BM collection. Clinical features, immunophenotypic markers using flow cytometry (Matutes score panel, CD38, CD138, CD27, CD80), conventional cytogenetic, FISH and SNP array data (n=46) were analysed. B cells from bone marrow and T cells from blood were isolated respectively using B cell isolation kit and Pan T isolation kit (Myltenyi Biotech). For DNA sequencing of exon 5 of MYD88, the exon 5 of MYD88 gene was amplified from genomic DNA by PCR.The purified PCR products were directly sequenced in both directions using BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems, CA, USA) and analyzed on the Applied Biosystems 3130xl Genetic Analyzer. BCWM1, MWCL1 (WM cell lines with MYD88 L265P mutation), MEC-1, RL, and MM.1S cell lines were used in this study. Cells were treated with a MYD88 inhibitor (MYD inh) ant its control peptide (Peptide crtl). Viability and cell growth of treated cells were determined using the MTS assay. Apoptosis was quantitated using annexin V - propidium iodide staining, mitochondrial membrane potential and caspase activity analysis using flow cytometry.
Results: MYD88 L265P mutation (MYD^{mut}) was observed in 79% of patients, including homozygous mutation in two patients (3%). MYD88 mutation was not identified in T lymphocytes isolated from 4 WM patients. We haven't observed any other mutation on exon 5. We then sought for other mechanisms of MYD88 gene alteration, such as copy number alteration (CNA) and copy neutral –loss of heterozygosity (CN-LOH) at MYD88 locus. We found an CN-LOH at MYD88 locus in solely one patient (2%), and haven't identified any deletion at 3p22. On the contrary, we observed a gain on chromosome 3 at 3p22 locus (including MYD88 gene) in 7/57 (12%) patients. Taking together, we identified alteration of the MYD88 locus in 85% of patients with WM, by either gain-of-function mutation (79%) or CNA (12%). Interestingly, we found gain on chromosome 3 more frequently in the MYD^{wild} group than in the MYD^{mut} group (P=0.02). Twenty one percent of the patients with WM had no mutation of MYD (MYD^{wild}), and were characterized with a female predominance, a splenomegaly, gain of chromosome 3 and CD27 expression. We did not observed difference in terms of survival according to the MYD88 mutation status. MYD88 mutation was not related to deletion 6q, gain of4, deletion 11q, deletion 17p, deletion 13q14 in our study. Overall, 63% of WM had at least one additional genetic alteration in the NF-kB pathway in our cohort, but no significant difference was observed according to the MYD88 mutation status. Inhibition of MYD88 signalling induced cytotoxicity and inhibited cell growth in cells expressing MYD88 L265P mutation. Inhibition of MYD88 homodimerization also significantly inhibited MYD88 signalling in the MYD^{mut} as compared to the MYD^{wild} cell lines, as exemplified by the marked downregulation of IRAK4 and STAT3 phosphorylation, downstream targets of MYD88.
Summary / Conclusion: Our results confirm a high frequency of MYD88 L265P mutation in WM that may become a useful biomarker for diagnostic in WM and may help better understand the physiopathogeny of WM. From a therapeutic standpoint, this data also suggested that direct targeting of MYD88 signalling may provide a novel approach for the treatment of MW in the future.

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MINIMAL RESIDUAL DISEASE (MRD) DETECTION BY NEXT-GENERATION SEQUENCING AND REAL-TIME QUANTITATIVE PCR: A METHODICAL COMPARISON IN ALL, MCL AND MM

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Background: Real-Time Quantitative (RQ)-PCR-based MRD detection using primers derived from the immunoglobulin heavy chain variable region (IGH) is an established disease monitoring tool in ALL, MCL and MM. It is highly sensitive and has been standardized in the context of international cooperative groups such as the European Scientific Foundation for Laboratory Hematooncology (ESHLO). However it has some limitations, including marker identification failure, and false negatives due to clonal evolution.
Aims: To verify if IGH-based next-generation sequencing (NGS) might overcome some of these limitations of RQ-PCR and further increase sensitivity, specificity, accuracy and reproducibility, we have performed comparison of the two methods on diagnostic (DG) and post-treatment follow-up (FU) samples on a panel of 55 pts.
Methods: 378 samples (62 DG, 316 FU) were collected from 55 pts enrolled in clinical trials (15 ALL, 30 MCL, 10 MM). IGH-based RQ-PCR was carried out as previously described [Ladetto *et al*, BBMT 2000; Brüggemann *et al*, Blood 2006], according to the ESHLO criteria [van der Velden *et al*, Leukemia 2007], at two experienced MRD laboratories. NGS was performed at the Sequenta facilities. Using universal primer sets, we amplified IGH variable, diversity, and joining gene segments from gDNA. Amplified products were sequenced to obtain a high degree of coverage (14 reads per each IGH molecule) and analyzed using standardized algorithms for clonotype determination. Tumor-specific clonotypes were identified for each patient based on their high-frequency in DG sample and then quantitated in FU samples. A quantitative and standardized measure of clone level among all leukocytes was determined using internal reference DNA. NGS analysis was performed independently under blinded conditions. Comparability of results by RQ-PCR and NGS was assessed by bivariate correlations between methods (software R 2.15.1 package irr). Discordances were classified as follows: a positive/negative discordance was defined as major when the positive result was >1 E-05 and minor when ≤1 E-05; a quantitative discordance was defined as the presence of two positive results with a quantitative discrepancy >1 log.
Results: 51 pts (93%) were evaluable with at least one tool (RQ-PCR 45, NGS 49), 43 (78%) with both and 4 (7%) with none. Disease-specific success rates are shown in Table 1. Sequences identified with both tools were identical or nearly identical in 41 cases and unrelated in two. Overall, 330 samples (87,3%) were evaluated with at least one tool (RQ-PCR 279, NGS 316) and 265 (70%) with both. In terms of MRD output, concordance was significant (P<0.001) and 214/265 (80,8%) samples had an optimal concordance (96 MRD neg and 118 MRD pos). Of these major discordances were 16 (6%); minor discordances were 24 (9,1%); quantitative discordances were 11 (4,1%). In 2 ALL clonal evolution hampered straightforward MRD assessment. In one case IGH RQ-PCR underestimated MRD while a second RQ-PCR marker (TCRD) overlapped NGS. In a second case NGS did not detect the tumor diagnostic clone due to loss of the complete IGHV at relapse whereas the preceding IGHDJ was preserved and detected by RQ-PCR.
Summary / Conclusion: NGS is an effective tool for MRD monitoring in ALL, MCL and MM. Good concordance was seen in the vast majority of cases. However, disease-specific pitfalls (clonal evolution, somatic hypermutations, frequency of complete IGH rearrangements) have to be considered for both methods. Prospective comparative analysis of unselected cases is required to verify the clinical impact of NGS-based MRD assessment.

Table 1. Rates of success of RQ-PCR abd NGS among ALL, MCL, an MM by patient.

Disease	Patients	Patients evaluable PCR	Patients evaluable NGS	Patients evaluable with both tools	Patients evaluable with at least one tool	Patients not evaluable
ALL	15	15	15	15	15	0
MCL	30	22	26	22	26	4
MM	10	8	8	6	10	0
TOT	55	45	49	43	51	4

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B-CELL REPERTOIRE ANALYSIS OF INDOLENT MCL WITH SPLENIC PRESENTATION : A DISTINCT ONTOGENY FROM CLASSICAL MCL?

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Background: Mantle-cell lymphoma (MCL) is a heterogeneous but well-defined lymphoid malignancy characterised by the presence of a translocation, the t(11;14)(q13;q32), inducing a dysregulation of the cyclin D1 expression, a key protein for the cell cycle at the G1-S phase transition. MCL is characterized by a rapid and pejorative clinical evolution. Recently, it has been recognized that some patients experience a particularly indolent course with a prolonged survival without therapy. These patients generally present with splenomegaly, blood involvement and minimal lymphadenopathy. However, indolent cases with a splenic presentation (splenic iMCL) remain incompletely characterized, especially regarding the B-cell reprtoire.
Aims: The aim of our study was to characterize the clinical and biological features of splenic iMCL, including their IGHV-IGHD-IGHJ repertoire and mutational status.
Methods: Genomic DNA of 20 patients with splenic iMCL extracted from tissue specimen (blood, bone marrow biopsy, spleen) was used to characterise the IGHV-IGHD-IGHJ gene repertoire, to analyse somatic hypermutation (SHM) features, and to look for common CDR3 motifs.
Results: Patients characteristics include a median age of 65 years (range : 48-84), 5 females and 15 males, ECOG-PS ≤ 1 for all patients, MIPI score low in2, intermediate in 8 and high in 8. Median leucocyte count was 14.4G/L. Anemia was present in 6 patients, thrombocytopenia in 12, LDH > UNL in 4. The median follow up was 83 months (6-213). At time of analysis, 11 patients were alive without receiving polychimiotherapy. Immunophenotype was available in 19 patients: Matutes score was ≤ 3 (0-1 n=12, 5 n=2, 3 n=2), CD5 was negative in 4 patients, IgM (+/-IgD) expression was reported in 13 patients/13 (IgG in 0), κ and λ in 12 and 7 cases respectively. A monoclonal productive IGH rearrangement was obtained for 16 patients. There was a preferential usage of IGHV4-34 (4/16 cases) and IGHV3-7 (3/16 cases) genes. Interestingly, IGHV1-2 and IGHV3-21, the most frequent genes used in splenic marginal zone lymphoma (MZL) and classical MCL respectively, were not (IGHV1-2) or seldom (IGHV3-21, 1/16) represented. While the IGHD repertoire had no particularity, there was an over-representation of the IGHJ4 gene (9/16) and an absence of IGHJ5. The vast majority of cases (14/16) had undergone SHM; the mean value for percentage of identity to germline IGHV sequences was 96%, and 10/16 sequences could be considered as truly mutated (<97% identity). The median CDR3 length was 13 (6-20) and no stereotypic CDR3 motif could be identified.
Summary / Conclusion: Although this constitutes a small series, our results suggest that iMCL with splenic presentation might have a different IGH repertoire than those of and classical MCL as well as splenic MZL. Further insight into the potential of splenic iMCL to form a distinct entity is currently being investigated by comparative genomic analyses.

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NIPA DEFICIENCY LEADS TO ACCELERATION OF THE LYMPHOMA DEVELOPMENT IN EUMYC MICE

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Background: Timely degradation of proteins that control cell proliferation and apoptosis is an essential mechanism in keeping normal growth from turning into runaway malignancy. We previously reported the cloning of NIPA (Nuclear-Interaction-Partner-of-ALK) and characterized it as a F-Box-protein that defines an oscillating E3-ubiquitin-ligase.
Aims: To study *in vivo* function of the G_2/M checkpoint NIPA in greater detail, we inactivated the gene encoding *Nipa* using a conditional-knockout strategy.
Results: Nipa$^{-/-}$ animals are viable, but sterile due to a block of spermatogenesis. Our studies demonstrate that loss of Nipa has no substantive effect on physiological cell cycle progression of primary MEFs indicating that this cell cycle checkpoint is inactive under optimal proliferation conditions. Interestingly, Nipa checkpoint control can be unmasked by oncogenic c-Myc-transformation. Here we show significant differences in c-Myc-induced transformation: Focus formation ability of c-Myc-infected Nipa$^{-/-}$ MEFs was greatly reduced. Moreover, Nipa-deficiency leads to premature senescence in cultured primary MEFs. Ectopic reexpression of Nipa resulted vice versa in delayed senescence of knockout MEFs. Next, we sought to know, whether increased apoptosis in Nipa$^{-/-}$ c-Myc-transduced MEFs is dependent on a functional p53-Axis. Interestingly, the effect of Nipa deficiency on c-Myc-mediated transformation was totally abolished by p53-knockdown. We observed no differences in focus formation ability or growth behaviour in Nipa$^{-/-}$ MEFs with inactivated p53, suggesting the importance of p53 in Nipa-induced cell death. Looking in more detail on the c-myc-p53 axis we detected a substantial increase in Arf-p19 levels in Nipa$^{-/-}$ cells. Moreover, Nipa-knockdown in Zn-inducible-Arf-NIH/3T3 cells lead to stabilization of Arf p19. To test the impact of these findings in a relevant *in vivo* model we intercrossed Nipa$^{-/-}$ animals with a transgene EμMyc-Strain. Nipa$^{-/-}$EμMyc$^{TG/wt}$ animals developed lymphomas within a significantly shorter latency than Nipa$^{+/+}$EμMyc$^{TG/wt}$ animals. Furthermore, lymphomas of knockout animals were more aggressive. FACS- and biochemical-analyses showed no gross differences between Nipa$^{-/-}$ and wt lymphomas except highly elevated Arf-p19 levels in Nipa$^{-/-}$ lymphomas, pointing to an important role of Nipa in Myc-p19-signalling.
Summary / Conclusion: Taken together our results highlight the functional importance of the Nipa-p53-axis in cell cycle regulation and suggest that deregulation of the protein provides a substantial contribution during the process of tumorigenesis.

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INFLUENCE OF THE B-CELL RECEPTOR (BCR) SIGNALING PATHWAYS ON CD20 LEVELS IN TUMOR CELLS AND ANTITUMOR ACTIVITY OF ANTI-CD20 MONOCLONAL ANTIBODIES

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Background: Anti-CD20 monoclonal antibodies (mAbs) are widely used in the treatment of non-Hodgkin's lymphomas (NHL) and chronic lymphocytic leukemia (CLL). Combining new agents with already used anti-CD20 mAbs seems to be a reasonable approach to further improve current therapeutic options. It seems that signaling via the aberrantly activated B-cell receptor (BCR) plays a key role in the pathogenesis of B-cell tumors. Blocking BCR signaling complex network holds a great therapeutic potential in both NHL and CLL. Several trials are currently being conducted to investigate the effects of combination of BCR- targeting agents with anti-CD20 mAbs. To improve these therapeutic approaches it is utterly important to decipher actual mechanisms of interactions between BCR-targeted therapies and anti-CD20 mAbs in established *in vitro* models.

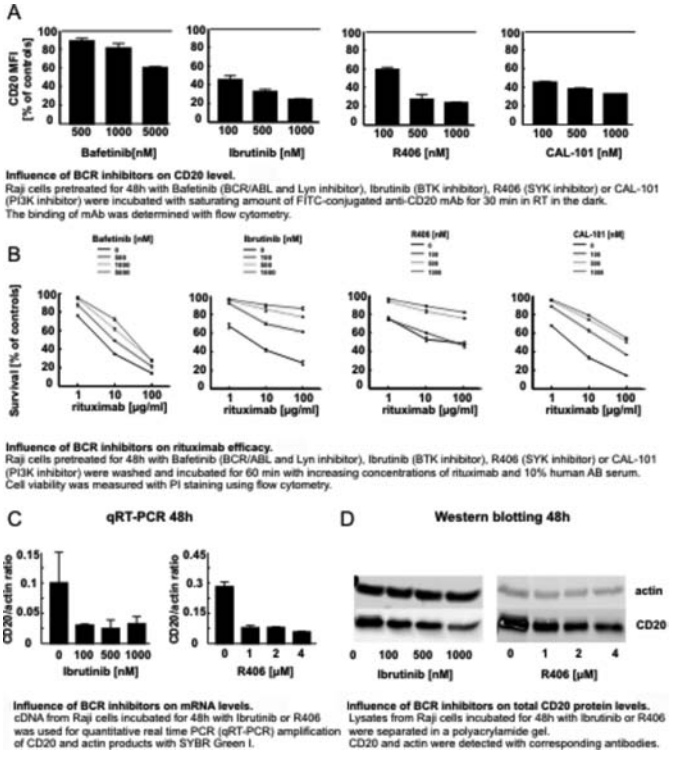

Figure 1.

Aims: The aim of this study is to elucidate the role of BCR signaling pathways in the regulation of CD20 levels in tumor cells and antitumor activity of anti-CD20 mAbs.
Methods: The project is realized fully in *in vitro* settings in the models of human lymphoma cells as well as primary cells from patients with B-cell tumors. Cells are pre-incubated for 48h with inhibitors of BCR signaling (SYK, BTK, PLC, PKC, PI3K, AKT) and subsequently tested using flow cytometry for their contribution to antitumor effect of anti-CD20 mAbs (Figure 1B). Membrane level of CD20 antigen is assessed with FITC-conjugated anti-CD20 antibody staining (Figure1A), total level of CD20 protein is assessed in Western blotting (Figure 1D). Transcription processes are monitored with quantitative real time PCR (qRT-PCR) (Figure 1C), ChIP and EMSA. Moreover, stably transduced lymphoma cells with silenced or overexpressed proteins of interest are employed.
Results: The results of our preliminary experiments show that blocking BCR network at many stages of the signaling cascade with specific chemical inhibitors or selective shRNA-mediated silencing of SYK or BTK results in considerable down-regulation of CD20 level as determined with flow cytometry. Moreover, a 48-hour incubation with BCR inhibitors leads to a substantial impairment of antitumor activity of anti-CD20 mAbs. Selected inhibitors of BCR signaling considerably decrease CD20 protein level in total cellular lysates as analyzed using Western blotting. In Raji cells incubated with selected BCR inhibitors qRT-PCR shows a significant decrease in CD20 mRNA level. The preliminary results from EMSA and ChIP indicate that mRNA decrease is not dependent on CD20 promoter and activity of transcription factors known to regulate CD20 expression. Further studies elucidating the exact mechanisms of the observed phenomena will be performed.
Summary / Conclusion: Blocking BCR complex network on nearly every step of signal initiation and propagation considerably down-regulates CD20 levels what might have extremely important consequences for the anti-cancer therapy that is based on the use of anti-CD20 mAbs. These studies should provide us with extensive knowledge on the biology of CD20 protein and pathways involved in CD20 regulation. In light of our recent experiments therapeutic combinations of BCR inhibitors and anti-CD20 mAbs-based modalities should be rationally and consciously introduced into clinic in optimized therapeutic schemes. We hypothesize that results of our experiments may lead to identification of the most beneficial therapeutic modalities that would improve the quality of life of patients suffering from B-cells originating tumors.

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GENETIC VARIATION IN INTERFERON REGULATORY FACTOR 4 AND INTERLEUKIN 10 GENES, AND THE RISK FOR DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Immune alteration is a major risk factor for non-Hodgkin lymphoma (NHL), but the specific immune mechanisms responsible remain unresolved. In a large consortial study, it has been recently reported that an increased risk for NHL, especially the major lymphoma
Aims: We provide a comprehensive analysis of IRF4 and IL10 polymorphisms in a case-control study and risk association with DLBCL in Koreans.
Methods: The case-control series consisted of 192 *de novo* DLBCL treated at five hospitals throughout Korea from August 2001 through August 2009 and 192 individuals from the population with age and gender matched healthy volunteers. The DNA samples were genotyped using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (Sequenom, Inc., San Diego, CA). We genotyped 15 haplotype-tagging SNPs (htSNPs) of IF4 (rs1877175, rs1877179, rs2001508, rs2666957, rs2671422, etc.) and 7 htSNPs of IL10 (rs1518111, rs1800871, rs1800872, rs1800890, rs3021094, rs3024490, rs3790622) genes, respectively. We determined if the allelic distribution of the nine SNPs was consistent with Hardy-Weinberg equilibrium using the □2 test. All the genotype frequencies were in accordance with Hardy-Weinberg equilibrium. The allele and genotype frequencies of these SNPs were compared between the cases and controls using the 2 test or Fisher's exact test. Unconditional logistic regression was done to estimate the odds ratios (OR) and 95% confidence intervals (95% CI) of the individual SNPs. The haplotypes were reconstructed according to the genotyping data and the linkage disequilibrium status of these nine SNPs.
Results: We did not find significant associations between IRF4 htSNPs and the risk of DLBCL. However, the minor allele heterozygotes of the rs3021094 htSNP of IL10 showed an increased risk of DLBCL (adjusted odds ratio = 1.453, P=0.034). On 10-million permutation testing, this haplotype including rs3021094 variant allele was significantly associated with an increased risk of DLBCL (P=0.001).
Summary / Conclusion: This study presents several novel aspects of the genetic susceptibility to develop DLBCL. Our data do not statistically support the association between IRF4 htSNPs and risk for DLBCL. However, we demonstrate that elevated DLBCL risk is associated with the rs3021094 htSNP of IL10. Larger studies that will focus on the role of the rs3021094 htSNP of IL10 for developing DLBCL are needed in the future.

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PROGNOSTIC IMPACT OF EZH2 EXPRESSION, H3K27 TRIMETHYLATION AND DNA METHYLATION IN DIFFUSE LARGE B CELL LYMPHOMA

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Background: International Prognostic Index (IPI) score is used to predict the prognosis in diffuse large B cell lymphoma (DLBCL) for around 20 years. However, few molecular prognostic factors were proposed. Overexpression of Enhancer of zeste homolog 2 (EZH2) and decreased histone 3-lysine 27 trimethylation (H3K27me3) are associated with poor prognosis in many cancers. Promoter CpG island hypermethylation of tumor-suppressor genes is a common hallmark of all human cancers. 5-methylcytosine (5-mC) levels in cancer cells can reflect the DNA hypermethylation status and measurement of 5-hydroxymethylcytosine (5-hmC) levels may estimate the DNA demethylation status in cancer cells. A unique EZH2 Tyr 641 somatic mutation was detected in DLBCL and follicular lymphoma. However, the clinical implications of DNA methylation status, DNA hydroxymethylation, EZH2 expression, the extent of H3K27me3 and EZH2 Tyr 641 somatic mutation in DLBCL patients have not been studied in a comprehensive or integrated way.
Aims: We aim to see significant impact of DNA methylation, DNA hydroxymethylation, EZH2 expression levels and mutation status, and H3K27 trimethylation on the clinical and biological presentation of DLBCL.
Methods: We enrolled the 110 DLBCL patients with complete remission (CR) after standard chemotherapy. These patients included 45 consecutive patients in Far Eastern Memorial Hospital and 65 consecutive patients in Chi Mei Medical Center (diagnosed between 2002 and 2009). The demographic data, treatment regimens, and response of disease were reviewed retrospectively. Immunohistochemistry (IHC) was used to examine 5-mC, 5-hmC, EZH2 expression and the extent of H3K27me3 in formalin-fixed, paraffin-embedded biopsy specimens of DLBCL. DNA extraction from the tissues was examined for EZH2 Tyr641 mutation. Statistical analysis was performed with the Stata statistical software (Small Stata, version 11.0, Stata Corp, College Station, TX).
Results: Statistical analysis was performed in 110 CR patients with Rituximab-CHOP (R-CHOP) and CHOP regimen. Totally we recruited 70 male (64%) and 40 female (36%) with a median age 57 years. Sixty-three percent of the patients had stage I/II disease. According to the IPI, 76% of the patients were classified as low/low-intermediate risk (IPI=0-2). Thirty patients received CHOP-like regimen and eighty patients received R-CHOP-like regimen as first-line chemotherapy. The median observation time for overall survival (OS) in the 110 patients was 49 mo. The clinical parameter of IPI score was correlated with OS. None of 48 patients harbored *EZH2* mutation at Tyr641. Low expression 5-mC tended to have lower H3K27me3 expression (P=0.019). And low expression 5-mC tended to have lower 5-hmC expression (P=0.002). There was no obvious relationship between the expression of EZH2 and degree of H3K27me3 in DLBCL tumor cells. There was no significant prognostic impact in single epigenetic marker. Then we subdivide the patients by combination H3K27me3 with 5-hmC. In this new-classification analysis, the high H3K27me3/ low 5-hmC patients were associated with longer OS in univariate (P=0.013) and multivariate analysis (P=0.017).
Summary / Conclusion: High H3K27me3/ low 5-hmC expression was a possible favorable prognostic factor in DLBCL patients with CR after standard chemotherapy. The incidence of *EZH2* mutation at Tyr641 in our cohort is much lower compared with western countries. Low expression 5-mC tended to have lower H3K27me3 expression and low expression 5-mC tended to have lower 5-hmC in DLBCL tumor cells. Our study suggests epigenetic markers may be an informative biomarker for prognosis prediction in DLBCL.

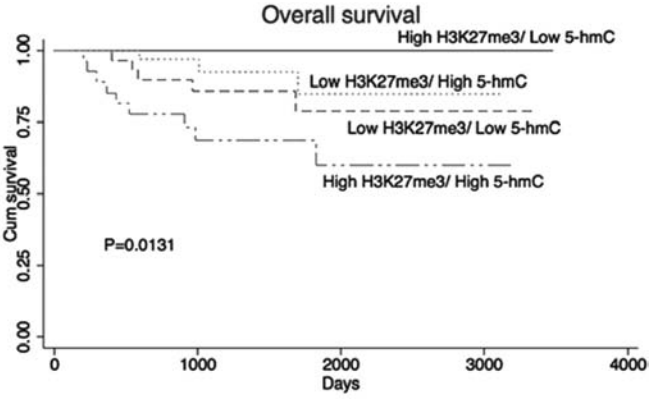

Figure 1.

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BMI1 EXERTS ITS ONCOGENIC EFFECTS VIA ENHANCEMENT OF ANTI-APOPTOTIC POTENTIAL IN MANTLE CELL LYMPHOMA

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Background: Mantle cell lymphoma (MCL) is categorized as an indolent $CD5^+$ B cell lymphoma and is associated with numerous genomic copy number alterations, including 9p21 deletion (*CDKN2A*) and 10p12 amplification (*BMI1*). The target gene of the 10p12 amplification is a proto-oncogene, *BMI1*. Its overexpression is recurrently observed in the blastoid variant of MCL, which suggests *BMI1* may play an important role in MCL tumorigenesis. Deletion of 9p21 is seen in 40-50% of MCL cases and results in dysregulation of the tumor suppressor gene *CDKN2A*, which encodes $p16^{INK4a}$ and $p14^{ARF}$. Earlier studies have shown that *BMI1*/Bmi1 exerts its oncogenic effects, at least in part, by silencing the *CDKN2A* tumor suppressor locus, thereby promoting cell cycle progression and suppressing apoptosis. Thus, Bmi1 has been thought to regulate *CDKN2A* in MCL. However, previous report showed that MCL recurrently exhibited both homozygous deletion of 9p21 (*CDKN2A*) and amplification of 10p12 (*BMI1*), suggesting that in that case, *BMI1* regulates other tumor suppressors.
Aims: The aim of this study is to determine the role of *BMI1* in MCL, especially in relapsed cases. And we identify a functional role of apoptotic regulation of *BMI1*.
Methods: We infected MCL cell lines with a robust inducible single-lentiviral vector knockdown reagent, pLKO-Tet-On, encoding either a non-targeting control siRNA or a Bmi1-targeting siRNA (siBMI1). Then using puromycin selection, stable polyclonal lines were generated. Chip assays were performed by use of SimpleCHIP™ Enzymatic Chromatin IP Kit (Cell Signaling Technology) according to manufacturer's protocol.
Results: We first examined *BMI1* expression in primary cases and found that its expression was significantly higher at recurrence than at initial diagnosis. Next, we found that transfection of siBMI1 induced apoptosis despite the absence of *CDKN2A*. Chip assay showed that Bmi1 interacted with pro-apoptotic genes (*BCL2L11*/Bim and *PMAIP1*/Noxa), which were recently shown to be Bmi1 targets. We also found that Bcl2 was up-regulated in MCL.Finally, we found that bortezomib, which is known to be a proteasome inhibitor, induced apoptosis with down-regulation of Bmi1 and up-regulation of Bim and Noxa in MCL.
Summary / Conclusion: We for the first time showed that *BMI1*/Bmi1 is associated with disease progression in MCL. We found that Bmi1 negatively regulates *BCL2L11*/Bim and *PMAIP1*/Noxa to exert an anti-apoptotic effects in MCL cells, which carry homozygous 9p21 (*CDKN2A*) deletions.

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THE ROLE OF CXC–CHEMOKINES IL-6, IL-8 AND CXCR2 RECEPTOR IN LYMPHOPLASMACYTIC LYMPHOMA: CORRELATIONS WITH MICROVASCULAR CHARACTERISTICS AND CLINICAL FEATURES

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Background: Increased neovascularisation is a vital process underlying the development and progression of malignant neoplasms. Limited information on the subject is available for Waldenstrom's macroglobulinaemia/Lymphoplasmacytic lymphoma (WM/LPL), a rare and usually indolent B-cell lymphoma.
Aims: To evaluate the microvessel characteristics and IL-6, IL-8, CXCR2 and VEGF expression in the bone marrow of WM/LPL patients and investigate any possible correlation with disease characteristics and outcome.
Methods: Sixty-three patients were studied (47 WM and 16 LPL) for whom paraffin-embedded tissue from bone marrow trephine biopsies performed at diagnosis, before treatment was available. The microvascular characteristics were evaluated using CD34 stained slides. Microvessel density (MVD), total vascular area (TVA) and several other size- and shape-related parameters were quantified in the region of most intense vascularization using a computerized image analysis software. Moreover, slides were immunostained with IL-8, IL-6, CXCR2 and VEGF and evaluation was performed blindly using light microscopy. A Histo-score (H-score) based on the percentage of stained neoplastic cells multiplied by staining intensity was calculated.
Results: Thirty-two, 25 and 6 patients were classified into low, intermediate and high risk respectively, according to the IPSSWM staging system. Seventy-six percent of the patients required treatment while 24% were asymptomatic and were regularly followed. Median patients' follow-up was 77 months. Microvascular characteristics, i.e microvessel density (MVD), total vascular area (TVA) and several other size- and shape-related parameters, were evaluated in CD34 stained bone marrow slides using computerized image analysis. A Histo-score (H-score) based on the percentage of immunopositive neoplastic cells multiplied by staining intensity was calculated for IL-6, IL-8, CXCR2 and VEGF immunohistochemical expression. MVD ranged from 7 to 393 μm^2 (median 36,5) and TVA from 2681 to 102480 μm^2 (median 32427). IL-6, IL-8, CXCR2 and VEGF expression was observed in 84%, 43%, 89% and 90% respectively. A positive correlation between IL-6 and CXCR2 H-scores was observed; IL-6 and VEGF H-scores correlated with hypoalbulinemia and increased serum β_2-microglobulin respectively and both with bone marrow lymphoplasmacytic infiltration and MVD. The degree of angiogenesis, microvessel shape, VEGF expression and CXCR2 expression correlated with a shorter time to first treatment in multivariate analysis.
Summary / Conclusion: In WM/LPL, increased expression of cytokines implicated in neovascularization and microvessel augmentation confer a more aggressive disease, reflected by an earlier need of treatment.

Non-Hodgkin Lymphoma - Clinical 1

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HIV STATUS DOES NOT INFLUENCE THE OUTCOME OF PATIENTS DIAGNOSED WITH DLBCL TREATED WITH R-CHOP IN THE HAART ERA

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Background: The prognosis of patients with HIV and Diffuse Large B-cell Lymphoma (DLBCL) has improved considerably since the advent of HAART, approaching that of patients with DLBCL in the general population when treated with the same chemotherapy regimens.
Aims: To analyze the outcome of patients diagnosed with DLBCL treated with R-CHOP in the HAART era according to HIV status.
Methods: From 2003 to June 2011, 294 patients were newly diagnosed with DLBCL at 5 university hospitals in London and treated with R-CHOP chemotherapy. The cohort comprised 208 HIV negative (HIV-) patients from St Bartholomew's Hospital and 86 HIV positive (HIV+) patients from that hospital and 4 other London Hospitals. In the same study period, a further 90 patients (48 with HIV infection) were diagnosed with DLBCL and offered different regimens (R-CODOX M-IVAC: 22 HIV+/20 HIV-). CNS prophylaxis was given according to each centre policy. Clinical features according to HIV status were compared using chi-square or Fisher's exact test. Survival analysis was performed using the Kaplan-Meier method and log-rank test. Multivariate analysis was done using the Cox regression proportional hazards model.
Results: Patients characteristics according to HIV status are shown in Table 1. HIV+ patients had significantly more B-symptoms and had more extranodal sites of disease at diagnosis, with more common involvement of the gastrointestinal tract, lung and spleen than HIV- controls. Central nervous system involvement was rare and similar between groups. The proportion of patients with high risk disease according to the International Prognostic Index was similar between groups. The HIV viral load (VL) was undetectable at the time of DLBCL diagnosis in 25 of 82 (30%) patients with available data. Forty-seven patients (57%) had a CD4 count <200/mL and 15% a CD4 count <50/mL. The majority (80/86; 93%) of patients with HIV infection received HAART concomitantly during chemotherapy. Response rate and median duration of response were similar for both cohorts. After a median follow-up of 45 months, 30 patients relapsed after achieving CR, including four HIV+ patients. Ninety-five patients have died (18 HIV+ patients), 72 of them due to DLBCL. Four patients (2 HIV+ and 2 HIV-) died due to treatment toxicity. Survival was similar with 2-year event-free survival (EFS) of 65% (95%CI: 56–72) for HIV+ and 59% (95%CI: 46–69) for HIV- cohort (P=NS). Five-year overall survival (OS) was 88% (95%CI: 80–92) and 79% (95%CI: 67–87) for HIV+ and HIV- patients respectively (P=0.05). HIV status did not predict OS or EFS on multivariate analysis including all variables comprising the IPI and HIV status.
Summary / Conclusion: In this retrospective study, patients diagnosed with DLBCL in the setting of HIV infection have more systemic symptoms and extranodal involvement. However this did not translate in a larger proportion of patients with high risk disease according to the IPI. HIV status does not influence the outcome of patients diagnosed with DLBCL treated with R-CHOP in the HAART era.

Table 1. Clinical characteristics of the patients included in the study.

	HIV+ (n= 86) No.(%)	HIV- (n= 208) No.(%)	p
Male	69 (80)	119 (57)	<0,001
Age, y (median, range)	48 (20 – 67)	64 (17 – 91)	
Age > 60	8 (9)	131 (63)	<0,001
Stage III-IV	63 (73)	134 (64)	NS
B-symptoms	54 (63)	60 (29)	<0,001
≥ 2 extranodal sites	45 (52)	46 (22)	<0,001
Bone marrow	5 (6)	29 (14)	NS
GI tract	20 (23)	23 (11)	0,01
Lung	20 (23)	14 (7)	<0,001
Spleen	15 (17)	0 (0)	<0,001
CNS involvement	2 (2)	7 (3)	NS
High LDH	35 (41)	105 (51)	NS
ECOG PS ≥2	33 (38)	33 (16)	<0,001
IPI ≥3	32 (37)	84 (42)	NS
Response Rate (%)	73	73	NS
Relapse (from CR/CRu)	4 (6)	26 (17)	NS
Duration of response (median, months)	41	38	NS

P302

ALLOGENEIC-STEM CELL TRANSPLANTATION UPFRONT IN THE TREATMENT OF PATIENTS WITH ADVANCED T-CELL LYMPHOMA: AN INTENTION-TO-TREAT ANALYSIS FROM A SINGLE INSTITUTION.

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Background: Non-cutaneous T-cell lymphomas (TCL) have a dismal outcome (except for ALK + anaplastic T-cell lymphoma) compared to B-cell lymphomas. Polychemotherapy regimens like CHOP remain standard of care but usually fail to cure most patients. Autologous stem cell transplantation (ASCT) upfront has been evaluated in several retrospective and few prospective studies. Results are heterogeneous and not comparable from one study to another. Experience regarding allogeneic-stem cell transplantation (allo-SCT) in TCL is still sparse. Allo-SCT is often, if not always, proposed as a salvage option for heavily pretreated relapsed or refractory patients. Thus, its role in the therapeutic arsenal of TCL remains to be established. However, a previous large study suggested that allo-SCT can cure relapsed/refractory patients (Le Gouill *et al*, JCO 2008).
Aims: We initiated a single centre homogenous and pre-defined treatment algorithm addressing the role of allo-SCT upfront for patients with advanced TCL. All patients aged <70 y. (except alk + ALTC) with stage >I disease at time of diagnosis that were referred to our institution were systematically planned to receive CHOP or CHOP-like induction chemotherapy followed by allo-SCT upfront from an HLA-matched related or unrelated donor or cord blood cells (CBU).
Methods: From November 2004 to December 2012, 49 patients met these criteria. Diagnosis were PTCL-NOS in 33 cases, AITL in 4 cases, Alk neg ALTCL in 7 cases; T/gd lymphoma in 2 cases or other entities in 3 cases. Median age at diagnosis was 50y (range, 29-67y). Thirty-one patients were males. IPI score was >or= to 2 in 35 cases, while 20 patients presented with at least two extranodal localizations. An HLA-matched donor (sibling in 15 cases, unrelated in 17 cases or suitable CBU units (n=7)) could be found for 39 patients. Overall, 15 patients (20%) reached CR and 25 patients (50%) reached PR. Eighteen patients had to receive salvage chemotherapy (high-dose aracytine in 8 cases; pentostatin in 4 cases and other in 6 cases). After salvage chemotherapy, two patients reached CR and 8 reached PR. Among these patients, 7 could proceed to allo-SCT. In all, 29 patients (60%) out of the total 49 patients proceeded to allo-SCT (mainly using a reduced-intensity conditioning regimen). 20 patients did not proceed to allo-SCT because of progressive disease (n=13), absence of matched donor (n=3), unfit patients with comorbidities (n=3) or patient refusal (n=1). Disease status prior to allo-SCT was CR1 in 12 cases and PR1 in 17 cases. Of note, three patients underwent ASCT prior allo-SCT because graft was not available on time.
Results: In the whole series of 49 patients, median follow-up (mFU) was 16.3 months. 1 and 2 years-PFS rates calculated from diagnosis were 56 % (CI95%; 43.6-71.5) and 51.3% (CI95%; 39-67.5), respectively. One and 2-years-OS were 60 % (CI95%; 47.7-75.2) and 50.3 % (CI95%; 40.6-69.2), respectively. For allo-SCT patients (n=29), 1- and 2y-PFS calculated from time of allo-SCT (mFU= 32.2 months) were 64.7% (CI 95%; 49.2-85.0) and 60.4 % (CI95%; 44.5-81.9); 1- and 2y-OS were 71.6 (CI95%; 56.7-90.4) % and 71.6 (CI95%; 56.7-90.4), respectively. Nine transplanted patients (including 2 patients in CR at time of allo-SCT) patients died. Causes of death were: disease progression (n=6) or toxicity (n=3). TRM at 1 year was 8.2% (CI95%; 0-18.5). In the non-transplanted group (n=20), only 6 patients are still alive.
Summary / Conclusion: In conclusion, the current series suggests that allo-SCT is feasible with low toxicity rate when performed upfront in responder patients and it can provide long term disease control. However, the intention-to-treat analysis suggests that only 60% of patients that were planned to undergo allo-SCT at diagnosis could be actually transplanted. Absence of response after induction chemotherapy was the major reason for patients not undergoing allo-SCT, underlining the urgent need for new therapeutic options to increase response rates in this yet incurable disease.

P303

INTRATHECAL METHOTREXATE IS NOT EFFECTIVE IN PREVENTING CENTRAL NERVOUS SYSTEM RELAPSE IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS TREATED WITH R-CHOP

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Background: Central nervous system (CNS) relapse is a serious complication of diffuse large B-cell lymphoma (DLBCL), occurring in approximately 5% of patients. Rituximab (R) does not strongly penetrate the blood-brain barrier, and therefore, CNS relapse continues to be of great concern even in an era when R is routinely administered.
Aims: To evaluate the extent to which CNS prophylaxis is achievable using intrathecal methotrexate (IT-MTX) in DLBCL patients.
Methods: A total of 362 patients with newly diagnosed DLBCL received 6 cycles (maximum 8 cycles) of full-dose R-CHOP therapy between 2003 and 2009. Of these patients, 322 who achieved complete remission (CR) were enrolled in this study. Patients more than 70 years old with an Eastern Cooperative Oncology Group performance status (PS) greater than 1 were excluded if they were treated with a reduced dose but were included if they were treated with full-dose R-CHOP, at the discretion of the attending physician. Reduction of the initial therapy dose by more than 20% because of a major comorbidity was also an exclusion criterion. CNS prophylaxis was consisted of 4 doses of 15mg/body IT-MTX with 25 mg hydrocortisone administered once CR was achieved. In general, CNS prophylaxis was administered to patients with at least one of the following factors at presentation: 1) a lactate dehydrogenase (LDH) level equal to or more than twice the upper normal limit; 2) the presence of a bulky mass of at least 10cm in diameter; 3) a PS of more than 1; or involvement of the 4) bone marrow, 5) skin, 6) testicles, 7) nasal/paranasal tissue, 8) bone, 9) or breast. Although CNS prophylaxis was generally performed only for patients aged less than 70 years received prophylaxis at the discretion of the attending physician. CNS relapse was defined as the detection of malignant cells in cytocentrifuged preparations of cerebrospinal fluid or intracranial mass on computed tomography or magnetic resonance imaging.
Results: The median age of the 322 patients (male, 188: female, 134) was 64 years (range, 18-80 years). The median follow-up time was 61 months. Forty patients (12%) received CNS prophylaxis (group A) and the remaining 282 patients (88%) did not (group B). CNS relapse during the first complete remission (1CR) was noted in 3 patients in group A (8%) and in 8 patients in group B (4%) although this difference was not statistically significant. The median time between the initiation of R-CHOP and the CNS relapse was 8.2 months (range, 3.5-34.0 months). The cumulative incidence of CNS relapse was 3.6% at 3 years. The 3-year survival rate of the 11 patients with CNS relapse during 1CR was 62%. CNS relapse was of the parenchymal type in 2 patients and of the combined (leptomeningeal and parenchymal) type in 1 patient in group A and of the leptomeningeal type in 1 patient, of the parenchymal type in 5 patients, and of the combined type in 2 patients in group B. In subgroup analyses of patients with each risk factor for CNS relapse described above the incidence of CNS relapse was not statistically different between groups A and B. Furthermore, in subgroup analyses of patients with advanced stage disease, high/high-intermediate risk as defined by the International Prognostic Index, an elevated LDH level, and involvement of at least 2 extranodal sites, the incidence of CNS relapse was not statistically different between groups A and B.
Summary / Conclusion: In DLBCL patients treated with R-CHOP, IT-MTX administration was not an effective prophylaxis for CNS relapse. Alternative strategies such as intravenous MTX should be evaluated because most CNS relapses were of the parenchymal type.

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R-CHOP21 VS R-CHOP14 IN 1024 DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: RESULTS FROM A MULTICENTRE RETROSPECTIVE STUDY OF FONDAZIONE ITALIANA LINFOMI (FIL)

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Background: Diffuse large B cell lymphoma (DLBCL) is the most common histotype of non-Hodgkin's lymphoma. R-CHOP21 (C21) is considered the standard therapy but a large number of studies tested the dose dense regimen R-CHOP14 (C14).
Aims: The aim of our multicenter retrospective study was to evaluate the efficacy in terms of Overall Survival (OS) and Progression free survival (PFS) of the two regimens C21 and C14 in a large cohort of patients (pts) with a diagnosis of DLBCL or follicular grade IIIb lymphoma, treated with curative intent.
Methods: Patients diagnosed between January 2002 to December 2011 were considered for the study: 1024 pts were collected in thirteen Italian Haematoloy Departments, all pts included in the analysis were treated consecutively over a period of time decided by each centre.
Results: 702 were treated with C21 and 322 were treated with C14. The two cohorts of pts were balanced for all clinical characteristics excepted for age higher than 60 years (66% in C21 vs 37% in C14 (P 0.000)), high-intermediate and high risk IPI (33% C21 vs 28% C14; P 0.01). All pts in C14 used primary prophylaxis with G-CSF, and patients treated with C21 used G-CSF as secondary prophylaxis. After induction therapy 817 pts (80%) obtained a complete remission: 553/702 (79%) after C21 and 264/322 (82%) after C14. After a median period of observation of 36 months 101 pts out of 817 CR pts relapsed, 69/553 (12,4%) in the C21 arm and 32/264 (12.1%) in the C14 arm. OS at 3 years was 81% in C21 and 85% in C14 (P:0.1); PFS was 70% in C21 and 72% in C14 (P:0.4). Univariate statistical analysis showed that OS was significantly superior in younger pts (<60 year), Ann Arbor stage I-II, absence of B-symptoms, no bulky disease, negative bone marrow biopsy, low and low-intermediate risk IPI; PFS was significantly superior for the same characteristics. Multivariate analysis showed that OS was affected by age (P .002) and IPI (P .0000) and PFS by stage (P .002) and IPI (P .0000). The results of univariate analysis performed stratifying for therapy shown that C14 is able to overcome some negative prognostic factors as symptoms and bulky disease at diagnosis. No differences in haematological or extra-haematological toxicities were observed in the two arms; four deaths for sepsis were observed (1 in C14 and 3 in C21).
Summary / Conclusion: Our results confirm that C14 does not improve either OS or PFS in comparison with standard C21 in the whole lymphoma population analysed. However, we observed in univariate analysis that the intensified therapy reduced the prognostic impact on OS of some important factors such as symptoms and bulky disease in comparison with standard C21, suggesting that C14 could be useful in a subset of pts. Due to the retrospective nature of this study these results should be confirmed in prospective multicentre studies.

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SINGLE-AGENT LENALIDOMIDE IN RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA: A COMBINED ANALYSIS FROM THE MCL-001, NHL-002, AND NHL-003 STUDIES

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Background: Mantle cell lymphoma (MCL) patients (pts) with relapsed/refractory disease have a poor overall prognosis and limited treatment options following bortezomib. Lenalidomide is an immunomodulatory agent with consistent clinical activity and a predictable safety profile, as shown in multiple phase II studies of relapsed/refractory pts with aggressive non-Hodgkin lymphoma, including MCL following bortezomib treatment.

Aims: Evaluate the combined efficacy and safety of single-agent lenalidomide in pts with relapsed/refractory MCL from 3 phase II studies: MCL-001 (n=134), NHL-002 (n=15), and NHL-003 (n=57).
Methods: Lenalidomide was given at 25 mg/day PO on days 1-21 of each 28-day cycle in pts with relapsed/refractory MCL as tolerated for 52 weeks (NHL-002) or until disease progression (NHL-003 and MCL-001). Efficacy data were examined by investigators for NHL-002 and independent central review for MCL-001 and NHL-003 studies.
Results: Relapsed/refractory MCL pts (N=206) had a median age of 67 years (range 33-84); 63% were ≥65 years, and 91% had stage III/IV disease. Pts received a median of 4 prior therapies (range, 1-13); 51% received ≥4 prior regimens and 76% had prior bortezomib. The efficacy data across studies are shown on Table 1: ORR was 32% (10% CR/CRu), median TTR of 2.1 months, and median DOR 16.6 months (not reached for CR/CRu pts at 38.9+ months). Kaplan-Meier estimates showed a median PFS of 5.4 months and OS of 23.9 months. The mean daily dose of lenalidomide was 21 mg. The most common grade 3/4 adverse events (AEs) were 44% neutropenia, 29% thrombocytopenia, 11% anemia, 7% fatigue. Other any-grade AEs included 7% tumor flare reaction, 7% venous thromboembolic events, and 3% invasive second primary malignancies. Lenalidomide treatment provided a consistent ORR across all subgroups except in patients with high LDH.
Summary / Conclusion: Relapsed/refractory MCL pts showed a consistent and predictable safety profile among 3 phase II studies of single-agent lenalidomide. The response to lenalidomide was rapid and durable in heavily pretreated pts, including pts who received prior bortezomib.

Table 1. Efficacy of lenalidomide in relapsed/refractory MCL.

Efficacy outcomes	(N=206)
ORR, % (95% CI)	32 (25-38)
CR/CRu, % (95% CI)	10 (6-15)
Median TTR, mo (95% CI)	2.1 (1.6-24.2)
Median DOR, mo (95% CI)	16.6 (9.2-32.4)
Median PFS, mo (95% CI)	5.4 (3.7-6.7)
Median OS, mo (95% CI)	23.9 (19.0-34.9)

CR/CRu, complete response/CR unconfirmed; DOR, duration of response; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; TTR, time to response.

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PHARMACOKINETIC/PHARMACODYNAMIC MODELING OF THE BISPECIFIC T-CELL ENGAGER ANTIBODY BLINATUMOMAB IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA

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Background: Blinatumomab (AMG 103) is a novel, anti-CD19/anti-CD3 bispecific, T-cell engager (BiTE®) antibody that redirects T-cells to $CD19^+$ B-cells, resulting in target-specific B-cell lysis.
Aims: Using a pharmacokinetic/pharmacodynamic (PK/PD) modeling approach, we evaluated the exposure-response relationship in patients with non-Hodgkin's lymphoma (NHL) who received blinatumomab in a phase 1 study.
Methods: Patients (N=76) with NHL received blinatumomab by continuous intravenous (cIV) infusion at doses ranging from 0.5−90 $\mu g/m^2$/d in 4- or 8-week cycles. All patients provided written informed consent. PK parameters of blinatumomab were determined. Pharmacodynamic responses during blinatumomab treatment included peripheral B- and T-lymphocyte counts, serum cytokine levels, and sum of the products of the greatest diameters of tumor size in lymph nodes (SPD) at the end of treatment. Blinatumomab concentration in the serum at steady state (C_{ss}) and the cumulative area under the concentration–time curve (AUC_{cum}) over the period before the evaluation of SPD were used to determine the exposure-SPD relationship.
Results: Blinatumomab exhibited linear pharmacokinetics at the doses tested. The early pharmacodynamic response to blinatumomab was characterized by B-cell depletion, T-cell redistribution, and transient cytokine release. Following cIV infusion at doses from 0.5−90 $\mu g/m^2$/d, B-cells declined at a first-order rate with a dose-dependent rate constant ranging from 0.16−1.0 h^{-1}, resulting in complete B-cell depletion within 48 hours at doses ≥5 $\mu g/m^2$/d. T-cell counts decreased in a dose-independent manner within 24 hours of initiation of infusion, completely returning to baseline within 2 weeks. Cytokine elevation of greater frequency and magnitude was observed only in some patients who received higher blinatumomab doses. Cytokine elevation was transient and levels were highly variable across patients but diminished within 48 hours after start of infusion. The blinatumomab exposure-SPD relationship was best described by an inhibitory E_{max} model ($E = E_0-(I_{max}*C)/(IC_{50}+C)$). According to the model estimation, a C_{ss} of 2141 pg/mL and AUC_{cum} of 1381 μg· h/L would result in a 50% reduction in SPD. These exposure levels could be achieved with a blinatumomab dose of 54 $\mu g/m^2$/d administered over 4 weeks.
Summary / Conclusion: Blinatumomab doses ≥5 $\mu g/m^2$/d completely depleted B lymphocytes from the circulation, with a depletion rate proportional to the dose. Blinatumomab C_{ss} and AUC_{cum} were correlated with tumor reduction. Tissue accessibility and tumor microenvironment may explain why higher doses and a longer time were required for tumor reduction, compared with peripheral B-cell depletion. This PK/PD model can be used to inform dose selection for future studies of blinatumomab in NHL.

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INFERIOR OUTCOME OF ELDERLY DLBCL PTS WITH 25-OH VITAMIN D DEFICIENCY TREATED WITH CHOP PLUS RITUXIMAB: RESULTS OF THE RICOVER-60 TRIAL OF THE GERMAN HIGH-GRADE NON-HODGKIN LYMPHOMA STUDY GROUP DSHNHL

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Background: Vitamin D deficiency was shown to be is associated with a worse outcome in patients with non-Hodgkin's lymphoma (Drake *et al*., 2010).
Aims: To study whether this observation could be confirmed in patients with aggressive B-cell lymphomas treated uniformly within a prospective trial, we analyzed 25-OH vitamin D serum levels in patients treated within the RICOVER-60 trial of the DSHNHL.
Methods: 25-OH Vitamin D serum levels were determined with a commercial chemoluminescence immunoassay in the serum from elderly patients of the RICOVER-60 trial which compared 6 or 8 cycles of CHOP, both with and without rituximab.
Results: 193 of 359 pts (53.8%) had vitamin D deficiency (<10 ng/mL) and 165/359 patients (46.0%) had vitamin D insufficiency (10-30 ng/ml) according to current definitions. When treated with R-CHOP, patients with vitamin D levels ≤8 ng/ml had a 3-year EFS of 59% compared to 79% of patients with vitamin D serum levels >8 ng/ml; the respective figures for 3-year overall survival were 70% and 82%, respectively. In R-CHOP pts these differences were significant in a multivariable analysis adjusting for IPI risk factors with a hazard ratio (HR) of 2.1 (P=0.008) for EFS and a HR of 1.9 (P=0.040) for OS. In pts treated without R effects of vitamin D deficiency were significant only for OS (HR 1.8; P=0.025), but not with respect to EFS (HR 1.2; P=0.388). These results were confirmed in an independent validation set of 63 patients treated within the prospective RICOVER-noRx study.
Summary / Conclusion: Vitamin D deficiency is with a significantly worse outcome of patients with DLBCL treated with R-CHOP. The stronger adverse effect of vitamin D deficiency in patients receiving rituximab suggests that vitamin D deficiency interferes with the mechanisms of action of this antibody. A prospective study evaluating the effects of vitamin D substitution on outcome of patients receiving R-CHOP is warranted.
S*upported by Deutsche Krebshilfe.*

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SUBCUTANEOUS RITUXIMAB AND CHEMOTHERAPY ACHIEVES SIMILAR TROUGH LEVELS, SAFETY, AND RESPONSE AS INTRAVENOUS RITUXIMAB IN FIRST-LINE FOLLICULAR LYMPHOMA: STAGE 1 RESULTS OF THE PHASE 3 SABRINA STUDY

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Background: Rituximab (R) plus chemotherapy induction followed by maintenance R is the backbone therapy for follicular lymphoma (FL). Intravenous R (R^{IV}) administration time and patient convenience could be improved by the development of a subcutaneous formulation (R^{SC}).
Aims: R^{SC} is expected to have similar efficacy if it achieves non-inferior serum

C_{trough} levels compared with R^{IV}.
Methods: SABRINA (BO22334; NCT01200758) is a 2-stage phase 3 study of R^{SC} or R^{IV} plus chemotherapy (≤8 cycles CHOP [cyclophosphamide, doxorubicin, vincristine, prednisone] or 8 cycles CVP [cyclophosphamide, vincristine, prednisone]) followed by R^{SC} or R^{IV} maintenance in patients with FL. All patients gave informed consent. The stage 1 primary objective was to estimate the mean serum $C_{trough,SC}$:$C_{trough,IV}$ ratio at day 21 of induction cycle 7 for R^{SC} 1400 mg and R^{IV} 375 mg/m^2 given every 3 weeks. The non-inferiority limit was Θ_L=0.8. Here we report stage 1 end-of-induction outcomes.
Results: Patients with previously untreated confirmed CD20-positive grade1,2, or 3a FL (n=127) were randomized to R^{SC} (n=63) or R^{IV} (n=64). Patients were stratified by Follicular Lymphoma International Prognostic Index score, chemotherapy, and region. In each arm, approximately 63% received CHOP and 37% received CVP chemotherapy. R^{IV} was given in the first induction cycle regardless of randomization. At day 21 of induction cycle7, geometric mean serum C_{trough} levels were 134.6 µg/mL for R^{SC} (n=48) and 83.1 µg/mL for R^{IV} (n=54). $C_{trough,SC}$:$C_{trough,IV}$ was 1.62 (90% confidence interval [CI]: 1.36–1.94); SC non-inferiority was demonstrated by the lower limit of the CI exceeding the non-inferiority limit of 0.8. The geometric mean area under the curve (AUC) ratio (AUC_{SC}:AUC_{IV}) was 1.378 [90% CI: 1.241–1.530]. After a median follow-up of approximately 9 months, each arm had similar rates of all-grade adverse events (n=57 [92%] SC; n=57 [88%] IV) and grade 3/4 adverse events (n=29 [47%] SC; n=30 [46%] IV). Neutropenia was the only grade 3/4 adverse event in >10% of patients (26% SC, 22% IV) and was not associated with increased infection rates. Administration-related reactions (ARRs) occurred in 31 (50%) R^{SC} patients and 21 (32%) R^{IV} patients. 1 and 3 patients in the R^{IV} and R^{SC} arms, respectively, had a grade 3 ARR; none had a grade 4 ARR. ARRs (all grades) in ≥5% of R^{SC} and R^{IV} arms were: erythema (8% vs 3%), pruritus (6% vs 3%), chills (3% vs 6%), injection site erythema (10% vs 0%), and vomiting (3% vs 6%). Investigator-assessed overall response rates were 90.5% (95% CI: 80.4–96.4) in R^{SC} patients and 84.4% (95% CI: 73.1–92.2) in R^{IV} patients. Complete response (confirmed/unconfirmed) rates were 46.0% (29/63 patients, 95% CI: 33.4–59.1) for R^{SC} patients and 29.7% (19/64 patients, 95% CI: 18.9–42.4) for R^{IV} patients. Stable and progressive disease rates were similar in each arm. Consistent with the investigator assessment, results of an independent response review indicate that the switch to SC administration does not impair the anti-lymphoma activity of R.
Summary / Conclusion: The observed serum R^{SC} C_{trough} level was non-inferior to the R^{IV} C_{trough} level at day 21 of induction cycle 7. R^{SC} demonstrated comparable investigator-assessed ORR and CR rates, which were in line with those obtained upon independent review. The safety profile was similar. Stage 1 patients are continuing maintenance treatment; stage 2 has begun recruitment. In conclusion, subcutaneous R administration (1400 mg) is feasible, with a safety profile and efficacy similar to those obtained from intravenous administration.

P309

A MULTICENTER, RANDOMIZED PHASE III STUDY OF RITUXIMAB AS MAINTENANCE TREATMENT VERSUS OBSERVATION ALONE IN PATIENTS WITH AGGRESSIVE B-CELL LYMPHOMA: THE AGMT NHL13 TRIAL

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Background: The clinical impact of maintenance treatment after intensive induction immunochemotherapy in diffuse large B-cell lymphoma (DLBCL) is still unclear. Rituximab (R) maintenance was not superior to observation in the ECOG 4494 study (Habermann TM *et al*. J Clin Oncol. 2006;24:3121-7), which was unfortunately not fully powered for this analysis.
Aims: The Austrian Study Group (AGMT) initiated a clinical trial (NHL13, Eudract Nr. 2005-005187-90, http://www.clinicaltrials.gov/ ct2/show/NCT00400478?term=ML+18223&rank=1) to investigate the value of R maintenance in patients with DLBCL and follicular lymphoma grade 3B (FL G3B) in complete or unconfirmed complete remission (CR, CRu) after induction with R-CHOP-like chemotherapy (Figure 1).
Methods: In the NHL13 multicenter, prospective trial 683 previously untreated adult patients with DLBCL (N=662) or FL G3B (N=21) recruited in 27 countries (163 sites) between June 2004 and September 2008 were randomized. Inclusion criteria were DLBCL at all stages in CR or CRu after treatment with 4 to 8 cycles of R-CHOP like therapy. Patients were randomized between R maintenance (375 mg/m^2 every 2 months for 2 years) (N=338) and observation only (N=345). The last patient received R in September 2010. The study was closed after 148 events had been reached in March 2012.
The primary endpoint of this study was event-free survival. Secondary endpoints included progression-free survival and overall survival. Data will be analysed using a Cox regression model.
Results: Both arms were well balanced regarding clinical presentation, sex or prognostic indices at study entry. No major safety signals were seen in 2 planned interim analyses. In the interim analysis in 2010 a significantly higher rate of infections (mainly NCI CTC V 2.0 grade 1 or 2) was noted in female patients in the R arm (P=0.004). Final analysis will be performed in March 2013 and the data will be presented at the meeting.
Summary / Conclusion: The AGMT NHL13 trial should provide a definitive answer to the question whether Rituximab maintenance therapy is beneficial for patients with DLBCL and FL G3B in general or in particular subgroups.

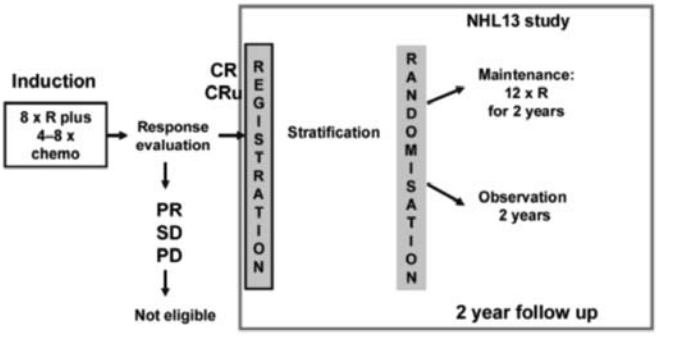

Figure 1. NHL13 Srudy design.

P310

PIXANTRONE MONOTHERAPY IN HISTOLOGICALLY CONFIRMED, RELAPSED OR REFRACTORY AGGRESSIVE B-CELL NON-HODGKIN LYMPHOMA: POST HOC ANALYSIS FROM A PHASE III TRIAL

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Background: Pixantrone (PIX) is conditionally approved by the European Medicines Agency for the treatment of multiply relapsed or refractory aggressive B-cell NHL, and is the only therapy to be granted approval for these patients. The Phase III EXTEND trial was conducted in patients with aggressive *de novo* or transformed NHL who had previously received ≥2 regimens. Analyzed by ITT, PIX was associated with a higher rate of CR or unconfirmed CR (CRu) compared with physicians' choice of monotherapy (20.0% vs 5.7%, P=0.021) and longer PFS (5.3 vs 2.6 months [Pettengell R *et al*. Lancet Oncol 2012]).
Aims: To evaluate the efficacy of PIX in the subset of EXTEND patients who had aggressive B-cell lymphoma, as confirmed by central independent pathological review (histologically confirmed ITT [HITT] B-cell population). Within this group, we also investigated efficacy in clinically relevant subgroups: those with 2–3 previous regimens (i.e. excluding patients with ≥ 4 previous regimens) and those with and without previous rituximab.
Methods: EXTEND was a Phase III multicenter, open-label randomized trial of PIX (85 mg/m² PIX dimaleate intravenously on days1, 8, and 15 of a 28-day cycle, for up to 6 cycles) vs physicians' choice of monotherapy (vinorelbine, oxaliplatin, ifosfamide, etoposide, mitoxantrone, gemcitabine or rituximab). The primary endpoint was CR or CRu. For this *post-hoc* subgroup analysis, patients with DLBCL, follicular grade III lymphoma or transformed indolent lymphoma were retrospectively identified through consensus of two (three in case of disagreement) independent pathologists.
Results: A total of 140 patients (PIX/comparator; n=70/70), based on the site diagnoses, were randomized into the EXTEND study. In the HITT B-cell population after central independent histological review, (PIX/comparator; n=50/47), mean age was 59.6/55.3 years; 62.0%/51.1% were male; median previous regimens was 3.0/3.0; previous rituximab was recorded in 60.0%/55.3%. Diagnosis was DLBCL in 82.0%/87.2%, transformed indolent lymphoma in 14.0%/10.6%, follicular lymphoma grade III in 4.0%/2.1%. Compared with com-

parator, PIX was associated with significantly higher rates of CR at end of treatment (P=0.027) and at end of study (up to 18 months follow-up, P=0.013) and longer median PFS (Table). In the HITT B-cell population with 2–3 previous regimens, at the end of the study, PIX was associated with significantly higher rates of CR/CRu (P=0.047), CR (P=0.012), ORR (P=0.005) and improvement in PFS (Table 1). In patients with 2–3 prior regimens without prior rituximab (PIX/comparator; n=19/21), PIX was associated with a CR/CRu rate of 15.8% vs 4.8% (not significant, NS) and median PFS was 6.1 vs 3.5 months (HR 0.36, 95% CI 0.18–0.73). In those with prior rituximab, PIX (n=20) and comparator (n=18) led to CR/CRu rate of 30.0% vs 5.6% (NS) and a median PFS of 5.4 vs 2.8 months (HR 0.52, 95% CI 0.26–1.04). In all three subgroup analyses, OS was longer with PIX vs comparator, but did not reach statistical significance.
Summary / Conclusion: Compared with comparator monotherapy, PIX provided significantly improved efficacy (as measured by CR/CRu, ORR, and PFS) in patients with histologically confirmed aggressive B-cell lymphoma treated with 2–3 previous regimens. The results in this subgroup were similar to those in the ITT population.

Table 1.

End of Study	HITT aggressive B-cell (2–9 previous regimens)		HITT aggressive B-cell (2–3 previous regimens)	
	PIX	Comp	PIX	Comp
n	50	47	39	39
CR/CRu, n (%)	9 (18.0)	4 (8.5)	9. (23.1)*	2 (5.1)
CR, n (%)	7 (14)*	0	7 (17.9)*	0
ORR	18 (36.0)*	8 (17)	17 (43.6)*	5 (12.8)
Median PFS, months	5.6	2.5	5.7	2.8
HR (95% CI)	0.51 (0.33, 0.78)		0.44 (0.27, 0.71)	
Median OS, months	8.1	6.3	11.9	7.0
HR (95% CI)	0.72 (0.45, 1.13)		0.67 (0.4, 1.12)	

Pix, pixantrone; comp, comparator. * pixantrone vs. comparator: p < 0.05

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SOX11 CAN BE USED AS A MINIMAL RESIDUAL DISEASE MARKER FOR MANTLE CELL LYMPHOMA IN LONGITUDINAL STUDIES

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Background: Mantle cell lymphoma (MCL) is an aggressive subtype of Non-Hodgkin Lymphoma (NHL). It is characterized by the translocation t(11;14)q(13:32) which leads to the over-expression of Cyclin D1 (*CCND1*), although a subgroup of less than 5% does not express *CCND1*. Recent studies have also found the transcription factor *SOX11* to be a possible diagnostic marker for MCL, independent of *CCND1* expression. The advantage of *SOX11* is that it is not expressed in healthy donors, as opposed to *CCND1*, and can therefore be used as a sensitive minimal residual disease (MRD) marker as well as for diagnostic purpose.
Aims: To quantify the *SOX11* expression in a longitudinal study to verify its potential as an MRD marker for MCL using a highly sensitive and specific *SOX11* RT-qPCR assay.
Methods: A *SOX11* specific RT-qPCR assay with a sensitivity of $2x10^{-4}$ has been established without risk of genomic DNA contamination (1) which enables us to use *SOX11* as a molecular marker for detection of MRD in MCL. The *SOX11* expression is reported per 1000 reference genes (RG) copies (RG: *β2M* and *GUS*). The detection limit for *SOX11* was defined to Cq = 40. For *CCND1* the normal limit was defined as 5.5 copies per 1000 RG copies (Hamborg *et al*., Eur J Haem, 2012). In this longitudinal study we followed 16 patients, diagnosed with MCL, during their clinical disease course. The expression of *SOX11* and *CCND1* was quantified in peripheral blood (PB) and bone marrow (BM) and subsequently compared to the clinical status of the patients.
Results: The 16 patients were followed by simultaneous PB and BM sampling together with routine clinical evaluation and CT scan. In our study 15 out of 16 patients were *SOX11* positive in PB at either diagnosis or at relapse contrary to *CCND1* where only 11 out of 16 patients were above the defined normal limit of 5.5 *CCND1* copies per 1000 RG copies as shown in Figure 1A. Data from our study are merged with data from (Hamborg *et al*., Eur J Haem, 2012) and show *SOX11* mRNA level in diagnostic PB (n =44) and BM (n=19) samples, complete remission PB (n=30) and BM (n=22) samples; and relapse PB (n=11) and BM (n=6). We calculated the MRD level based on *SOX11* expression per 1000 RG copies and found a close relationship of *SOX11* expression and clinical status based on routine pathological examination of 16 patients diagnosed with MCL as exemplified in Figure 1B. In general, we saw a tendency for higher expression of *SOX11* in PB compared to BM at diagnosis. This could suggest that PB sampling for MRD monitoring could be used preferably to BM aspiration, which is more extensive and often a painful procedure.
Summary / Conclusion: By using an RT-qPCR assay, specifically targeting *SOX11* mRNA, we found that the over-expression of *SOX11* could be a useful marker for MRD quantification in MCL resembling the clinical status of the patients. This was demonstrated in patients with longitudinal sampling of blood and bone marrow. Furthermore, we postulate that the application of *SOX11* as an MRD marker in MCL could be precious in those cases where *CCND1* is not over-expressed at the time of diagnosis.

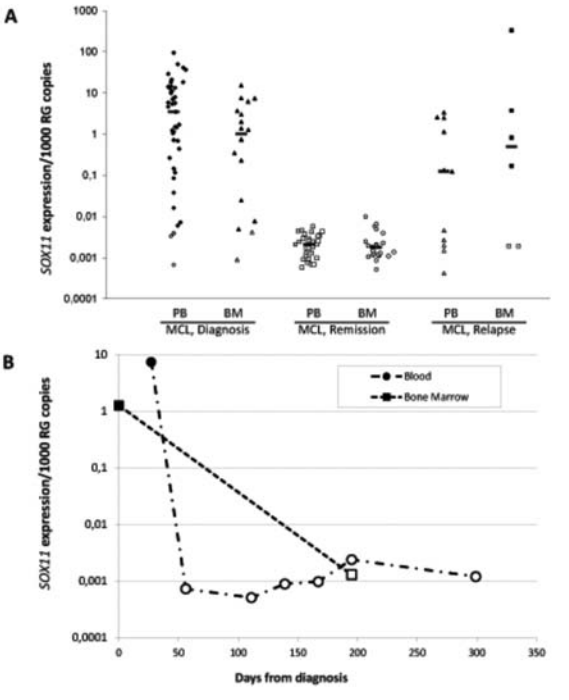

Figure 1.

P312

CONTRIBUTION OF CEREBROSPINAL FLUID SCD19 LEVELS TO THE DETECTION OF CNS LYMPHOMA: IMPACT ON DISEASE OUTCOME

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Background: Flow cytometry (FCM) is more sensitive than conventional cytology (CC) for detection of occult leptomeningeal lymphoma. However, still FCM-negative patients exist which show central nervous system (CNS) recurrence and/or CNS parenchymal disease.
Aims: Here we evaluated the cerebrospinal fluid (CSF) levels of a large panel of B-cell associated markers and its contribution to the diagnosis of CNS lymphoma.
Methods: We investigated the CSF levels of CD19, CD21, CD22, CD24, CD38, CD44, CD72, free light chain (FLC)-kappa, FLC-lambda, IgA, IgG, IgM and b_2-microglobulin in 114 B non-Hodgkin lymphoma (NHL) -92 diffuse large B-cell (DLBCL) and 22 Burkitt lymphomas (BL)- patients at risk of CNS disease who gave their informed consent to participate in the study, and determined their utility as surrogate markers for CNS lymphoma and patient outcome.
Results: From all markers investigated, only CD19 and to a less extent b_2-microglobulin were associated with CNS disease. Higher soluble CD19 (sCD19) CSF levels were associated with a greater frequency of neurological symptoms, parenchymal CNS lymphoma, lymphocytosis, poorer performance status and extra-nodal disease; conversely, sCD19 CSF levels showed no significant association with the degree of involvement of lymph nodes, blood, bone

marrow, liver and the spleen. When combined with occult CNS disease by FCM, sCD19 CSF levels emerged as an independent predictor for event-free and/or overall survival.
Summary / Conclusion: Presence of lymphoma cells by FCM and/or increased sCD19 CSF levels, are highly suggestive of leptomeningeal and/or parenchymal CNS lymphoma and are associated with a poorer event-free and/or overall survival of DLBCL and BL patients at risk of CNS disease.

P313

RITUXIMAB-PECC FOLLOWED BY 90Y-IBRITUMOMAB TIUXETAN CONSOLIDATION IN RELAPSED OR REFRACTORY DLBCL PATIENTS WHO ARE NOT ELIGIBLE FOR OR AFTER ASCT: PRELIMINARY RESULTS FROM A PHASE II HOVON STUDY
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Background: Patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) after- or not eligible for autologous stem cell transplantation (ASCT) have a poor prognosis. The optimum salvage therapy for these patients is not known. Treatment results with second-line chemotherapy have generally been disappointing. In some centers in the Netherlands PECC (Prednisone, Etoposide, Chlorambucil and Lomustine) combination chemotherapy is used as salvage treatment for such patients. PECC is a completely oral schedule. The efficacy and toxicity of PECC in this patient category has not been evaluated systematically before. ^{90}Y-ibritumomab tiuxetan (Zevalin®) showed clinical activity as a single agent in relapsed DLBCL.
Aims: We conducted a prospective multi-center phase II study evaluating induction therapy with Rituximab (R)-PECC followed in responsive patients by ^{90}Y-ibritumomab tiuxetan consolidation.
Methods: Patients aged ≥ 18 years with refractory CD20 positive DLBCL or first or second relapse, more than one year after or not eligible for ASCT, were enrolled. Treatment consisted of R-PECC (Prednisone 40 mg/m^2 po D1-5; Etoposide 100 mg/m^2 po D1-5; Chlorambucil 8 mg/m^2 po D1-5; Lomustine 80 mg/m^2 po D1 and Rituximab 375 mg/m^2 iv D1), every 28 days for 4 cycles. Patients with progressive disease after 2 cycles went off protocol. Patients in complete or partial remission after 4 cycles received consolidation treatment with ^{90}Y-ibritumomab tiuxetan at the standard single dose of 15 MBq/kg (0.4 mCi/kg) 6 to 12 weeks after start of the last R-PECC cycle. Response to treatment was evaluated according to the revised 2007 Cheson criteria.
Results: Between November 2008 and February 2012 62 patients were enrolled. We report data for response and toxicity after the R-PECC induction for all patients. Median age was 70 years (range, 45-82). All patients received CHOP at first-line, 12 without rituximab. Prior therapies consisted of (R)-CHOP (65%), R-CHOP and R-DHAP/VIM (24%) or R-CHOP and R-DHAP/VIM plus ASCT (11%). Fourteen patients (23%) were refractory to the last prior therapy. Progressive disease occurred in 20 patients (32%). Overall response rate (ORR) after R-PECC was 52% (34% CR,18% PR). ORR of relapsed patients vs refractory patients was 64% vs 9%, P=0.099. Median response duration was 7.4 months. One patient died because of therapy related toxicity due to sepsis and pneumonia after the first R-PECC cycle. The most common grade 3 or 4 adverse event was haematological toxicity (47%), followed by infection (18%), malaise (13%) and gastro-intestinal toxicity (8%). Forty-one serious adverse events (SAEs) occurred in 24 patients, Most SAEs were possibly or probably related to therapy (mainly hospitalisations due to infections). Approximately half of the patients (45%) have entered the ^{90}Y-ibritumomab tiuxetan consolidation phase of the study.
Summary / Conclusion: The R-PECC regimen shows good clinical activity in relapsed DLBCL patients. Its activity in refractory patients is low. This largely oral regimen provides patients not eligible for high-dose salvage treatment with a convenient treatment schedule with an acceptable safety profile.

P314

OUTCOME OF CHILDREN WITH MATURE B-CELL LYMPHOMA TREATED ON LMB-96 PROTOCOL; A CCHE REPORT
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Background: The outcome of childhood non-Hodgkin lymphoma (NHL) has improved steadily, yet the therapy offered in oncology units in low-income countries is usually less aggressive.
Aims: To investigate treatment outcome of newly diagnosed mature B cell NHL patients treated on LMB-96 treatment protocol at the Children's Cancer Hospital Egypt (CCHE).
Methods: Retrospective review of patient charts was performed. Study period was between July 2007 till December 2011, and patients were followed till end of December 2012. All patients were treated with FAB LMB 96 protocol with no dose reduction.
Results: A total of 315 children were included in this retrospective study. They were 74females (23.5%) and 241 males (76.5%). Median age was 5.4 years (ranged 36 days-*18*.0 years). Diagnosis was Burkitt's lymphoma in 249 patients (77.7%), B-Cell ALL (L3) in 44 patients (13.96%), diffuse large B-cell lymphoma in 17 patients (5.39%), mediastinal B cell lymphoma in 3 patients (0.95%), and mature B NHL (NOS) in 2 patients (0.63%). According to the St. Jude stagingsystem, 11 patients (3.5%) had stage I, 92 patients (29.2%) had stage II, 141 patients (44.8%) had stage III, and 71 patients (22.5%) had stage IV. According to the LMB-96 protocol, risk stratification: 24 patients(7.6 %) were group A, 220 patients (69.8 %) group B, 36 patients (11.4 %) group C, while 35 patients were group C CNS positive (11.1%). Out of the 220 group Bpatients evaluated after the COP pre-phase; CR/PR was obtained in 186 patients (84.5 %), while 22 patients (10%) had Stable/Progressive disease. Of the 315 patients included in our study, 57 patients (18.1%) died (52 in active disease, while 5 died in CR). At the end of follow up period, 238 patients were alive in CR (76.3 %), 8 patients (2.5%) were alive but had a resistant disease, and 10 patients (3%) abandoned treatment. With a median FU period of 23.27m (range: 0.984 m- 59 m), overall survival was 81.1%. It was 100% for group A patients, 85.2% for Group B, and 61.8% for Group C patients. Event free survival was 77.5%. It was 100% for Group A, 81.3% for Group B, and 57.9% for Group C.
Summary / Conclusion: LMB-96 treatment protocol has proven to be effective for treatment of Egyptian mature B-cell NHL. Overall survival as well as EFS are comparable to international figures, and toxicity seen is within acceptable standards.

P315

INTERIM POSITRON EMISSION TOMOGRAPHY SCAN PREDICTS EARLY OUTCOMES OF DIFFUSE LARGE B-CELL LYMPHOMA IN THE POST-RITUXIMAB ERA
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Background: The role of interim positron emission tomography (PET) scan to predict outcomes remains equivocal. Recently, in an attempt to standardize reporting criteria for interim PET, Deauville five-point scale (5-PS) and the rate of reduction in maximal standardized uptake value (SUVmax) were proposed. However, Deauville 5-PS might still be related to false positive results in some patients and the rate of SUVmax reduction not be suitable for patients with low baseline or high interim SUVmax.
Aims: The aim of this study was to investigate the prognostic value of interim PET assessment combining 5-PS and the rate of SUVmax reduction in patients with diffuse large B-cell lymphoma (DLBCL) treated with R-CHOP.
Methods: Interim PET responses of 132 patients with newly diagnosed DLBCL and treated with R-CHOP were investigated between 2006 and 2011 in two Korean institutions. Interim PET scan was performed after 3 or 4 cycles of R-CHOP, before 1 week of the next cycle. All PET scans were read by 2 nuclear medicine physicians at each institution who were unaware of any subject information. Interim PET response was assessed by 5-PS based on the Deauville criteria and quantitative analysis of FDG uptake changes based on the percentage of SUVmax reduction between initial and interim PET scans. After using the receiver operating characteristics analysis, SUVmax reduction <78.6% was selected as optimal cut-off for positive PET.
Results: The median age was 62 years (range, 15-88) and 85 (64%) were male. 64 patients (49%) were presented as advanced stage disease and 30 (23%) had B symptoms. ECOG performance status was 0 or 1 in 100 (76%) and serum LDH level was elevated in 76 (58%). Thus, 44 (33%) were classified as high-intermediate to high risk of International Prognostic Index (IPI). 123 patients (93%) completed planned R-CHOP±involved-field radiotherapy. Using 5-PS and SUVmax reduction rate, 28 (21%) and 26 patients (20%) were positive on interim PET scan, respectively. 18 patients (14%) showed positive PET in agreement between 5-PS and SUVmax reduction rate. However, 10 (8%) of the 28 PET positive patients on 5-PS showed negative PET based on SUVmax reduction rate. Similarly, 8 (6%) among 26 positive patients on SUVmax reduction rate were negative on 5-PS. Thus, 18 patients showed discordant interim PET results between 5-PS and SUVmax-based assessment. With a median follow-up of 25.3 (range, 5.6-75.5) months, 2-year progression-free survival (PFS) was significantly worse in patients with positive interim PET according to 5-PS (27.4% vs. 88.2%, P<0.001) and SUVmax reduction rate (25.4% vs. 86.6%,

P<0.001) than those with negative results, respectively. Combining both visual and quantitative assessments, 2-year PFS was significantly different according to the point of positive results in each assessment (0 point, 90.4% vs. 1 point, 57.3% vs. 2 point, 9.3%, respectively, P<0.001). In multivariate analysis for PFS, high-intermediate to high risk of IPI (HR, 4.67; 95% CI, 1.96-11.14) and 2 points in the combined assessment of 5-PS and SUVmax reduction rate of interim PET (HR, 7.20; 95% CI, 2.81-18.46) were independent prognostic factors for worse PFS

Summary / Conclusion: Visual and quantitative SUV-based assessments in the interim PET appears to predict early outcomes of patients with DLBCL in the post-rituximab era. Moreover, combined assessments with 5-PS and rate of SUVmax reduction make it possible to more clearly differentiate the clinical outcomes in patients with DLBCL. Further studies are needed to validate our results.

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EFFICACY OF NON-PEGYLATED LIPOSOMAL DOXORUBICIN IN ELDERLY PATIENTS WITH AGGRESSIVE B-CELL NON HODGKIN LYMPHOMA: A MULTICENTRIC STUDY

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Background: Diffuse Large B-cell Lymphoma is predominantly diagnosed in the elderly. However, elderly patients often have a variety of comorbidities and age-related decrease in organ function resulting in worse clinical outcomes related to high rate of adverse events and reduced dose intensity chemotherapy. Nonpegylated liposomal doxorubicin, when substituted for standard formulation in the combination therapy R-CHOP, was found to be with similar efficacy but well tolerated, decreasing anthracycline-related toxicities and early withdraw of chemotherapy.

Aims: We conducted a multicenter, phase II, double-arm trial comparing the efficacy and the safety of 2-weekly and 3-weekly R-COMP regimen.

Methods: Patients with confirmed histological diagnosis of B-cell non-Hodgkin lymphoma, aged≥ 65 years and IPI≥ 1were eligible. The dose-intensity for all patients was established according to a functional assessment of frailty, Activities of Daily Living (ADL). Thirty-nine patients with an ADL=6 were assigned to receive 6 cycles of dose-dense R-COMP14 regimen, whereas 90 patients with an ADL<6 were assigned to receive six cycles of R-COMP21 regimen. The clinical features were comparable in both study arms, without statistical differences.

Results: The median age was 74 years (65-89) at diagnosis and the study included 21 (16%) very elderly patients (≥80 years). The international prognostic index (IPI) score was 4-5 in 12 (31%) patients and in 18 (21%), whereas the Ann Arbor stage was stage III-IV in 27 (69%) and in 67 patients (74%) in the R-COMP14 and R-COMP21 arm, respectively. The Performance status was WHO<2 in 70% of all patients, the median number of comorbidities was 2 (1-7) and the median LVEF was 60% with 16% of patients having LVEF ≤ 50%. The number of cycles administered was 234 in R-COMP14 and 481 in R-COMP21, with the relative dose-intensity for the regimens of 93% and 90%, respectively. Toxicity was mainly hematological in both groups, grade 3/4 neutropenia occurred in 11% and 22% of cycles in the R-COMP 14 and 21 groups and febrile neutropenia of 3% and 8% respectively. Only 8/129 patients (6%) presented a grade II-IV cardiotoxicity. All patients were evaluable for response. With a median follow-up of 24 months the overall response rate (ORR) in R-COMP14 arm was 85%, with complete response (CR) rate of 72%, whereas the ORR in R-COMP21 was 90%, with CR rate of 73%; the event-free survival was 67% and 69 %, respectively. Univariate analysis revealed that event-free survival was negatively impacted by high risk IPI (1-3 vs 4-5; P=0.02). The prognostic factors for shorter overall survival (OS) were: age>70 years (P=0.009), high IPI score (IPI=1-3 vs 4-5; P=0.04), advanced-disease stage III-IV (P=0.01) and impaired WHO performance status 2-3 (P=0.003). Multivariate analysis showed that WHO performance status 2-3 (P=0.02) and age> 70 years (P=0.03) negatively impact the overall survival in both arms.

Summary / Conclusion: The functional assessment of frailty, with Activity of Daily Living (ADL), has allowed to identify really elderly, frail patients. The overlapping ORR and EFS between the dose-dense R-COMP 14 regimen and the standard R-COMP 21 regimen suggest that the use of NPLD may overcome the impact of a dose-dense immunochemotherapy, representing a therapeutic opportunity for frail elderly patients, not suitable for a dose-dense treatment.

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THE ROLE OF PET/CT AFTER RITUXIMAB-CHOP (R-CHOP) IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA (PMLBCL): RESPONSE ASSESSMENT, PROGNOSTIC SIGNIFICANCE AND IMPLICATIONS FOR SUBSEQUENT RADIOTHERAPY (RT)

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Background: R-CHOP is superior to CHOP in PMLBCL. PET-Scan is an accurate method to detect the presence of active disease in diffuse LBCL after R-CHOP, as well as in Hodgkin lymphoma. PET-scan has not been evaluated separately in PMLBCL, where consolidation RT is also debatable. Thus, PET-Scan is used in PMLBCL based on the projection of data derived from Hodgkin and aggressive B-cell lymphomas.

Aims: The evaluation of PET findings in patients with PMLBCL who have responded to R-CHOP, the assessment of their prognostic significance and their impact on the decision for subsequent RT.

Methods: Among 94 consecutive patients with PMLBCL, who were treated in 11 Greek Centers, 71 underwent PET-scan after having responded to R-CHOP (CR, CRu, PR). The remaining 23 patients were not included in the study for the following reasons: documented progressive disease (n=10), PET done after RT (n=5), no PET availability (n=8). The endpoint was Progression Free Survival (PFS), measured from the time of PET examination.

Results: The median post-PET follow-up was 26 months for patients who have not progressed (up to 92). Among 71 evaluable patients, 42 were PET-neg (59%) and 29 (41%) PET-pos. Among 42 PET-neg patients, 24 (57%) did not receive RT and 18 (43%) were irradiated at a median dose of 3480 cGy. Three (3/24) non irradiated patients relapsed (mediastinum and 2 isolated CNS relapses respectively) vs. 0/28 irradiated patients. The 2-year PFS for PET-neg patients who were irradiated or not was 100% vs. 87% (P=0.11). If isolated CNS relapses, which could not be prevented by mediastinal RT, were censored, the corresponding figures were 100% vs. 95% (P=0.34). Among 29 PET-pos patients, 27 (93%) were irradiated at a median dose of 4000 cGy, one was not irradiated and one was forwarded to high dose therapy and autologous transplant: 6/29 patients relapsed (all irradiated). The 2-year PFS was only marginally better in PET-neg vs. PET-pos patients (93% vs. 73%, P=0.06). However, among PET-pos patients, SUVmax appeared to discriminate a subgroup with adverse outcome: The 2-year PFS was 92% vs. 53% (P=0.05) for patients with SUVmax<5 (1/16 relapsed) and SUVmax≥5 (5/13 relapsed) respectively.

Summary / Conclusion: PET-scan remains positive in a substantial proportion of PMLBCL patients who achieve a radiographic response with R-CHOP. In the majority of them, however, 18-FDG uptake is relatively low. Persistence of a positive PET only marginally associated with inferior outcome, when additional RT was administered, although higher SUVmax values might predict a substantially higher risk of relapse. Among 24 non-irradiated, PET-neg patients, only 1 relapse would be preventable by RT. According to these data: (1) R-CHOP responders with PMLBCL should not be forwarded to ASCT simply based on a positive PET/CT; (2) RT can be spared in the majority of PET-neg pts, but selection criteria still need to be defined.

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PRIMARY B-CELL CENTRAL NERVOUS SYSTEM LYMPHOMA: CLINICOPATHOLOGIC CHARACTERISTICS AND TREATMENT OUTCOMES OF 88 PATIENTS AT A TERTIARY REFERRAL CENTER

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Background: Primary central nervous system B-cell lymphoma (PCNSL) is a

rare aggressive variant of diffuse large B cell lymphoma (DLBCL) with a poor prognosis. Optimal therapeutic strategies have not been defined. Currently, high dose Methotrexate (HD MTX) and Rituximab (R) have been shown to be effective chemotherapeutic agents in the treatment of PCNSL. The role of intrathecal (IT) therapy in patients with PCNSL remains unclear.

Aims: To review clinicopathologic characteristics, therapy and outcomes of 88 patients (pts) with primary CNS DLBCL.

Methods: This was a retrospective review of patients at Moffitt Cancer Center (MCC). Data were obtained from the MCC electronic records. Patients at the MCC with PCNSL were identified using our institutional database from January1, 2000 to September 30, 2011. Baseline demographics, clinical, pathological and treatment data were collected and analyzed. Treatment information was recorded including if initial regimens contained: HD MTX (>3.5 g/m^2), R therapy, IT therapy, and radiation therapy (XRT). Descriptive statistical analyses were utilized. Kaplan-Meier (KM) method was used to estimate median Overall Survival (MOS) and log rank test was used to compare the groups in HIV negative patients. All data was analyzed using SPSS version 21.0 statistical software.

Results: 88 patients who underwent therapy for PCNSL from January1, 2000 to September 30, 2011 were used in this analysis. Mean age at diagnosis was 58.7±15.5. 49 (55%) were male, 79 (89%) were Caucasian, 54 (61%) had a performance status of ECOG 0 or1, 48 (54%) were over the age of 60, and 8 (9.1%) were HIV positive. 43 (49%) presented with motor deficits, and 60 (70%) presented with cerebral lesions. The MOS was 36 months (95% CI 30-42) in the entire cohort of patients. In the HIV negative cohort of patients, initial treatment regimens containing HD MTX versus (vs) no HD MTX the MOS was 42 vs. 4 months, (P=0.003), initial R vs no R the MOS was 50 vs 34 months (P=0.034) and initial treatments containing HD MTX and R vs no HD MTX and R the MOS was 50 vs 34 months, P=0.032. There was no difference in MOS between patients who received initial treatments containing HD MTX vs HD MTX + R, 36 vs 50 months (P=0.15). There was no difference in MOS between patients who recieved initial IT therapy vs those without inital IT therapy, 41 vs 36 months (P=0.60). Treatments containing initial XRT vs no inital XRT did not improve MOS, 37 vs 36 months (P=0.34).

Summary / Conclusion: Initial treatment with a regimen including HD MTX, R, or the combination improved MOS patients. Initial regimens containing IT or XRT therapy with or without chemotherapy did not improve MOS in our cohort of patients. Multicenter collaborative trials are needed to further assess the best initial therapy in this rare disease.

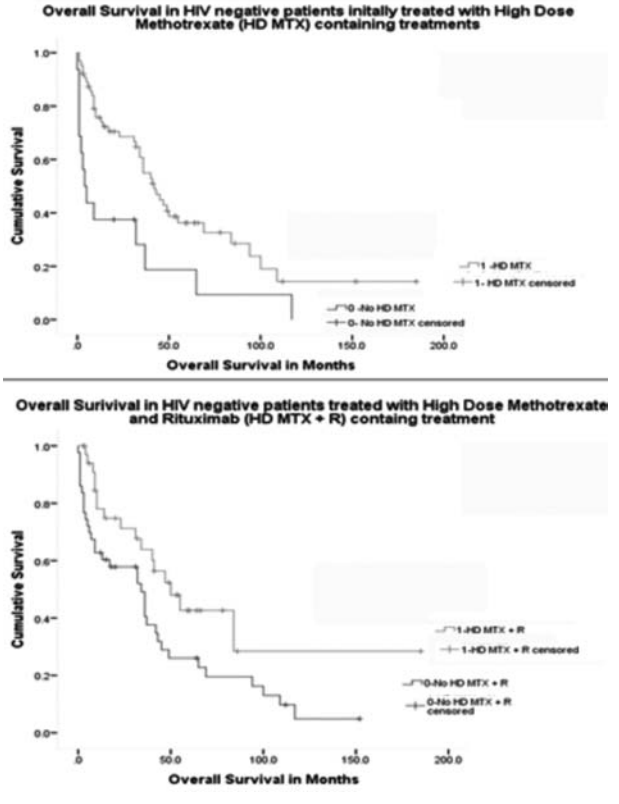

Figure 1.

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THE LYMPHOCYTES TO MONOCYTES RATIO IDENTIFIES A PATIENT SUBGROUP AMONG HIGH RISK DLBCL THAT MAY BENEFIT FROM UPFRONT INTENSIVE TREATMENT WITH AUTOGRAFT

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Background: At diagnosis, a peripheral blood lymphocytes to monocytes ratio (LMR) lower than 2.6, identifies a group of DLBCL patients with a poor prognosis when treated with R-CHOP (Rambaldi *et al*, ASH 2012). No data are available for patients treated upfront with Rituximab containing high dose sequential chemotherapy programs (R-HDS) and autologous stem cell transplantation (AST)

Aims: To investigate whether LMR ratio may identify a high-risk patient subgroup that benefits from a primary high-dose program with ASCT

Methods: We analysed LMR ratio at diagnosis in a series of DLBCL patients enrolled into a trial comparing R-CHOP 14 with R-HDS associated with AST (R-HDS 0305, Clinical Trials.gov.number NCT00355199 by GITIL). Patients characteristics: DLBCL without CNS involvement, with an age between 18–60 years and an High IPI (stage > II B-bulk with ECOG-PS=0-3 and age adjusted IPI (aaIPI) 2–3 or age 61–65 years with ECOG-PS = 0–2 and IPI > 3). R-CHOP 14 (8 cycles) or R-HDS regimen and AST were carried out as previously reported(Tarella C *et al*, Leukemia 2007)

Results: LMR data were collected in 216 evaluable DLBCL patients enrolled into this trial. We identified two groups of patients according to baseline LMR: 144 patients (67%) had a low LMR (≤2.6), while 72 patients (33%) had a high (>2.6) LMR. The two groups were comparable for age, gender, stage, ECOG, extranodal sites, bone marrow infiltration while high LDH level was associated with a low LMR (P= 0.009). In multivariate analysis OS and EFS corrected by age, gender, stage, ECOG, LDH, extranodal sites, BM involvement and treatment arm, resulted significantly improved by R-HDS and AST only in the low LMR group with an hazard ratio (HR) of 0.41 (95% IC 0.2-0.81), P=0.011. In the same patient population with a low LMR, a high ECOG was associated with a two times higher risk of events (HR 2.55, 95% IC 1.26-5.16, P=0.009). After a median observation of 35.4 months (0.3–89.2), the OS and EFS of patients treated by R-CHOP 14 or R-HDS and AST were 71% versus 86% (P=0.022) and 63% versus 83% (P=0.008) respectively.

Summary / Conclusion: The analysis performed among high-risk DLBCLpatients enrolled in a prospective, randomized study, confirms the negative impact of a low LMR at diagnosis in patients treated with R-CHOP. R-HDS and AST improved OS and EFS and overcome the prognostic value of LMR.

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DELAYS IN DIAGNOSIS AND TREATMENT OF DIFFUSE LARGE B-CELL LYMPHOMA DO NOT AFFECT SURVIVAL

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Background: Although diagnostic and treatment delays in solid tumors are known to negatively impact on outcomes, little is known with respect to hematological malignancies. Diffuse Large B-Cell Lymphoma may present with a wide array of symptoms, thus rendering initial diagnosis challenging and time consuming.
Aims: We evaluated disease-specific, patient-related and socioeconomic factors leading to delays in DLBCL diagnosis and treatment and the respective impact on overall and progression-free survival.
Methods: We studied a comprehensive clinical database of 278 patients with a new diagnosis or new presentation of transformed DLBCL treated at our center between 2002 and 2010. All patients received at least one cycle of R-CHOP chemotherapy (median 6). We defined various time intervals based on Cancer Care Ontario guidelines as follows: **patient associated delay** – time from symptoms onset to first known contact with a primary care physician (PCP); **diagnostic delay** – >6 weeks from first PCP contact to initial hematology consultation; and **treatment delay** – >4 weeks between hematology consultation and first R-CHOP cycle.
Results: In the population studied (n=278), the median age was 63 and 46% were female. Patients waited a median of 4 weeks (IQR 2-13) before seeking medical attention. A further median of 8 weeks (IQR 4-17) was required for the PCP to diagnose DLBCL or at least to achieve enough clinical suspicion for referral to hematology. From initial hematology consult, a median of 3 weeks (IQR 1-4) passed until chemotherapy was initiated. In univariate analyses, patients who lacked bone marrow involvement (P=.005), had lower IPI scores (P=.031), higher Charlson comorbidity index (P=.048) and who had initiation of treatment in the outpatient setting (vs. inpatient; P=.021), were more likely to experience diagnostic delays >6 weeks. In multivariable logistic regression analysis, only bone marrow involvement (OR=0.41, P=.018), Charlson comorbidity index (OR=1.42, P=.017) and requirement for urgent inpatient chemotherapy administration (OR=0.40, P=.012) remained associated with diagnostic delays. With respect to treatment delays, in univariate analyses patients who did not have pathology diagnosis upon initial hematology consultation (P<.0001) and had B symptoms (P=.039) were more likely to experience treatment delays >4 weeks. On multivariable analysis, lack of pathological diagnosis at the time of hematology referral was the only factor that remained associated with treatment delays (OR=8.25, P<0.001). No socioeconomic factors (low income, level of education, and cohabiting alone) predicted for either diagnostic or treatment delays. On Cox multivariable regression analyses, we found that only IPI score and number of R-CHOP cycles significantly impacted overall survival (HR=1.82, P<.001; HR=0.70 P<.001) and progression free survival (HR=1.56, P<.001; HR=0.82, P=.004).
Summary / Conclusion: Selected disease and patient-related factors may be associated with delays in management of DLBCL. However, unlike in solid tumor presentations, we can reassure patients that waiting a reasonable time frame to complete diagnostic and staging milestones should not affect their disease course, as long as appropriate chemotherapy dosing is administered.

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NON-INTERVENTIONAL STUDY BE-1ST: FIRST LINE THERAPY WITH BENDAMUSTINE IN ADVANCED INDOLENT NON-HODGKIN LYMPHOMA (NHL) IN TREATMENT ROUTINE

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Background: Bendamustine has become a standard for treatment of indolent non-Hodgkin lymphoma (NHL) in the past years. Current treatment recommendations and results derive from interventional clinical trial experience. The non-interventional study Be-1st reveals treatment and outcome in daily practice in Germany between 2010 and 2011.
Aims: To gain further insight into therapy, efficacy and safety of Bendamustine in clinical routine.
Methods: From 04/2010 until 10/2011 324 patients (pts) were enrolled at 57 study centres in Germany. Tumor entities to be observed were follicular lymphoma and other indolent NHL (except CLL) as well as mantle-cell lymphoma. Treatment modalities were electronically recorded for 6 months until 12/2011. Data sets include demographic information, treatment regime, efficacy and side effects. Major inclusion criteria are advanced indolent NHL according to WHO-classification, patients requiring 1st-line treatment with Bendamustine, no previous chemotherapy, no pre-treatment with Interferone or Rituximab. Exclusion criteria are contraindications according to the applicable SmPC.
Results: Data of 307 pts were analyzed for treatment modalities and efficacy. Concerning safety, data of 323 pts with at least one documented treatment cycle were evaluated.
Gender was balanced, with a mean age of 69 years. Follicular lymphoma (FL) were most frequent (50%), followed by marginal-zone lymphoma (MZL, 17% incl. MALT-lymphoma), immunocytoma (IC, 15%) and mantle-cell lymphoma (MCL, 12%). The combination of Bendamustine/Rituximab (BR) was most common (94%), complemented by Dexamethasone or Prednisone in 11% of these pts. Most pts received Bendamustine during the first 2 days of the cycle (87%). In the majority of cases, treatment was administered at 4-week intervals. The median dose of Bendamustine was 88,4 mg/m^2 d1+2 as recommended in current consensus guidelines. The median treatment duration was 6 cycles (n=277, evaluable pts). The overall response rate (ORR) was 85%, for pts with completed documentation (n=281). Complete response (CR) was 42,7%, partial response (PR) 43,1% and stable disease (SD) 6%. Progressive disease occurred in 10 pts (3,6%). In 161 of 323 pts (50%) a total of 429 side effects of Bendamustine of all CTCAE grades were documented. Most frequently reported CTCAE categories were blood/bone marrow (35% of pts), gastrointestinal (13% of pts), constitutional symptoms (8% of pts), dermatology/skin (5% of pts), neurology (3% of pts) and infections (2% of pts). A total of 74 grade 3/4 adverse events or 17% of all cases occurred in 54 out of 323 pts (17%) - with 65 of grade 3 (15%) and 9 of grade 4 (2%). The majority of cases were leucopenia/neutropenia (12%). Two grade 5 toxicities were documented. One death was associated with thrombocytopenia. The reason of death in another case was unknown.
Summary / Conclusion: Be-1st reflects the treatment routine of Bendamustine for indolent NHL in Germany in 2010/2011 and provides valuable information on administration, outcome and safety. Bendamustine is mostly applied in combination with Rituximab. CR and ORR are comparable to reported data values. The observed adverse reactions were in accordance to the SmPC. In addition to experiences from interventional trials, the favorable efficacy and toxicity profile of Bendamustine could be demonstrated in clinical routine as well.

Table 1.

	All Tumor Entities		FL	MZL	IC	MCL	Other Entities
No. of pts	**281**		**142**	**46**	**45**	**33**	**15**
Best Response	n	%	%	%	%	%	%
Complete Response	**120**	**42,7**	47,2	45,7	31,1	30,3	53,3
Partial Response	**121**	**43,1**	43,0	39,1	46,7	45,5	40,0
Stable disease	**17**	**6,0**	3,5	8,7	11,1	6,1	6,7
Progessive disease	**10**	**3,6**	3,5	-	2,2	12,1	-
Not evaluable	**13**	**4,6**	2,8	6,5	8,9	6,1	-
ORR	**239**	**85,1**	**90,1**	**82,6**	**75,6**	**75,8**	**93,3**
DCR	**257**	**91,5**	93,7	93,5	86,7	81,8	100,0

Non-Hodgkin Lymphoma - Clinical 2

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THE PROGNOSTIC ROLE OF EBV IN PERIPHERAL BLOOD OF PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

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Background: The Epstein-Barr virus (EBV) belongs to the herpes virus family and infects more than 90% of the human population establishing persistent latent infection in the host. Although EBV infection is benign in most individuals, it has been linked to the etiology of a number of lymphoproliferative diseases. 'EBV-positive diffuse large B-cell lymphoma of the elderly' was included as a provisional entity of DLBCL in the revised 2008 WHO classification. We recently showed that the plasma EBV-DNA load at HL diagnosis is an indicator of disease activity and biological characteristics associated with negative prognosis (*Hohaus et al,Clin Cancer Res,2011;17(9); 2885–92*).
Aims: We studied the role of EBV-DNA copy number in different blood compartments (whole blood, mononuclear cell fraction, and plasma) in patients with DLBCL at diagnosis, as a potential predictive indicator for the presence of EBV in lymphoma cells and as a prognostic marker in patients treated with immunochemotherapy (R-CHOP).
Methods: We analysed 136 patients with DLBCL (median age 62 years, range 15-92 years; 60 females and 76 males). EBV was detected using a commercial real-time PCR kit, amplifying a 191 bp region of the EBNA-1 gene (*Bio-Quant EBV, Biodiversity, Brescia, Italy*) in peripheral blood compartments (whole blood n=133, plasma n=55, and mononuclear cells n=52). Lymph node samples from 61 DLBCL patients were analyzed for EBV infection through in situ hybridization for EBV-encoded small RNAs (EBER).
Results: EBV was frequently detected in peripheral blood: 35 of 133 whole blood samples (26%) resulted positive. The copy number varied between 200 and 196000 copies. The presence and copy number of EBV in whole blood and mononuclear cells were correlated (P<0.05, P<0.01, respectively), while there was no correlation to the detection of EBV in plasma. We did not find any association between the presence or viral load of EBV-DNA in any blood compartment and the presence of EBV in the lymphoma cells of 61 patients studied with EBER–ISH (11 patients EBER pos). The presence and viral load of EBV in PB was not related to age or gender, and other disease characteristics as LDH level, stage, and IPI. In univariate analysis on 133 patients treated with R-CHOP, the presence of EBV-DNA in peripheral blood was associated with a significantly shorter event-free survival (EFS): 60% versus 79% at 2 years, P<0.04). As well, the EBV copy number was correlated with a worse outcome (hazard ratio of 1.86 for each logarithmic increase; 95% C.I, 1.17-2.97; P<0.009). Correcting for IPI in a multivariate Cox analysis, the presence of EBV-DNA in peripheral blood retained its prognostic significance (hazard ratio 2.02; 95% C.I., 1.03-3.95; P<0.04).
Summary / Conclusion: Our findings suggest that EBV can be frequently detected in peripheral blood at DLBCL diagnosis, which does not reflect the EBV status of the lymphoma cells, but associates with a worse outcome following standard immunochemotherapy. Further studies are needed to explore the mechanisms that permit the expansion of EBV-positive cells in peripheral blood of patients with DLBCL.

P323

RITUXIMAB AND CHLORAMBUCIL AS FIRST LINE THERAPY OF LOW-GRADE OCULAR ADNEXAL LYMPHOMAS: LONG TERM FOLLOW-UP RESULTS

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Background: Ocular adnexal lymphomas (OALs) represents 8% of primary extranodal lymphomas, 80% of OALs constitutes extranodal marginal zone lymphomas (EMZL). Radiotherapy is associated to high rates of local disease control, but also to the risk of relapse and immediate or delayed complications, such as xerophtalmy, corneal ulcerations, cataract and retinal damage. Single-agent chemotherapy with alkylating agents and rituximab are used for the treatment of low-grade lymphomas, including OALs.
Aims: We investigated the efficacy and the safety of a combination of chlorambucil and rituximab as first line treatment in patients (pts) with OALs.
Methods: Pts with histologically proven low-grade OALs were enrolled in this study. Staging included CT-scan (orbit, neck, chest and abdomen) MR of the orbit, Chlamydia psittaci (Cp) detected by PCR and bone marrow biopsy. Treatment consisted of chlorambucil (0,1 mg/Kg/die for 45 days, then on days 1 to 15 monthly for 4 months) and rituximab (375 mg/sqm weekly for 4 doses, then monthly for 4 infusions). At the end of therapy pts were restaged clinically and with a MR of the orbit.
Results: Since November 2003 to November 2012 22 consecutive OALs (20 EMZL, 2 follicular lymphoma, FL) have been treated according to protocol. The median interval between onset of symptoms and diagnosis was 13 months (range 4-36). Eight pts were male (36%) and 14 pts were female (64%). Median age at diagnosis was 68 yrs (range, 35-86 yrs). Disease was localized in the conjunctiva in 16 pts (72%), in the lacrimal glands in 3 pts (14%) and in other orbital sites in 3 pts (14%). Twenty-one pts presented a stage I disease, 1 stage IV, and no pts showed B-symptoms. LDH was within normal range in 19 of 22 pts (86%), ECOG-PS was 0 and IPI was low or low-intermediate in all pts. We evaluated PCR for Cp in the first 10 consecutive pts and it was negative. All pts completed the treatment without delay; there was no grade III-IV toxicities neither hospitalizations. Five pts had grade 1-2 rituximab infusion-related reactions usually during the first infusion and haematological toxicity was mild. At the end of treatment 21 pts (95%) resulted in CR, and 1 obtained a PR (5%). After a median follow-up of 62 months (range, 10-106) all pts are alive, 17 maintained CR and 4 relapsed after3,4,5 and 7 yrs. Three pts were retreated with the same protocol: one of them attained a new CR and 2 pts are currently under treatment; the other relapsed systemically as FL. The patient in PR after 1st line obtained CR with 2nd line therapy (rituximab fludarabine and cyclophosphamide). The median PFS was 50 months (range, 1-98). All pts performed ophthalmologic follow-up: we didn't report ocular toxicities, and all pts conserved a normal visual function, including acuity. No secondary myelodisplastic syndrome or neoplasms are reported.
Summary / Conclusion: After a long follow-up the combination of rituximab and chlorambucil proved to be low toxic, feasible and effective therapy for primary OALs. No delayed ocular or haematological complications are reported. For these reasons this regimen should be considered for first line of this indolent lymphoma.

P324

LYMPHOMAS WITH MYC-TRANSLOCATION OTHER THAN BURKITT'S: BAD PROGNOSIS DESPITE SPECIFIC IMMUNOCHEMOTHERAPY FOR BURKITT'S LYMPHOMA

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Background: *MYC* translocations involving chromosome 8q24 have been strongly associated with Burkitt's lymphoma (BL). However these translocations can occur in a wide variety of B-cell lymphomas, especially diffuse large B-cell lymphoma (DLBCL) and B-cell lymphoma unclassifiable with intermediate features between DLBCL and BL. Unlike BL, these lymphomas have bad prognosis and the standard treatment has to be established.
Aims: The objective was to study the clinical-biological characteristics and prognosis in a series of lymphomas with *MYC*-translocation other than BL focusing in the given treatment.
Methods: Retrospective study of patients with *MYC*-translocation other than BL treated in a single institution from 2006 to 2012. Cases with diagnosis of BL, Burkitt's-like lymphoma and any other lymphoma with *MYC*-translocation were reviewed by 2 pathologists and classified according to the WHO 2008 criteria. The status of *MYC*, *BCL2* and *BCL6* genes was evaluated by fluorescent *in situ* hybridization (FISH) using dual-colour break-apart commercial probes (LSI MYC DC BA, LSI BCL6 DC BA and LSI BCL2 DC BA; Abbot Molecular, Abbot Park, IL, USA) on whole tissue sections of formalin-fixed paraffin-embedded tissue. Main clinical and biological data were collected from the records.
Results: From 2006 to 2012, 27 patients with a median follow-up of one year (range 0.3-5.3) were included. Median age was 59 years (range 36-33) and 16 (53%) were male. ECOG score at diagnosis was ≥2 in 12 patients (46%), 11 (42%) had ≥2 extranodal sites involved, serum LDH was increased in 17 out of 25 (68%), Ann Arbor stage III/IV in 17 (65%) and B symptoms were present in 12 of 25 (48%). IPI was high or intermediate/high in 13 out of 25 (52%). Seventeen cases were diagnosed with DLBCL and 10 with B-cell lymphoma unclassifiable with intermediate features between DLBCL and BL. Isolated *MYC* rearrangement without *BCL2* or *BCL6* rearrangements was observed in 11 (41%) cases, 13 were double hit, and 3 triple hit lymphomas. Neither differences regarding the main clinic and biologic features between patients with *MYC* translocation alone and patients with double and triple hit were observed, nor between patients with DLBCL and those with intermediate features between DLBCL and BL. Sixteen cases were treated with R-CHOP (14 DLBCL and 2 intermediate), and 11 with a specific immunochemotherapy for BL (8 intermediate and 3 DLBCL) (P=0.003). Complete response (CR) was achieved in 7 out of 24 (29%) cases (5/15 treated with R-CHOP and 2/9 with Burkitt-type immunochemotherapy) (1 patient is still pending of reevaluation and 2 were not evaluable for response). Four of the 5 patients in CR after R-CHOP relapsed, versus none patients in CR after Burkitt-type immunochemotherapy. The 2-

year (95% CI) overall survival (OS) and progression-free survival (PFS) probabilities for the whole series were 34% (11%>57%) and 18% (1%>35%) respectively. Patients with isolated *MYC* translocation had worse OS probability at 2 years than those with double and triple hit: 17% (95% CI: 0%>45%) versus 44% (95% CI: 13%>75%) (P= 0.036); but PFS at 2 years were similar: 14% (95% CI: 0%>38%) for *MYC* translocation and 20% (95% CI: 0%>4%) for double and triple hit (P= 0.26). Two-year OS and PFS probabilities were not different between patients treated with Burkitt-type immunochemotherapy and those treated with R-CHOP: 52% (95% CI: 21%>83%) versus 36% (95% CI: 10%>62%) for OS; and 31% (95% CI: 2%, 60%) versus 16% (95% CI: 0%, 36%) for PFS (Figure 1).

Summary / Conclusion: Lymphomas with *MYC* translocations other than BL present aggressive characteristics at diagnosis and have poor prognosis despite treatment with a specific immunochemotherapy for BL.

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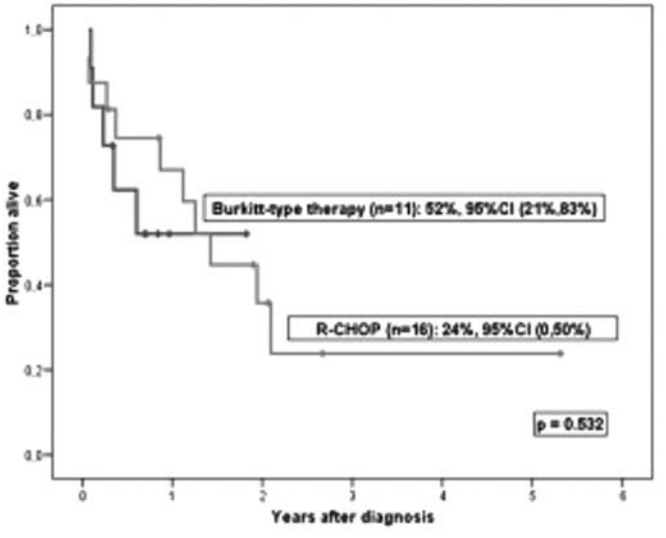

Figure 1.

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UBLITUXIMAB (TG-1101), A NOVEL ANTI-CD20 MONOCLONAL FOR RITUXIMAB RELAPSED/REFRACTORY B-CELL MALIGNANCIES

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Background: Anti-CD20 therapy (rituximab or RTX) in treating patients (pts) with B-cell lymphomas has resulted in significant improvement in treatment response and clinical outcomes. Despite advances, pts continue to relapse from, or are refractory to, RTX-based regimens. Ublituximab (UTX) is a novel, chimeric mAb targeting a unique epitope on the CD20 antigen. UTX has been glycoengineered to enhance affinity for all variants of FcγRIIIa receptors, and therefore demonstrates greater ADCC activity than RTX (Le Garff-Tavernier, 2011). UTX displayed greater antitumor activity than RTX in NHL *in vivo* models and in low CD20 expressing tumors (ASH 2011). A Phase I trial with UTX used as a single agent in pts with Rel/Ref CLL reported an ORR of 45%.

Aims: Herein we report on two ongoing Phase I dose-escalation studies of UTX in pts with RTX Rel/Ref B-cell malignancies: TG-1101-101, a single agent dose escalation study of UTX; and TG-1101-102 a dose escalation study of UTX in combination with lenalidomide (REV), an immunomodulator that has displayed single agent activity in pts with NHL and CLL, and through activating effects on NK cells has been shown to enhance the ADCC activity of anti-CD20 mAbs.

Methods: Eligible pts are Rel/Ref to a prior anti-CD20 regimen; have evaluable disease with confirmed CLL/SLL or NHL diagnosis with no active Hep B/C, and must provide informed consent to participate. For TG-1101-101 (single agent UTX), the Ph I dose-escalation uses a sequential 3+3 design in dose cohorts of 450, 600, 900, and 1200 mg. UTX is administered once weekly for 4 infusions followed by monthly maintenance therapy. In TG-1101-102 (UTX+REV), the dose escalation is a sequential 3+3 design with 4 cohorts of escalating UTX. REV is started at 10 mg and allowed to increase by 5mg per cycle (up to 20mg) if well tolerated. UTX is administered days1, 8, 15 of Cycles 1 & 2 (Cycle=28 days) with maintenance. REV is started Cycle 1/Day 9 and continued daily. In both studies, safety and efficacy are primary and secondary endpoints, respectively. PK and correlative PD data are also being collected in both studies.

Results: In TG-1101-101, 9 pts (5 FL, 3 MZL, 1 MCL) have been enrolled. Med age 63; Gender: 3M/6F. Med prior therapies = 4 (range 2-6). RTX refractory (44%). 8/9 pts are evaluable for safety and efficacy; no DLT's and no Gr 3/4 AE's to date. 8/9 pts have had at least one response assessment: 1 CR (RTX refractory MZL); 3 PR's (2 MZL, 1 FL); 2 SD (FL) and 2 PD (1 FL, 1 MCL). PK analysis ongoing. In TG-1101-102, 4 pts have been enrolled (3 CLL/SLL and 1 MCL). Med age 68; all male; Med prior therapies = 3. Two pts progressed: Cycle 2 (MCL) and Cycle 3 (CLL) – study was later amended to start monthly maintenance in Cycle 3. Two remain on study. No DLTs have been observed to date. Gr 3/4 AE's to date include: Gr 4 neutropenia and thrombocytopenia in 1 pt; Gr 3 anemia, leukopenia, dyspnea and UTI in 1 pt. Data evaluation is ongoing; lymphocyte depletion has been rapid and profound in all pts.

Summary / Conclusion: UTX has been well tolerated to date in both Ph I studies with demonstrated early clinical activity confirmed at all doses in TG-1101-101. 7/9 pts in TG-1101-101 continue to receive UTX treatment (range 3–28+ wks). 2/4 pts in TG-1101-102 continue to be treated and display remarkable B-cell depletion. Enrollment in a 900mg expansion cohort is now open in TG-1101-101 with an emphasis on RTX relapsed/refractory indolent or low CD20-expressing lymphomas, including MZL. Enrollment into the dose escalation phase of TG-1101-102 is ongoing.

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THE POTENTIAL OF CD127 AS A PROGNOSTIC AND RESIDUAL DISEASE MARKER IN CHRONIC ADULT T CELL LEUKEMIA/LYMPHOMA.

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Background: Human T cell Lymphotropic Virus type 1 (HTLV-1) is a complex delta retrovirus infecting 15-20 million people worldwide. Most are chronic asymptomatic carriers (AC) but a minority [2-6%] develop a mature T-cell neoplasm Adult T cell Leukemia/Lymphoma (ATL). ATL has been classified into 4 subtypes: smouldering; chronic leukemic; lymphoma and acute; the latter two being aggressive subtypes with poor prognosis. The diagnosis depends on clinical features, immunophenotype, demonstration of HTLV-1 infection and ideally of monoclonal proviral integration. Treatment options include zidovudine with interferon, combination chemotherapy and novel agents. There are significant challenges in diagnosis, monitoring and treatment of ATL. The typical immunophenotype of ATL, CD3+/wkCD4+CD8-CD25+CD7-, is neither specific nor reliable. The methods used to detect monoclonal proviral integration are labour-intensive and/or expensive and not widely available. T Cell Receptor studies lack sensitivity and specificity. There is a need for a rapid, specific, reliable, widely available and cost effective method for diagnosis and monitoring.

Aims: To develop a new flow cytometry based assay for diagnosis and monitoring of leukaemic ATL.

Methods: Patients attending the National Centre for Human Retrovirology at Imperial College (St Mary's Hospital), London, UK donated peripheral venous blood samples after informed consent and ethical approval. Samples from 2 HTLV-1 immortalized cell lines (MT-2 and C8166) and peripheral blood mononuclear cells (PBMCs) from 3 uninfected individuals, 25 HTLV-1 non-ATL and 12 ATL [2 cutaneous lymphoma,1 cutaneous lymphoma/chronic, 1 acute and 8 chronic] patients were analysed. Cells were subjected to 11 colour immunophenotyping, real-time HTLV-1 proviral load [PVL] quantification and ligation mediated polymerase chain reaction followed by high through-put sequencing [HTPS] for HTLV proviral integration site analysis.

Results: The non-ATL patients had CD127+ & CCR7-lo expression in circulating CD25+CCR4+ cells and polyclonal integration site pattern. Four ATL patients had similar CD127+ & CCR7-lo expression in circulating CD25+CCR4+ cells (2 chronic, 2 cutaneous). These patients have remained well with PUVA only or no ATL treatment. Where examined the integration site analysis in PBMCs resembled the non-ATL patients (N=3). Eight ATL patients had CD127-lo expression on CD4+ CD25+ CCR4+ cells [1 acute, 1 cutaneous/chronic and 6 chronic] with a mono/oligoclonal integration site pattern. These patients have all required treatment for ATL. One of nine chronic ATL and the one acute ATL patient had high CCR7 expression [defined as >50% positivity]. Foxp3 expression was variable. IRF4 was not expressed in any of the patients with chronic ATL and all responded to first line zidovudine/interferon-a therapy. Longitudinal study of 5 patients during treatment for chronic ATL found the frequency of CD25+CCR4+ to correlate with PVL whilst CD127 expression correlated with integration site analysis and disease remission status.

Summary / Conclusion: In non-malignant HTLV-1 infection CD25+CCR4+ cells are CD127+ and the integration sites are polyclonal. In ATL CD25+CCR4+ cells were CD127+ or CD127-lo with CD127+ expression associated with a polyclonal and CD127-lo a mono/oligoclonal integration site pattern. Increased CD127 expression during therapy correlated with remission, a return to a poly-

clonal integration site pattern. CD127 expression appears to be useful to identify patients needing treatment and for monitoring the treatment of chronic ATL.

P327

EVALUATION OF THE PROGNOSTIC SIGNIFICANCE OF THE BODY MASS INDEX (BMI) IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) RECEIVING RITUXIMAB-CHOP (R-CHOP) OR SIMILAR IMMUNOCHEMOTHERAPY

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Background: Recent data correlate high BMI with better outcomes in Caucasian male Americans with DLBCL. Despite the large number of patients, the study referred to a highly selected patient population, included patients who did not receive Rituximab and does not provide an explanation of the findings. Thus, the prognostic significance of BMI in DLBCL must be further studied in an unselected patient population treated with R-CHOP.
Aims: The evaluation of the prognostic significance of BMI in a large multicenter cohort of patients with DLBCL treated with R-CHOP or similar immunochemotherapy combinations.
Methods: We evaluated 457 patients with DLBCL with a median age of 64 years (18-91), who had received R-CHOP or similar chemotherapy combinations in 4 Greek Medical Centres. Freedom From Progression (FFP) was the selected endpoint, including toxic deaths as events, while unrelated deaths without prior treatment failure were censored.
Results: The median BMI was 26.5 (IQR 23.9-29.4). Overall, 97/457 (21%) of patients were obese (BMI≥30). Higher BMI was correlated with more advanced age, while lower BMI with PS≥2, ≥2 extranodal sites and stages III/IV. There was no correlation with gender, LDH and B-symptoms. In spite of these correlations, the incidence of obesity was not correlated with none of the above neither with IPI. The 7-year FFP for patients with BMI≥30 and <30 was 82% and 70% (P=0.03) respectively. Among 329 patients without B-symptoms, the 7-year FFP for the patients with BMI≥30 and <30 was 87% and 74% (P=0.03), while the corresponding results for 104 patients with B-symptoms were 79% and 55% (P=0.12). Among males, the corresponding percentages were 83% and 66% (P=0.04), while among females 81% vs 76% (P=0.52). For patients <60 years old, the 7-year FFP with BMI≥30 and <30 was 85% and 81% (P=0.37), while for older patients 80% and 61% (P=0.03) respectively. Multivariate analysis revealed that BMI<30 was marginally associated with inferior FFP (relative risk 1.79, 95% confidence interval 0.92-3.49, P=0.085).
Summary / Conclusion: The present study suggests that obese patients with DLBCL may have better prognosis under R-CHOP or similar immunochemotherapy. This was not due to the potentially adverse prognosis of patients with weight loss, since the observation was valid for patients without B-symptoms. The effect of BMI on prognosis is more pronounced for males and older patients. The study will be expanded to include a larger patient population and take into account the actually delivered dose-intensity of immunochemotherapy regimens in obese and non-obese patients.

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AIDS-RELATED LYMPHOMAS (ARL), OUTCOME AND PROGNOSTIC FACTORS

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Background: HIV infection correlates to an increased risk of lymphoma, mainly diffuse large B-cell lymphoma (DLBCL) and Burkitt's lymphoma (BL). Primary central nervous system lymphoma (PCNSL) is a particular group with very poor outcome. Hodgkin's lymphoma (HL), although not included in the definition of AIDS, has also been unquestionably linked to HIV infection.
Aims: Retrospective analysis of the prognostic factors in ARL, looking for variations amongst 3 main groups: HL, NHL and PCNSL.
Methods: We assessed the available data of 96 patients (pts), HIV-positive, diagnosed with lymphoma between 2000 and 2012.
Results: The median age at diagnosis was 41 years-old [23;79]; 81.3% of the pts were male. Nineteen (19.8%) pts had HL and 77 (80.2%) had NHL, of which 13 (16.9%) were PCNSL; histologically, NHL were mostly DLBCL (49.4%) and BL (18.2%). Most pts were diagnosed at an advanced (III/IV) stage (92.7%), had IPI ≥3 (65.1%) and ECOG performance status (PS) ≥2 (51.3%). Extra-node involvement was present in 79.2%: bone marrow (35.4%), liver (27.1%), gastrointestinal (27.2%), CNS (27.1%) and lung (12.5%). All PCNSL pts had prior AIDS-defined disease, and 69.2% of them had CD4+ lymphocytes <50/mm^3 (P=.034). Merely 36.5% of the pts were on anti-retroviral therapy (ART) at the time of diagnosis (68.4% HL, 32.8% NHL, 7.7% PCNSL; P=.001). HL group had a higher percentage of pts with undetectable HIV (by PCR) at diagnosis (47.4% HL, 7.8% NHL, 0% PCNSL; P<.0001). One fourth of the pts had no conditions to initiate curative treatment (26.3% HL, 10.9% NHL, 92.3% PCNSL). Chemotherapy schedules included: HL - ABVD (10), ChlVPP (2); NHL - CHOP (29), Da-EPOCH (8), HyperCVAD (3), M-BACOD (3) among others; PCNSL - HDMTX (1). Radiotherapy was part of the treatment (curative/palliative) in 11.5% of the pts. A complete (CR) or partial (PR) response was achieved in 30.2% of the pts (66.6% HL; 34.5% NHL; 100% PCNSL- 1patient). 67pts died: due to lymphoma (63) or other causes (4); 27pts are alive (22 free from lymphoma); 2 were lost to follow-up. Median overall survival (OS) for the complete series was 6 months. The 3 groups HL/ NHL/ PCNSL had very different outcomes (10-year OS: 40.6%/ 17.1%/ 7.7%; P=.039). Age and sex had no impact on survival. None of the patients with early stage disease (3) have died. There was worse survival for PS ≥2 (P=.002); extra-node involvement (P=.017); lung involvement (P=.007); low platelet count (P<.0001); and IPI ≥3 (P=.013) in NHL group. Low hemoglobin levels (P=.018) and leukopenia (P=.026) had an impact on survival only for the HL group. There was inferior outcome for those pts with prior AIDS-defined disease (1-year OS: 31.3% vs. 60.6%; P=.025) and lower CD4+ T-lymphocyte counts [1-year OS: 26.7%(<50/mm^3), 36.4% (50-200/mm^3), 67% (>200/mm^3); P=0.004], particularly in the NHL's group. ART previous to diagnosis showed impact on survival only for HL's group (1-year OS: 69.2% vs. 0%; P<.0001). Detectable HIV was not associated to worse survival. As expected, response to treatment (CR/PR) is a major prognostic factor, regardless of type of lymphoma (1-year OS: HL 87.5% vs. 20%, P=.019; NHL 94.4% vs. 23.3%, P<.0001; PCNSL 100% vs. 9.1%, P=.008). On multivariate analysis, CD4+ T-lymphocyte counts (P=.023) and response to treatment (P=.001) were independently associated to OS in the NHL group.
Summary / Conclusion: Prognostic factors in ARL differ from those of HIV-negative lymphomas and vary for each subtype. A considerable percentage of patients do not present with conditions to initiate curative treatment, hence the high short-term mortality; however, when response is achieved, they seem to behave similarly to those without HIV infection.

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HIGH RATE OF DURABLE REMISSIONS AFTER TOXICITY-ADAPTED INTENSIVE INDUCTION, AUTOLOGOUS STEM CELL TRANSPLANTATION AND RITUXIMAB MAINTENANCE IN MANTLE CELL LYMPHOMA PATIENTS.

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Background: Mantle cell lymphoma (MCL) is aggressive B-cell neoplasm diagnosed predominantly among elderly men. Upfront use of high-dose AraC (12 g/m^2 per course), autoSCT and rituximab at all stages of therapy is the most effective treatment but possible only with patients younger 60-65 years. Prominent efficacy of gemcitabine-oxaliplatin combinations in relapsed and refractory MCL patients allowed including these drugs in first-line therapy.
Aims: Toxicity and efficacy assessment of schemes R-DA-EPOCH/R-GIDIOX and R-DA-EPOCH/ R-HD-Met-AraC, autoSCT and R-maintenance in primary MCL patients.
Methods: Since May 2008 41 untreated MCL pts (median 54 years (29-64), M/F 73%/27%, MIPI$_b$: 32% low, 34% intermediate, 34% high risk) were enrolled. After first R-EPOCH course pts were stratified according to toxicity they had received either R-DA-EPOCH/R-HD-Met-AraC or R-DA-EPOCH/R-GIDIOX. In the absence of hematological toxicity gr.4 for more than 3 days, severe infection complications and signs of renal failure pts underwent R-HD-Met-AraC (R 375 mg/m^2 day 0, methotrexate 1000 mg/m^2 24 hours CI day$_1$, AraC 3000 mg/m^2 q 12 hrs days 2-3). If there was one of these complications pts underwent R-GIDIOX (R 375 mg/m^2 day 0, gemcitabine 800 mg/m^2 days 1 and$_4$, oxaliplatin 120 mg/m^2 day$_2$, irinotecan 100 mg/m^2 day$_3$, dexamethasone 10 mg/m^2 IV days 1-5, ifosfamide 1000 mg/m^2 days 1-5). Further these courses are rotated: either R-DA-EPOCH/R-HD-Met-AraC or R-DA-EPOCH/R-GIDIOX. Depending on the terms of response, pts received 6-8 courses (3-4 cycles) and autoSCT (BEAM-R) with *in vivo* purging by rituximab. Pts with residual tumor after autoSCT were consolidated with local radiotherapy. R-maintenance was performed every 3 months for 3 years. Since Nov. 2011 all pts received intrathecal CNS prophylaxis (including patients who had undergone autoSCT for one year before Nov. 2011). The protocol was approved by the local ethics committee. Pts were analyzed in an intent-to-treat basis.
Results: A median follow-up is 21 months (range 2-58). Toward Feb. 2013 34 patients underwent autoSCT: 20 from R-HD-Met-AraC arm and 14 from R-

GIDIOX arm. 1 induction death after first HD-Met-AraC course (acute renal failure and septic shock). R-maintenance therapy was completed in 4 pts. All pts achieved CR in R-HD-Met-AraC arm. In R-GIDIOX arm OR was 93%: 13 CR, 1 PR (without progression for 26 months after autoSCT) and 1 PD after 4 courses. Main non-hematological toxicity of R-GIDIOX was hepatic, with elevated aminotransferases grades 1-2 and 3-4 in 59,5% and 7,1% of courses respectively, without clinical signs. The sources of stem cells were PB in 30 patients and BM in 4 cases of harvest failure after 3 R-GIDIOX and 1 HD-Met-AraC. Hematological toxicity of R-GIDIOX course: leukopenia gr.4 was in 71,4% (medium duration was5,4 days, range 1-13), thrombocytopenia gr.4 was in 42,9%. The estimated 4-years OS for the R-GIDIOX arm and the R-HD-Met-AraC arm were 100% and 78±12%. The estimated 4-years EFS for the R-GIDIOX group and the R-HD-Met-AraC group were 85±10 % and 71±13 %. There were 3 CNS relapses in 6-14 months after autoSCT. One patient was from R-GIDIOX arm and 2 patients were from R-HD-Met-AraC arm. 2 patients died in 6 months after CNS relapses, and in one case, CR was achieved again with radiotherapy and intrathecal treatment (CR more than 13 months). After these 3 early CNS relapses we included CNS prophylaxis in this treatment protocol.
Summary / Conclusion: HD-Met-AraC scheme is highly toxic and its use is possible only in 2/3 of patients younger 65 years. R-GIDIOX scheme is less toxic than HD-Met-AraC and equally effective in response induction and mobilizing, so it could be recommended for those in whom high-dose AraC and methotrexate can potentially cause severe adverse consequences.

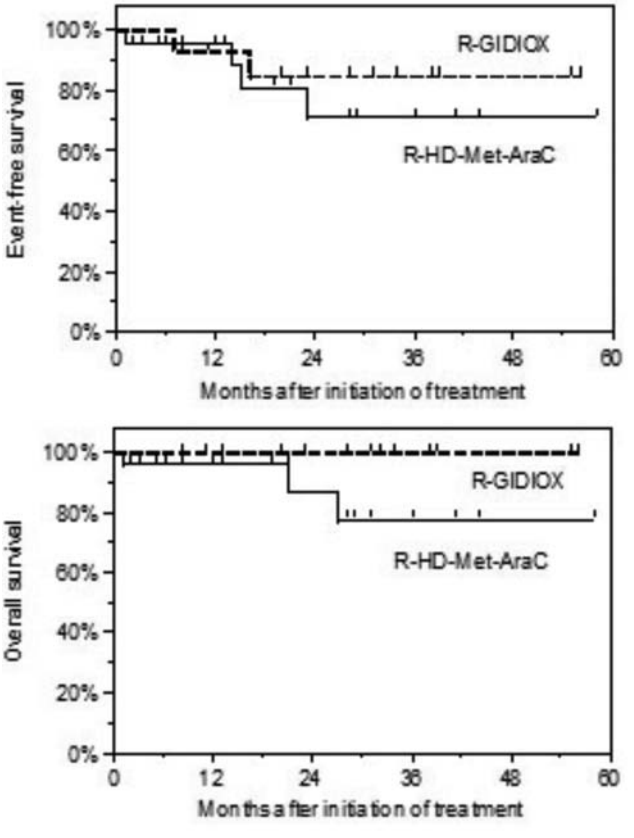

Figure 1.

P330

THE USE OF SYSTEMIC THERAPY DOES NOT IMPROVE THE RESULTS OF LOCOREGIONAL TREATMENT IN LIMITED STAGE (I-II) FOLLICULAR LYMPHOMA: STUDY OF 112 PATIENTS.

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Background: 20-25% of the patients with follicular lymphoma (FL) are in limited stage (I-II) at diagnosis. Involved field nodal or regional radiotherapy is the standard therapy but recurrence out of radiation field is the main cause of treatment failure.

Aims: The objective of this study was to investigate the value of the use of systemic therapy in a series of patients diagnosed with limited stage FL.
Methods: Retrospective study of patients with limited stage (I and II) FL diagnosed and treated in two Spanish hospitals. Clinical and biological characteristics, as well as therapy and response were collected.
Results: From 1989 to 2012, 112 patients were included. Median age was 57 years (range 17-93), 71 (63%) were females. ECOG score at diagnosis was <2 in 99 patients (91%) and B symptoms were present in only 7 (6%). Grade of FL histology was available in 105 cases: grade 1 in 39 (37%), grade 2 in 39 (37%) and grade 3a in 27 (26%). Ann Arbor stage: I in 61 (55%) (extranodal involvement in 15 of them) and II in 51 (45%) (extranodal involvement in 8). FLIPI score (available in 97 cases) was 0-1 in 73 (75%) and 2-3 in 24 (25%). Fifty-four patients (48%) were treated with a locoregional strategy (radiotherapy in 43, surgical resection in the remaining 11) and 58 (52%) received systemic chemotherapy (combined with radiotherapy in 21 [19%]); 31 cases received rituximab as part of their chemotherapy. The groups of locoregional (n=54) and systemic therapy±radiotherapy (n=58) were well balanced except for a higher number of patients with grade 3a histology and stage II in the group of systemic therapy±radiotherapy compared to locoregional treatment (46% vs 2%, P<0.001, and 64% vs 26%, P<0.001, respectively). Overall response was achieved in 95 (95%) of cases (CR in 90 patients [90%], 42 [96%] in locoregional therapy vs 48 [86%] in systemic therapy±radiotherapy), whereas 5 (5%) showed stable disease or progression. Thirty-one (35%) out of 90 patients achieving CR relapsed (18 in locoregional therapy vs 13 in systemic therapy±radiotherapy, P=0.181). DFS, PFS and OS probabilities at 8 years were, respectively, 58% (95% CI 45%>71%), 64% (95% CI 53%>75%) and 83% (95% CI 75%>91%) without differences according to treatment strategy (locoregional [n=54] vs systemic therapy±radiotherapy [n=58]), although patients treated with systemic therapy±radiotherapy showed a trend to longer CR duration (74% [95% CI 60%>88%] vs 48% [95% CI 27%>69%], P=0.07) (Figure 1).
Summary / Conclusion: Conclusions. Patients with FL in limited stage show good response to therapy. In this series no benefit from the use of systemic therapy was observed in terms of DFS, PFS and OS compared to locoregional treatment.
Supported in part by grant RD12/0036/0029 from RTICC, Instituto Carlos III, Spain.

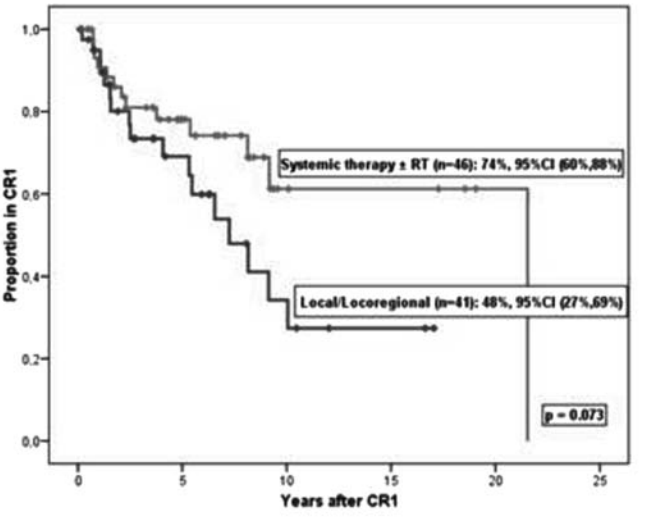

Figure 1.

P331

OCCULT BONE MARROW INVOLVEMENT DETECTED BY FLOW CYTOMETRY MAY AFFECT OVERALL SURVIVAL AND PROGRESSION FREE SURVIVAL IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

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Background: Diffuse large B cell lymphoma (DLBCL) is a common aggressive and potentially curable lymphoma. Histologic involvement of the BM at diagnosis affects disease stage and the International Prognostic Index (IPI) score that are both associated with outcome. In some cases flow cytometry (FC) detects occult marrow involvement which was not detected in microscopy. The clinical

relevance of such occult BM involvement is still not clear. Current guidelines lack clear recommendations as to the role of flow cytometry of BM aspirates in the staging of DLBCL.
Aims: To study the prognostic significance of occult BM involvement based on flow cytometry findings in DLBCL.
Methods: The medical charts of all consecutive DLBCL patients over the age of 18 that were diagnosed with DLBCL at a single center between 1994-2003 were reviewed. We defined 3 groups within the cohort of patients: 1. patients with histologic involvement of the BM as diagnosed by BM biopsy (BM+ group). 2. patients negative for histologic involvement or that BM results were inconclusive but that demonstrated cytometric evidence for involvemnt in BM aspirate (BM-FC+). 3. patients with neither histologic or cytometric evidence for lymphoma involvement in the BM (BM-FC-). Flow cytometry was performed on BM aspirates using two color flow cytometry. Data was aquired using the EPICS-XL (BECKMAN COULTER) flow cytometer from 1999 onwards, and the PROFILE II (BECKMAN COULTER) flow cytometer before 1999. B cell monoclonality was defined as either a ratio of immunoglobulin light chain expression of kappa:lambda >3:1 or lambda:kappa >2:1 in at least 2% of the gated population. BM biopsies were treated with standard procedures, stained with haematoxylin and eosin (H&E) and subjected to standard immunohistochemistry studies. Statistical analysis: Study endpoints included overall survival (OS) and progression free survival (PFS). Survival was studied using Kaplan-Meyer plots and compared using log rank test [Stata 12 (Texas)]. Patients were followed-up from time of diagnosis until death or April 2005, whichever came first.
Results: One hundred and one patients were included in the analysis. Median age of the cohort was 67 (range 21-90). Male and female were represented equally. Disease stages I to III were documented in 19%, 16% and 21%, respectively. Stage IV disease was documented in 33% of patients and stage was not available in 11% of patients. Low risk, low intermediate, high intermediate and high risk IPI were demonstrated in 38%, 10%, 20% and 20%, respectively. IPI was not available in 12% of patients. BM+ group included 13 patients (13%). The BM-FC+ group comprised of 16 patients (16%) and the BM-FC- included 72 patients (71%). Within the BM-FC-, BM-FC+ and BM+ groups IPI was Low risk in 42%, 38% and 15%; low intermediate risk in 12%, 6% and 0%; high-intermediate in 12%, 31% and 46% and high risk in 16%, 25% and 38%, respectively (P=0.01). Most patients were treated with CHOP or CHOP-like regimens (78%) and 5 patients were treated with palliative intent. Importantly, the vast majority of patients in this study were treated before rituximab was available and consequently only 10% of patients were treated with rituximab containing regimens. Median OS for the BM-FC-, BM-FC+ and BM+ were 4.6 years, 2.2 years and 0.88 years, respectively (log rank P=0.01). Median PFS for the BM-FC-, BM-FC+ and BM+ were 3.2 years, 1.4 years and 0.58 years, respectively (log rank P=0.01).
Summary / Conclusion: Occult involvement of the BM at diagnosis as assessed by FC of BM aspirates in patients with DLBCL identifies patients with inferior outcome. These data must be reproduced in the era of immunochemotherapy.

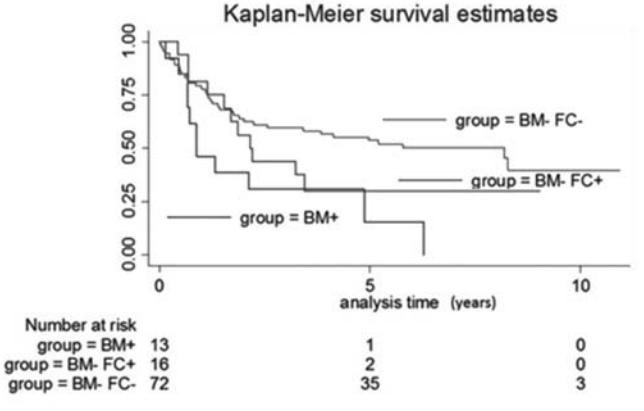

Figure 1.

P332

CLINICAL AND MOLECULAR SIGNIFICANCE OF TUMOR NECROSIS IN NEWLY DIAGNOSED PATIENTS WITH HODGKIN'S LYMHOMA AND DIFFUSE LARGE B CELL LYMPHOMA

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Background: Lymphoma of various types in newly diagnosed patients may exhibit necrotic areas in the tumor mass on imaging. To date, little is known about the clinical significance of this finding and even less is known about the mechanism of cell death in the necrotic tissue - apoptosis, molecular necrosis or autophagy
Aims: The objective of this study was to investigate the prognostic significance of tumor necrosis in newly diagnosed Hodgkin's lymphoma (HL) and diffuse large B cell lymphoma (DLBCL) patients and to define the molecular mechanism of cell death responsible for the necrosis.
Methods: Two hundred and four CT scan or CT-PET studies from newly diagnosed patients with DLBCL (131) and HL (73) were analyzed for the presence of tumor necrosis.Among 56 HL and DLBCL patients that underwent an open surgical biopsy of the tumor mass nineteen presented necrotic morphology in the biopsied tissues: 8 HL and 11 DLBCL. Biopsies without evidence of necrotic morphology (10 patients from the above 56) and reactive lymph nodes (4) were included as controls. All sections were stained for ki-67, a proliferation marker, activated caspase-3, apoptosis marker, and HMGB-1, a necrotic cell death marker.
Results: Radiographic appearance of necrosis was present in 47% of all patients, 34% of HL and 53% of DLBCL patients. A statistically significant correlation was found between necrosis and bulky disease (tumor mass size≥10 cm) ($P=0.0002x10^{-6}$) and also between necrosis and elevated LDH (P=0.00002). When DLBCL and HL patients were analyzed separately, we found significant correlation between necrosis and elevated LDH in DLBCL patients (P=0.0001) however in HL patients, there was no significant correlation between necrosis and LDH at the time of diagnosis. No statistically significant correlation was found between necrosis and age >60 or more advanced stage of disease. Surprisingly, we did not find correlations between patients' prognosis and tumor necrosis. There were no differences in interim response to treatment, disease-free survival and overall survival between patients with necrosis and without it. When DLBCL and HL patients were analyzed separately, we found better outcome in patients with tumor necrosis: Among DLBCL patients with elevated LDH there was a trend for better outcome, by disease-free survival and overall survival, in those with tumor necrosis (P=0.098 and 0.093, respectively). In HL patients, disease-free survival was greater in patients with necrosis compared to those without necrosis. More over, we found a significant correlation between tumor necrosis and disease stage- more HL patients without tumor necrosis had an advanced disease (stages III-IV) compared to those with necrosis (P=0.027). Our results show that in contrast to solid tumors, tissue necrosis in lymphoma is not a predictor of worse prognosis: though more patients with radiographic appearance of necrosis had also the known bad prognostic signs- elevated LDH and/or bulky disease, this group did not have worse outcome. We suggest that necrosis can lead to a better response to treatment in lymphoma.DLBCL cells showed high proliferation rate, with ki-67 index ranging from 40 to 90% and in HL cells the ki-67 index ranged from 5 to 50%. All HL patients were positive for HMGB-1 with no significant staining for caspase-3. Conversely, 10 DLBCL patient samples out of 11 were positive for activated caspase-3, none of them were positive HMGB-1. These results show a differential molecular mechanism of cell death in the tumor mass of newly diagnosed HL and DLBCL patients.
Summary / Conclusion: In summary, tumor necrosis in HL and DLBCL is correlated with bulky disease and high LDH, yet unlike solid tumors tumor necrosis is not correlated with worse prognosis. Rather, tumor necrosis seems to be associated with better outcome. The molecular mechanism of cell death differs between the two types of lymphoma.

P333

PREDICTIVE VALUE OF EARLY 18FDG POSITRON EMISSION TOMOGRAPHY (PET) IN HIGH-RISK AGGRESSIVE B-LYMPHOMAS TREATED WITH RITUXIMAB, INTENSIVE INDUCTION THERAPY AND AUTOLOGOUS TRANSPLANTATION

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Background: ^{18}FDG-PET results at the end of treatment have independent prognostic value in aggressive B-cell lymphomas. The value of early or interim PET is unclear. Also, it is unclear whether eventual negative prognostic impact of positive early PET can be reversed with intensified consolidation treatment.
Aims: We have studied the impact of early PET after 2 to 3 cycles of intensified induction therapy on prognosis of patients 18-62 years old with diffuse large B-cell lymphomas (DLBCL) and primary mediastinal B-cell lymphomas (PMBL) with age-adjusted prognostic index (aaIPI) 2-3.
Methods: Patients were treated in 2002-2011 with 3 cycles of high-dose R-CHOP (rituximab, 375 mg/m^2, cyclophosphamide, 3 g/m^2, doxorubicin, 75 mg/m^2, vincristin, 2 mg and Prednison, 300 mg m^2 + G-CSF), 3 cycles of R-ESHAP, and autologous transplantation (ASCT), with or without radiotherapy. PET was performed on 15th to 21st day of 2nd or 3rd cycle of high-dose R-CHOP. Overall survival (OS), progression-free survival (PFS) and lymphoma-free survival (LFS) was calculated according to Kaplan-Meier. Multivariable analysis for independent predictive factors was calculated according to Cox model.
Results: 91 patients with median age of 39 years (18-62) was treated. aaIPI was 2 in 56% of patients and 3 in 44% of patients. OS was 82±4 % at 3 years, PFS was 79±4 %, and LFS was 82±4 %. 36 patients (40 %) were early PET positive and 55 patients (60%) negative. Early PET negative patients had better 3-year LFS (90±4 % v. 70±8 %, P=0.03), and PFS (87±5 % v. 68±8 %, P=0.05) than early PET positive patients, but not better OS (88±5 % v. 73±8 %, P=0.21). Age less than 45 years and negative PET were the only independent variables for LFS and PFS on multivariate analysis.
Summary / Conclusion: Intensive treatment in early PET positive patients with high-risk aggressive lymphomas at least partially overcomes the results of positive early PET, however, early PET negative patients still have better PFS and LFS.
This work was supported by grants IGA MZ ČR NT 13072-4 and MSMT PRVOUK P27/LF1/1.

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CLINICOPATHOLOGICAL FEATURES AND BONE MARROW PATTERN ANALYSIS IN CASES OF HEPATOSPLENIC GAMMA DELTA T- CELL LYMPHOMA

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Background: Peripheral T- cell lymphomas account for approximately 10-15% of all Non Hodgkin lymphomas (NHL). Hepatosplenic gamma delta T-cell lymphoma (HSGDTCL) accounts for <1% of all NHLs. It is important to differentiate rarer HSGDTCL with a dismal prognosis from commoner T-cell acute lymphoblastic leukemia (T-ALL) with a good prognosis. Diagnosis may also be delayed due to lymphocytic morphology and subtle patterns of involvement in tissues.
Aims: Study clinicopathological features and morphological patterns in bone marrow (BM) biopsy of HSGDTCL.
Methods: Ours is a referral cancer hospital. Cases of HSGDTCL from Jan 2008 – Dec 12 were selected. Diagnosis was based on flow cytometric immunophenotyping (FCI) of peripheral blood (PB)/BM aspirate (BMA) in 14, hepatosplenic biopsy evaluation in5, FCI and hepatosplenic evaluation in1, and BM biopsy morphology with immunohistochemistry (IHC) in 1 case. Clinicopathological findings were recorded. BM aspirate was categorized on predominant tumor population as blastic, lymphocytic or mixed. BM biopsy was categorized on pattern of infiltration as intrasinusoidal, interstitial, both and diffuse. FCI was performed (4 to 8 color, FACS Canto II). Fluorescent in situ hybridization (FISH) was done for common mutation studies including isochromosome 7q. Response to treatment was noted, wherever available.
Results: We had 21 cases (0.22%) of HSGDTCL out of approximately 9478 cases of NHL. Age range was 5 – 51 years (median – 29 years) with 2: 1 M: F ratio. 16/19(84.2%) cases had hepatosplenomegaly, while 5(26.3%) had lymphadenopathy. Anemia and thrombocytopenia was seen in 13/16 (81.3%) and leukocytosis in 6(37.5%) cases. Diagnostic BM aspirate revealed mixed morphology in 12(57.2%), blastic in 4(19.1%) and lymphocytic in 5(23.8%) cases. BM infiltration pattern at presentation was both intrasinusoidal and interstitial in 10/21(47.6%), intrasinusoidal in 9(42.9%), interstitial and diffuse in 1 case each. Splenectomy specimens (3 cases) showed a diffuse red pulp involvement by CD3 positive tumor cells. Liver biopsy(4 cases) showed subtle intrasinusoidal CD3 positive T-cell infiltrate. FCI(18 cases) revealed mature aberrant T-cell immunophenotype. Tumor cells expressed CD3(18/18), CD7(18/18), TCR gamma-delta(15/15) and CD2(16/16). CD5 was expressed in 6/18(33.3%), CD8 in 6/18(33.3%) and CD56 in 7/18(38.9%) cases. Stains for CD4, CD34 and Tdt were negative in all.HLADR was positive in 1 case(5.6%). FISH studies revealed iso 7q in 8/11 cases(72.7%) with additional findings of trisomy 8(3 cases), t(7q;q22)(1 case), 7q;5q deletion(1 case), while 1 case showed no abnormalities. Median period of follow-up was 3 months with maximum survival of 11 months.
Summary / Conclusion: HSGDTCL is common in teenage and young adults (48% were <26 years). Hepatosplenomegaly was absent in 15.8% while lymphadenopathy was present in 26.3% cases. All cases were referred as ALL based on BMA morphology. Mature lymphoid cells were admixed with blast like cells. Expression of surface CD3 along with Tdt negativity raised a high suspicion for HSGDTCL. CD8 expression was noted in 33.3% cases as against common belief of double negative T-cells. CD5 was also expressed in 33.3% cases. Common patterns in BM biopsy were both intrasinusoidal and intersitial. IHC stain for CD3 was mandatory to highlight subtle infiltrates in trephine, liver and spleen biopsies. Iso 7 was seen in 72.7% cases. Chemotherapy protocols included CHOP, DHAP and SMILE; however, most patients were lost to follow up due to the cost involved and poor prognosis explained.

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CLINICAL RELEVANCE OF INTERNATIONAL PROGNOSTIC INDEX AS A PREDICTOR FOR VENOUS THROMBOEMBOLISM IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: RESULTS OF A SINGLE CENTER PROSPECTIVE COHORT STUDY

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Background: Venous thromboembolism (VTE) is a life threatening condition in lymphoma patients. Although high rates of VTE have been reported in patients with lymphoma, the majority of data were from retrospective studies with heterogeneous subtypes. Thus, it is still not clear about the risk factors for VTE in lymphoma patients. Although Khorana risk score has been proposed as a predictive model for cancer-associated thrombosis, there is few data prospectively validating the role of this risk model in lymphoma patients.
Aims: we explored risk factors influencing the occurrence of VTE in diffuse large B-cell lymphoma (DLBCL) patients who were uniformly treated with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone).
Methods: We analyzed the incidence of VTE from DLBCL patients enrolled in our prospective cohort study (NCT00822731). VTE was defined as pulmonary embolism (PE) and deep vein thrombosis (DVT). VTE was diagnosed with radiologic imaging studies including CT scan and ultrasound imaging. All patients were newly diagnosed with DLBCL and initially treated with R-CHOP via central venous catheter between July 2008 and December 2011.
Results: A total of 352 patients were analyzed, and prospectively monitored regarding the occurrence of VTE with the median follow-up duration of 22.6 months. There were 36 cases of VTE including DVT (19/36, 52.8%), PE (13/36, 36.1%) and both (4/36, 11.1%). The median time to VTE was 2.35 months, so the majority of VTE occurred within 6 months after diagnosis. The actuarial incidence of VTE at one year was 10.3%. A half of patients with VTE (18/36, 50.0%) were symptomatic and anticoagulation therapy was used for 22 patients with symptomatic DVT or PE. However, incidental cases of DVT or PE found during imaging follow-up for evaluation of tumor response did not require therapy. There were two deaths-related with VTE including refractory hypoxemia due to PE and bleeding due to anticoagulation. Age older 60 years and poor performance status (≥ ECOG grade 2) were significantly associated with VTE. However, other host factors including co-morbidity, body mass index, and gender were not related (P>0.05). Disease-related factors representing high tumor burden such as extranodal involvements, and Ann Arbor stage III/IV were significantly associated with VTE (P<0.05). However, the involvement of a particular extranodal site including stomach, pancreas, intestine, and mediastinum did not influence the occurrence of VTE. Khorana score-based risk model including cancer type, body mass index, prechemotherapy white blood cell, hemoglobin, and platelet count failed to predict VTE. The multivariate analysis showed that international prognostic index (IPI, high or high-intermediate risk) was the only factor that independently associated with the occurrence of VTE (OR 2.74, CI 1.27-5.88, P=0.01).
Summary / Conclusion: The IPI score is a predictive factor for the occurrence of VTE in DLBCL patients in the era of rituximab.

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PERIPHERAL T CELL LYMPHOMA AND ROLE OF AUTOLOGOUS TRANSPLANTATION

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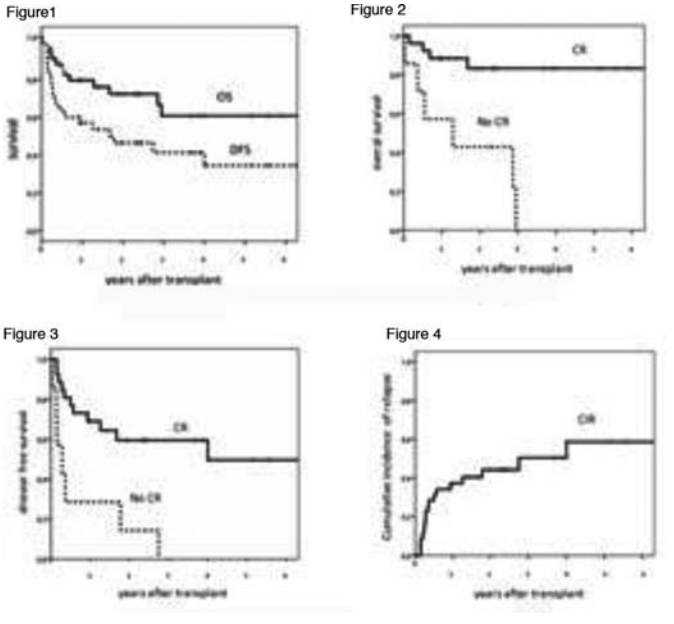
Background: Peripheral T cell is a rare type of lymphoid malignancy with incidence of approximately 15% in most US and European series The most common histopathologic subtypes are PTCL not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AILT), and anaplastic large-cell lymphoma (ALCL), with or without anaplastic lymphoma kinase (ALK) expression. The median age at diagnosis is 60 years, with increase male prevalence (2:1) and it carries a poor prognosis with DFS in first remission of no more than 35% in most series In chemosensitive disease, CHOP like chemotherapy followed by autologous hematopoietic transplant (auto-HCT) has shown to improve the response rates with increase in OS to 40-50% at 3 years, with a low non-relapse mortality. We have performed a retrospective analysis of 37 patients who underwent autologous hematopoietic stem cell (Auto-HCT) transplantation as a part of first line therapy our centre, to determine overall survival (OS), disease free survival (DFS), non-relapse mortality (NRM) and to analyse factors affecting the outcome.
Aims: To assess the role of disease status in patients with T- cell lymphoma patients undergoing autologous hematopoietic stem cell transplant
Methods: Thirty seven consecutive patients with diagnosis of PTCL who underwent Auto-HCT as part of their primary therapy between 1998 to 2011 were analysed. The clinicopathological characteristics of the patients are summarized in Table 1. Median age at transplantation was 51.5 yrs. 30 patients (81%) received CHOP as first line chemotherapy. Disease status on 33 patients prior to auto-HCT was confirmed but the pre-transplant disease status of remaining 4 patients was unavailable. Of these 33 patients, 26 were in first CR at the time of auto- HCT and 7 were in PR. 6 patients had received a 2nd line of chemotherapy to induce CR prior to transplant
Results: With a median follow up of 868 days (267-4725), the 3 year OS for the whole cohort was 58.3%, with a 3 year DFS of 41.3% (Figure 1) and a CIR of 50.4% 2 patients (5%) died due to transplant related complications within 1 year. Patients in CR at the time of auto-HCT (n=26) had an OS of 83.3% at 3 years (Figure 2) and DFS of 59.5% (Figure 3) compared to patients not in CR (n=7) (P=0.000 and P=0.001 respectively). The cumulative incidence of relapse was also higher in patients who were not in CR at the time of transplant (Figure 4). No differences in the outcome on univariate analysis was found taking into account age, CD34+ cells infused or number of chemo lines prior to transplant.
Summary / Conclusion: Patients in 1st CR at the time of auto- HCT have an excellent OS and DFS. This outcome was independent of age, CD34+ cells infused or number of chemotherapy lines prior to transplant and relates only to remission status at the time of transplant. On the basis of this analysis and other published data, auto-HCT is recommended for peripheral T cell lymphoma patients in 1st CR but not for those not achieving CR who should be considered for alternative treatment strategies

Table 1. Clinicopathological characteristics of Patients				
			Patients	
Characteristics		No		%
Age, years				
Median			51.5	
Range			27-76	
Sex				
	Male	24		65
	Female	13		35
Histological subtype				
	PTCL	13		34
	AILT	8		21
	ALCL-ALK +	2		5
	ALCL- ALK-	7		19
	T cell NOS	3		10
	Hepatosplenic	2		5
	EATL	2		5
B Symptoms		25		65
Elevated LDH		30		79
Stage III or IV		22		58
BM Involvement		21		55
ECOG status	>2	28		74
Chemotherapy				
	CHOP Like	30		81
	Non-CHOP	7		19
Lost follow up		5		13
Complete Remission (CR)		26		70
No Complete Remission		7		19

Table 1.



Figures 1,2,3,4.

P337

RETROSPECTIVE ANALYSIS OF DIFFERENT TREATMENT APPROACHES TO PATIENTS WITH EXTRA-GASTRIC MALT LYMPHOMA

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Background: MALT Lymphoma is a relatively common Non-Hodgkin Lymphoma. In contrast to gastric MALT lymphoma, which can be effectively treated with Helicobacter pylori (HP) eradication therapy, treatment strategies for extra-gastic diseases are less well defined and several different treatment strategies, including surgery, systemic chemotherapy, radiation therapy, and antibiotic therapy have shown efficiency in previous studies.
Aims: To investigate if one treatment modality might be superior.
Methods: 185 patients treated at our institution were retrospectively analyzed. Since most MALT lymphoma patients do not die from lymphoma, we used time to progression and therapy free interval as surrugate endpoints for effectiveness.
Results: 72 male and 113 female patients with a median age of 63 (IQR: 50-74) years at diagnosis had a median follow up time of 49 (IQR: 18-103) months. 81 (44%) patients received systemic chemo/immunotherapy, 34 (18%) radiation therapy, 30 (16%) surgery, and 15 (8%) antibiotic therapy. 13 (7%) were followed closely without receiving therapy. Patients undergoing received surgery had significantly less (P=0.002) stage IV disease. Patients having either surgery (100%), chemo/immuno therapy (85.5%), or radiation (80%) had significantly (P=0.01) higher response rates than patients treated with antibiotics (33.3%). However, significantly (P=0.018) more patients had progressive disease after radiation (58%) compared to chemo/immuno therapy (33.3%), or surgery (27.6%). There was no significant difference in the median time to progression (P=0.141) but the estimated time to progression (P=0.023) as well as the estimated therapy free interval (P=0.021) were significantly different among the various cohorts. Stage had no influence on the estimated time to progression (P=0.442). Furthermore, stage (P=0.442), elevated LDH (P=0.141), decreased hemoglobin (P=0.069), elevated beta 2 microglobulin (P=0.570), plasmacellular differentiation (P=0.839), monoclonal gammopathy (P=0.836), or autoimmune disease (P=0.127) did not influence the occurrence of disease progression.
Summary / Conclusion: Surgery for patients with early stages and chemo/immuno therapy for early and advanced stages seem to be superior to radiation therapy. Antibiotic therapy seemed to be the least effective treatment for extra-gastic MALT lymphoma.

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LOWER INCIDENCE OF EBV-POSITIVITY IN ELDERLY DIFFUSE LARGE B-CELL LYMPHOMA: POSSIBLE NEW SUBGROUP OF ELDERLY B-CELL LYMPHOMA

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Background: Diffuse large B-cell lymphoma (DLBCL) has been considered as a heterogeneous aggressive lymphoma. Recently, Epstein-Barr Virus (EBV)-positive DLBCL of the elderly has introduced as a new category of DLBCL by Japanese investigators (Asano N, *et al*. Blood 2009; 113: 2629-36), and documented in the 4th edition of WHO classification. EBV-associated DLBCL has characterized as age over 50 years, and the background of this subgroup of DLBCL disease has strongly affected by the underlying immunodeficiency. It has reported that these patients have a worse prognosis than those with EBV-negative DLBCL.
Aims: Recently, the population of EBV-associated B-cell lymphoma has been increased among patients aged over 50 years according to aging process. However, little is still known about clinical and biological characters in these patients; therefore, the aim of this study was to clarify the clinicopathological features and EBV infection in 125 patients with DLBCL in aged over 50 years.
Methods: Clinical and pathological data on 125 DLBCL patients aged over 50 years, who were diagnosed in our institute from January 2008 to January 2013, were retrospectively analyzed. Diagnoses were confirmed by immunohistochemistry performed on paraffin-embedded tissue sections, using selected members of a panel of monoclonal antibodies including CD3,4,5, 8, 10, 20, and 30, and the all cases were examined for the positivity of EBV by the *in situ* hybridization detection technique, and for EBV early RNAs (EBER) by using a REMBRANT detection kit (Zymed, San Francisco, CA). The patients diagnosed as transformation from low grade lymphomas such as follicular lymphoma and mantle cell lymphoma to aggressive B-cell lymphoma were excluded. The comparison between EBV-positive ratio by the detection of EBER in tumor samples and each clinical data according to age in every 10 years in aged over 50 years were carried out.
Results: Of 125 DLBCL patients, mean age was 72 years (51-97) and sex ratio was 69:56 (M:F). The patient population was 23 (50-59 y/o), 28 (60-69 y/o), 43 (70-79 y/o), and 31 patients (more than 80 y/o), respectively. The average positivity of EBER by using immunohistologic studies for the EBV-latent gene products on paraffin sections was 6.8% in all patients, and the ratio of EBER in each aged group was 6.3 (50-59 y/o), 0 (60-69 y/o), 16 (70-79 y/o), and 3.6% (more than 80 y/o), respectively. We also performed CD5 immunostaing as well, and the positive ratio in each aged group was 13 (50-59 y/o), 7.1 (60-69 y/o), 4.7 (70-79 y/o), and 6.5 (more than 80 y/o), respectively. These data on CD5 positivity showed that this cohort of DLBCL was same as general population.
Summary / Conclusion: It has reported that the incidence of EBV-associated lymphoproliferative disorders (LPD) increases with advancing age, and EBER-positive DLBCL in aged over 50 years showed more than 20% of patients (Shimoyama Y, *et al*. Pathol Int. 2009; 59: 835-43). However, in our analysis, only 6.8 % of patients with DLBCL in aged over 50 years showed EBER-positivity. Although the peak of EBER-positive ratio was shown in the group of 70-79 years (16%) as described in the previous report, other 3 aged groups showed a lower incidence of EBV-positivity (6.3% in 50-59-years, 0% in 60-69-years, and 3.6% in 80-years, respectively). The incidence of CD5 positivity was almost similar among each aged group; therefore, the patient population used in this study was not deviated. In conclusion, the incidence of age-related EBV-positive DLBCL is low as reported and it might be existed in new subgroup of B-cell lymphoma. This study was performed in a single institute; thus, it will be necessary to accumulate the patients with precious EBV-associated DLBCL in aged over 50 years, and we are now analyzing the clinical outcome of EBV-negative elderly DLBCL compared to that of EBV-positive cases.

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POLYMORPHISMS OF GLUTATHIONE S-TRANSFERASE MU 1 (GSTM1), THETA 1 (GSTT1) AND PI 1 (GSTP1) GENES AND THEIR CORRELATIONS IN DIFFUSE LARGE B CELL LYMPHOMAS

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Background: The role of genetic polymorphisms in the pathogenesis of lymphomas is not clear. The polymorphisms in detoxification enzymes of the glutathione S-transferase T1 and M1 (GSTT1 and GSTM1) family have been associated with development and the outcome of several lymphomas.
Aims: To study the influence of the GST polymorphisms Mu1 *(GSTM1), Theta1 (GSTT1)* and *Pi1 (GSTP1* Ile(105)Val) in 94 patients with DLBCL, comparing to distribution of 94 control cases and correlate with clinical, prognostic factors and survival.
Methods: Our prospective analysis included 94 patients, diagnosed between July 2009 to July 2012. Median age 59 years old (19-89) and 47 males. DLBCL was diagnosed according to the WHO classification and staged by Ann Arbor criteria. At diagnosis 41 (43.6%) patients had stages I/II and 53 (56.4%) stages III/IV; 33 (35.1%) patients with International Prognostic Index (IPI) >2. All patients were treated with R-CHOP. Genomic DNA from peripheral blood of all individuals and control was analysed by the multiplex-PCR for identification of the GSTM1 and GSTT1 genotypes and PCR-RFLP for identification of genotypes of the GSTP1 Ile(105)Val.

Table 1. *GST* genotypes in DLBCL patients and controls.

Genotypes	Patients Number (%)	Controls Number (%)	*P* value	OR* (95% CI)
GSTM1				
Null	33 (35)	43 (45)	0.14	0.64 (0.35–1.15)
Present	61 (65)	51 (55)		reference
GSTT1				
Null	13 (14)	18 (19)	0.33	0.67(0.31-1.47)
Present	81 (86)	76 (81)		Reference
GSTP1				
Ile/Ile	30 (32)	43 (45)		1.8 (0.99–3.25)
Ile/Val	55 (59)	46 (49)	0.05	
Val/Val	09 (9)	05 (6)		reference

Results: The frequencies of the *GSTM1, GSTT1* and GSTP1 Ile(105)Val genotypes among DLBCL patients and controls is presented in Table 1. No statistical difference was found between DLBCL patients and controls concerning the frequency of GSTM1and GSTT1, while that of GSTP1 ile/ile, ile/val and val/val were subtly different among patients and control.
When DLBCL patients were compared to clinical and diagnostic factors, it was found a higher frequency of older patients in GSTM1 deleted (P=0.02); female gender and IPI index > 2 in GSTT1 undeleted (P=0.03 in both factors); and the presence of B symptoms in GSTP1 (P=0.004). Median follow-up:17 months (1-47) and 77% (95%CI 69-85%) of overall survival. GSTM1 and GSTT1 had no statistical different survival. Patients with GSTP1 ile/val had a better survival than those with val/val and ile/ile (P=0.02).The disease -free survival showed no statistical differences among these groups.
Summary / Conclusion: our analysis revealed that GSTP1 ile/ile genotype conferred a worse overall survival, but not a longer disease- free survival. The other polymorphisms had no influence in development and outcome of the patients. Hence, these genes in the pathogenesis of DLBCL seems to influence the survival and can be another key for understanding the interaction between the risk of acquisition of DLBCL and the genetic polymorphism.

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SERUM ALBUMIN RETAINS INDEPENDENT PROGNOSTIC SIGNIFICANCE FOR SURVIVAL IN DLBCL IN THE POST-RITUXIMAB ERA

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Background: Serum albumin (SA) levels may serve as a good indirect measure of general health, co-morbid conditions, and/or disease biology, facilitating its use as a prognostic marker when assessed prior to chemotherapy in patients with diffuse large b-cell lymphoma (DLBCL) treated in the post rituximab (R) era. SA has been shown to be a prognostic marker in patients with Hodgkin lymphoma, splenic marginal zone lymphoma, and was shown to be a prognostic marker in patients with DLBCL prior to the introduction of R. There has been conflicting evidence of SA as a prognostic marker in the post R era of DLBCL treatment.
Aims: To evaluate if SA at diagnosis can predict clinical outcomes in patients with DLBCL in the post R era of treatment.
Methods: Patients at the Moffitt Cancer Center (MCC) between 2007 and 2010 who presented for diagnosis or treatment for DLBCL were identified using an institutional database. Only patients who received primary treatment at MCC with rituximab, cyclophosphamide, vincristine, doxorubicin, and prednisone (R-CHOP) were included in the study. Patients who were referred for second opinions or for relapsed/refractory disease were excluded. Clinical and treatment data was recorded including SA levels at diagnosis. Survival time was estimated using the Kaplan Meier (KM) method, and a Cox Proportional Hazard model was used to identify potential risk factors for the time to event data. A p-value< 0.05 was considered significant.
Results: 295 patients were initially identified. 171 patients were excluded for not having primary treatment at MCC (n=161) or because they did not receive standard induction with R-CHOP (n=10). Mean age at diagnosis was 56 years, 56 (46%) were age older than 60 years, 77 (62%) were male, 113 (91%) were Caucasian, 10 (8%) were HIV positive, 45 (36%) were Ann Arbor Stage 1 or2, 47 (38%) were international prognostic index (IPI) score 0-1, and 33 were IPI intermediate high or high risk (27%) . Overall survival (OS) and progression free survival at 4 years were estimated to be 65% and 58%, respectively. Median follow up was estimated to be 36 months. In univariate analysis SA ≥ 3.7g/dL was found to be a statistically significant predictor for OS in our series with a HR of 0.3 (95% CI 0.16-0.59, P<0.001). Initial lymphocyte count (HR 0.45, 95% CI 0.25-0.81, P=0.008), initial serum lactate dehydrogenase (HR 3.58, 95% CI 1.57-8.20, P=0.003), IPI-Risk (HR 1.95, 95% CI 1.43-2.66, P<0.001) also were found to be statistically significant predictors for OS. Age >60 years (HR 1.72, 95% CI 0.93-3.17, P=0.08) trended towards being a statistically significant predictor for OS. The KM survival curve for SA ≥3.7g/dL is shown in the Figure 1. Further, the prognostic significance for SA on OS was maintained after adjusting for IPI-risk.
Summary / Conclusion: SA ≥ 3.7g/dL was associated with a significant reduction in the risk of death among those with DLBCL treated with R-CHOP at MCC. This effect was maintained after adjusting for the IPI-risk, suggesting that SA may account for unmeasured elements of disease biology, such as proliferative rate, and/or inflammatory profile, as well as possible co-morbid conditions incompletely assessed by age or performance status. These data highlight the need for further analysis of SA in the context of larger prospective studies.

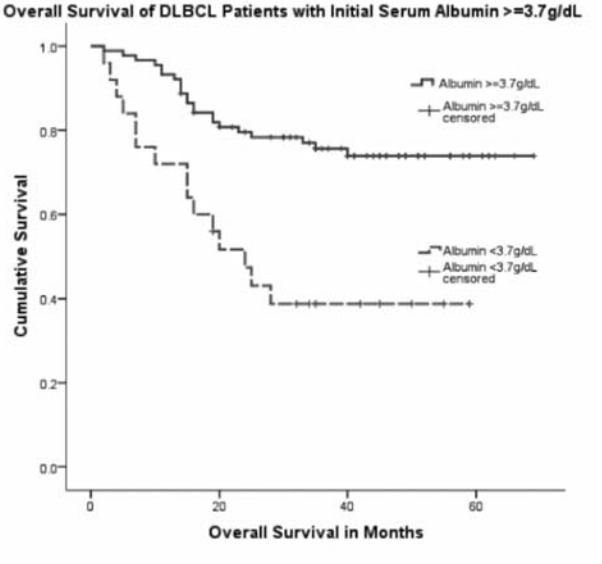

Figure 1.

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UTILITY OF F-18 FDG PET/CT FOR THE DIAGNOSIS AND THERAPEUTIC MANAGEMENT FOR EXTRANODAL NATURAL KILLER (NK)/T CELL LYMPHOMA, NASAL TYPE

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Background: Extranodal natural killer (NK)/T-cell lymphoma, nasal type (ENKTL) is quite rare in western populations, but relatively common in East Asia (especially China) and Latin America. ENKTL is an aggressive disease with poor prognosis, requiring risk stratification. Similar symptoms, such as nasal obstruction and purulent nasal discharge, are found in patients with nasal NKTL and in patients with chronic rhinosinusitis. The images from contrast-enhanced computed tomography (CT) frequently show no prominent mass. As a result, the diagnosis is often delayed because it is frequently misdiagnosed as sinusitis. F-18 FDG PET/CT is a powerful imaging tool for diagnosis, staging, and evaluation of therapeutic effect in oncology.
Aims: This study was designed to investigate the value of FDG PET/CT in the therapeutic management of extranodal natural killer (NK)/T-cell lymphoma, nasal type.
Methods: A total of 26 patients with NK/T-cell lymphoma, nasal type were diagnosed according to morphologic and immunophenotypic criteria as specified in the World Health Organization (WHO) classification. All patients underwent FDG PET/CT and clinical information was obtained by review of medical records.
Results: In 26 cases, all nasal/extranasal lesions were FDG-avid evidented to be neoplasm on CT scan and histopathological examinations. FDG-avid lesions in nasal/maxillary areas were uniformly more localized than demonstrated on CT scan, suggesting soft tissue masses on CT were partly due to inflammatory reaction. Among the 26 patients with definite diagnosis, 9 patients were restaged on the basis of F18 FDG PET/CT with 5 patients down-staged and 4 patients up-staged. Statistical difference of the standardized uptake values (SUV) after 6 courses of chemotherapy and/or radiotherapy between the complete remission (CR) group and the partial remission (PR) group can be found (4.1±2.3 versus 7.8±1.7, P=0.006). The SUV value between pre-reatment and post-treatment were also of statistical significance in 22 patients (11.4±6.2 versus 5.6±2.1, P=0.000).At a follow up of 18 months, patients got CR had a longer survival time than those got PR, stable disease (SD) or progress disease (PD) in 20 patients out of 26 patients. (median survival:310 days vs 284 days, 95% CI: 0.7156 to 1.365, P<0.05).
Summary / Conclusion: Our preliminary study suggests that FDG PET/CT can provide more accurate information on the diagnosis, staged and therapeutic response assessment in extranodal natural killer (NK)/T-cell lymphoma, nasal type. FDG PET/CT can be an invaluable imaging modality in this clinical setting. Further investigation with large patients enrollment is warranted.

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THE PROGNOSTIC IMPACT OF CONCORDANT AND DISCORDANT BONE MARROW INVOLVEMENT IN DIFFUSE LARGE B-CELL LYMPHOMA, INDEPENDENT OF THE REVISED INTERNATIONAL PROGNOSTIC INDEX AND CELL OF ORIGIN

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Background: Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disorder with various outcomes. Many studies have suggested prognostic factors, such as International Prognostic Index (IPI), gene-expression profiles, and cell of origin (COO) such as germinal center B-cell (GCB) and non-GCB subtypes. Prior studies suggested bone marrow (BM) involvement histology, such as concordant and discordant, could be prognostic factor. However, the impact of discordant BM involvement remains unclear, and there are few data about multivariate analysis of BM involvement type and COO type.
Aims: The aim of this study was to evaluate the prognostic impact of BM involvement type in patients with DLBCL treated with R-CHOP after controlling for the revised IPI (rIPI) score and COO.
Methods: Patients with DLBCL treated with R-CHOP at Gachon University Gil Medical Center, between November 2004 and December 2011 were enrolled. Sex, rIPI using five factors such as age > 60, LDH, ECOG performance status, Ann Arbor stage, and extranodal involvement were collected retrospectively. If there were ≥ 5 large, non-cleaved cells per HPF on BM biopsy sections, it was considered as concordant BM involvement (cBMi), otherwise it was considered as discordant BM involvement (dBMi). COO type was defined using immunohistochemical stain of CD10, BCL-6, and MUM-1 according to the algorithm proposed by Hans *et al*. Clinical characteristics were compared using the Chi-square test (data not shown). The overall survival (OS) and progression-free survival (PFS) were assessed using Kaplan-Meier method and the log-rank test was used for comparison between groups. Multivariate analysis was performed using Cox regression model to assess the independent effect of prognostic variables on outcome.
Results: In total of 152 patients with DLBCL, 126 (82.9%) had negative BM, and 26 (17.1%) had BM lymphomatous involvement. 15 (9.9%) had cBMi and 11 (7.2%) had dBMi. In 107 patients classified by COO, 40 patients (37.4%) had GCB type, 67 patients (62.6%) had non-GCB type. Compared to patients with negative in BM, the PFS was significantly shorter for patients with either concordant (log-rank P <0.001) or discordant (log-rank P =0.002) BM involvement. OS was significantly shorter for patients with cBMi compared to those with negative BM (log-rank P <0.001). However, there was no significant difference in OS between patients with negative BM and dBMi (log-rank P =0.065). In addition, there was no significantly difference in PFS and OS between patients with cBMi and dBMi (log-rank P=0.336, 0.159, respectively). In 107 patients, patients with non-GCB type showed significantly shorter PFS compared to those with GCB type (log-rank P =0.043). In multivariate analysis, both cBMi and dBMi remained as a significant negative prognostic factor for both PFS and OS independent of the rIPI score and COO. Non-GCB type did not have significant prognostic impact on the PFS and OS after controlling for the rIPI score and BM involvement (Table 1).**Summary / Conclusion:** Both concordant and discordant BM involvement had adverse prognostic impact for PFS and OS; it was independent of the rIPI score and COO (non-GCB). The outcomes of patients with cBMi were poorer than those with dBMi, but there was no statistically significance. However cBMi had higher relative risk for OS than dBMi in multivariate analysis (RR=5.9 for cBMi, 3.3 for dBMi).. Although patients with non-GCB showed shorter PFS than those with GCB in log-rank test, non-GCB type did not have significant prognostic impact on the PFS after controlling for the rIPI score and BM involvement.

Table 1. Cox regression model of concordant BM involvement rIPI factors for PFS an OS.

	PFS			OS		
Variable	P	RR	95% CI	P	RR	95% CI
Concordant vs negative BM						
BM involvement, COO, and rIPI score						
Concordant BM	.002	7.0	2.0 to 24.3	< .001	5.9	2.2 to 15.8
COO (non-GCB)	.638	1.4	0.4 to 5.6	.377	0.6	0.2 to 1.8
rIPI (1-3)	.458	1.4	0.6 to 3.1	.015	2.5	1.2 to 5.2
BM involvement, COO, and individual rIPI factors						
Concordant BM	.003	8.7	2.1 to 35.5	.002	5.1	1.8 to 14.4
Age > 60 years	.677	0.8	0.3 to 2.5	.285	1.7	0.7 to 4.2
LDH > 485 U/L	.375	0.5	0.1 to 2.4	.929	0.9	0.2 to 3.3
ECOG PS ≥ 2	.024	4.2	1.2 to 14.7	.013	3.3	1.3 to 8.3
Stage III, IV	.370	2.0	0.4 to 8.8	.303	1.9	0.6 to 6.2
Extranodal sites > 1	.771	1.2	0.3 to 4.7	.871	1.1	0.4 to 3.1
COO (non-GCB)	.750	1.3	0.3 to 5.5	.250	0.5	0.2 to 1.6
Discordant vs negative BM						
BM involvement, COO, and rIPI score						
Discordant BM	.002	7.0	2.0 to 24.3	.036	3.3	1.1 to 10.1
COO (non-GCB)	.638	1.4	0.4 to 5.6	.796	0.9	0.3 to 2.3
rIPI (1-3)	.458	1.4	0.6 to 3.1	.011	2.7	1.3 to 5.8
BM involvement, COO, and individual rIPI factors						
Discordant BM	.031	6.6	1.2 to 36.8	.109	3.5	0.8 to 16.1
Age > 60 years	.892	1.1	0.3 to 3.5	.082	2.7	0.9 to 8.0
LDH > 485 U/L	.357	0.4	0.1 to 2.5	.842	0.9	0.2 to 3.1
ECOG PS > 2	.126	4.0	0.7 to 24.1	.016	4.5	1.3 to 15.4
Stage III, IV	.418	1.9	0.4 to 9.4	.472	1.6	0.5 to 5.3
Extranodal sites > 1	.509	0.6	0.1 to 2.6	.746	0.8	0.2 to 2.6
COO (non-GCB)	.298	2.0	0.5 to 7.6	.530	0.7	0.2 to 2.0

Abbreviations: CR, complete response; uCR, unconfirmed CR; NA, not available; rIPI, revised international prognostic index; ECOG PS, Eastern Cooperative Oncology Group performance status; PFS, progression-free survival; OS, overall survival; RR, relative risk; CI, confidence interval

Stem cell transplantation - Clinical 1

P343

TYROSINE KINASE INHIBITORS IMPROVE OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PH(+) ALL IN FIRST COMPLETE REMISSION: A STUDY FROM THE ACUTE LEUKEMIA WORKING PARTY OF EBMT

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Background: The introduction of Tyrosine kinase inhibitors (TKIs) has likely modified the natural history of Philadephia chromosome Acute Lymphoblastic Leukemia (Ph+ALL). Indeed, TKIs used pre and post- allogeneic hematopoietic stem cell transplantation (allo-HSCT) may allow for improved outcome. However, data are still scarce in this field.
Aims: The current study aimed to assess outcomes in a cohort of 473 Ph+ALL patients who received allo-HSCT in first complete remission (CR1) between 2000-2010 and focused on the role of TKIs in this setting.
Results: In this series, median age was 42 (range 18-70) and 55% were males. Median time from diagnosis to CR1 was 44 days and from diagnosis to allo-HSCT 158 days. 225 patients (47.4%) received allo-HSCT from an HLA-identical sibling, while 249 (52.6%) received an HLA-matched unrelated graft. 79% patients underwent a myeloablative conditioning (MAC) regimen and 21% underwent a reduced intensity conditioning (RIC) regimen. 329 patients received a TKI at time of induction therapy- mainly imatinib (90%)- and 157 patients received TKI in the post transplant period. The overall incidences of acute GVHD and chronic GVHD (cGVHD) were 40% and 53% respectively. At 3 years, overall survival (OS) and leukemia-free survival (LFS) were 53% and 42%. The relapse incidence (RI) was 35% and non-relapse mortality (NRM) 23%. In multivariate analysis, 2 predictive factors were associated with LFS: TKI use during induction therapy improved LFS (P=0.03 HR=0.76, 95%CI, 0.59-0.97) while age>42y was associated with lower LFS (P=0.02 HR=1.01, 95%CI, 1.01-1.02). In multivariate analysis for relapse, 2 factors were associated with decreased relapse: use of TKI in pre-allograft treatment (P=0.04 HR=0.71, 95%CI, 0.52-0.98) and use of an unrelated donor (UD) vs. identical sibling (P=0.001, HR=0.6, 95%CI, 0.44-0.81). Also, a RIC regimen was associated with an increased incidence of relapse (P=0.007 HR=1.64, 95%CI, 1.14-2.35). Concerning NRM, age>42 and UD were factors associated with higher NRM (P=0.02, HR=.02, 95%CI, 1.01-1.03;P=0.0 HR=1.46,95%CI,1.01-2.12), while a RIC regimen was the only factor associated with lower NRM (P=0.02 HR=0.51,95%CI=0.3-0.89). Concerning the incidence of cGVHD, peripheral blood stem cell vs bone marrow was the only risk factor of developing a cGVHD (P=0.005 HR=1.63,95%CI=1.16-2.28). Interestingly, post-transplant TKI was a protective factor of cGVHD development (P=0.006 HR=0.64 95%CI=0.47-0.88).
Summary / Conclusion: Overall, this large study demonstrated that the introduction of TKIs in Ph+ALL treatment paradigm (pre and post transplant) allowed for a significant outcome improvement after allo-HSCT including LFS, relapse, but also incidence of cGVHD, suggesting that further prospective studies are needed to refine the use of TKIs after allo-HSCT.

P344

LONG TERM SURVEY OF 180 PATIENTS TRANSPLANTED AFTER RIC REGIMEN USING THREE DOSES OF ATG: A BELGIAN HEMATOLOGY SOCIETY (BHS) NATIONAL PROSPECTIVE TRIAL

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Background: The use of RIC allows older patients (pts) and those with comorbidities to be treated by allogeneic SCT. However, the optimal conditioning remains debatable and particularly the role of Anti-thymocytes globulin (ATG).
Aims: We report the 10 yrs follow-up of a prospective multicenter dose-finding study where the aim was to determine the optimal dose of ATG in terms of engraftment, acute and chronic GVHD, and event-free survival (EFS).
Methods: From Jan 2000 to Dec 2007, 184 pts (not eligible for myeloablative transplant (MAC) due to older age or comorbidities) were transplanted with a HLA identical sibling after RIC containing fludara (30 mg/m²/d x 4 days) and cyclophosphamide (CPA) (1 g/m²/d x3 days). GVHD prevention consisted of cyclosporine A and rabbit ATG (Fresenius). The first cohort of patients (n=26) receiving rATG 10mg/kg/day x4, was stopped because of protocol stopping rules (50% relapse, 37% TRM). The second group receiving 10 mg/kg/day x2, (n=20) was prematurely closed because of 50% of acute GVHD. A third group of patients didn't receive any rATG but additional MMF (n=33). The fourth cohort received 2 days of 10 mg/kg ATG and mycophenolate mofetil (MMF)(n=101). The whole study included 184 pts: 26 AML, 18 MDS, 21 CLL, 12 CML, 46 MM, 45 NHL, 3 MPN, 8 HL and 1 AA.
Results: 180 pts were analyzed. Median age was 54 (13-74) yo. Median follow-up was 8 yrs (90 mos). 43 pts (24%) are in CR before alloSCT and 92 (51%) are in CR after SCT with 30% remaining in continuous CR. The overall survival (OS) is 42%/ 8 yrs. There was no difference in OS according to disease or doses of rATG. EFS is 29%/8 yrs. Pts with MM had worse EFS compared to other patients (15% vs 48% for CLL, 38% -for NHL, 27% for AML and MDS). Concerning the role of rATG, pts who received 4 days of rATG had a worse EFS compared to the other patients (11% vs 30 -35% for the other rATG groups, P=0.09). There were no significant differences in EFS according to age. AGVHD was related to the dose of rATG: 52% of grade 2-4 aGVHD in the rATG 2 vs 25% in the rATG 4. cGVHD was not statistically different according to the doses of rATG but the group treated with rATG 2 and MMF had 34% of cGVHD but only 8% of extended cGVHD. Pts who developed extensive chronic GVHD had a better outcome than patients without chronic GVHD (relapse rate: 16.7% vs 53%). TRM was lower in the rATG 2-MMF group (20%). TRM was higher above 60 yo (46% vs 18% P=0.02).
Summary / Conclusion: Fluda/CPA/ATG-based RIC-SCT enables transplants in older pts or pts unfit for MAC with excellent long term outcome (45% OS, 29% EFS/8 yrs). Two doses of rATG combined with CSA and MMF allow limited cGVHD and lower TRM and was thus chosen for subsequent trials. This approach resulted in 48 and 38% of cure in CLL and NHL but is not sufficiently effective in MM, AML and MDS.

P345

SPECTRUM OF EPSTEIN-BARR VIRUS-ASSOCIATED DISEASES IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Epstein-Barr virus (EBV)infection may result in a spectrum of diseasesin recipients ofallogeneic hematopoietic stem cell transplantation (allo-HSCT), including fever, post-transplant lymphoproliferative diseases (PTLD) and endorgan diseases(pneumonia, encephalitis/ myelitis, hepatitis and so on).Now PTLD has been widely studied; however, there are only scattered reports about EBV-associated diseases other than PTLD.
Aims: To investigate the incidence, clinical characteristics and prognosis of the spectrum of EBV-associated diseases.
Methods: A total of 263 recipients undergoing allo-HSCT were enrolled. The blood EBV-DNA loads were regularly monitored by quantitativereal-time polymerase chain reaction.
Results: The 3-year cumulative incidence of total EBV-associated diseases, PTLD, EBVfever and EBV endorgan diseases were 15.6%±2.5%, 9.9%±2.0%, 3.3%±1.3% and 3.3%±1.2%, respectively. In the EBV endorgan diseases, the 3-year cumulative incidence of EBV pneumonia,encephalitis/ myelitisand hepatitis were 2.2%±1.0%, 1.6%±0.8% and 0.9%±0.6%, respectively. Of the 36 patients with EBV-associated diseases, 7 onlyhadfever without tissue involvement, and 29 had tissue involvement including 19 with extranodal involvement. The involved area included the lymph nodes (n=18),central nervous system (CNS, n=14), lung (n=9), tonsil (n=6), liver (n=4), spleen (n=3) and nasalcavity (n=1). Thirty patients presented with fever, 4 with lymphadenectasis and 2 with CNS symptoms as the initial manifestations. Fever wasthe most common symptom of EBV-associated diseases. The median time to onset of PTLD and EBV endorgan diseases was 61 (range, 22-337) days and 60 (range, 43-95) days post-transplantation, respectively (P=0.209). The EBV-DNA loads of secretions (cerebrospinal fluid, bronchoalveolar lavage fluid,hydrothorax and ascites) were significantly higher than that of blood (39620±8875 copies/mL versus 17619±5275 copies/ml, P=0.030).Thecell immunophenotype of secretions was consistent with histopathology of affected tissue. Patients with PTLDhad better response rate torituximab-based treatments, compared to those with

EBV endorgan diseases (including PTLD accompanied by EBV endorgan diseases)(P=0.014).The 3-yearoverall survival was 37.3%±13.7%, 100.0% and 0.0%±0.0% in patients with PTLD, EBV fever and EBV endorgan diseases, respectively(P=0.001).
Summary / Conclusion: EBV-associated diseases other than PTLD are not rare in recipients of allo-HSCT. The clinical manifestations and onset time of EBV endorgan diseases and PTLD are similar.EBV detection and cell immunophenotypic analysis in the secretions of affected tissues could be proposed as an alternativemethod of diagosis for patients unsuitable for biopsy. EBV endorgan diseases do not respond well to rituximab-based therapy, compared with PTLD.

P346

ENTERAL VERSUS PARENTERAL NUTRITIONAL SUPPORT POST ALLOGENEIC HAEMATOPOIETIC CELL TRANSPLANTATION – RESULTS OF A RANDOMIZED CONTROLLED TRIAL

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Background: Nutritional support during allogeneic haematopoietic progenitor cell transplantation (HPCT) is imperative to prevent malnutrition and resultant inferior patient outcomes. However, there is currently no high quality studies or consensus in the literature as to the most efficacious approach to supplemental feeding post HPCT, leading to wide variation in practice in the use of enteral (EN) or parenteral (PN) feeding across HPCT units.
Aims: To determine the tolerability and efficacy of EN *versus* PN in patients undertaking HPCT.
Methods: Randomized controlled study in patients undertaking HPCT with either myeloablative or reduced intensity conditioning (RIC). Informed consent was obtained from patients before commencing conditioning and they were randomized to receive either EN or PN after commencing HPCT if they could not maintain adequate oral nutritional intake, defined as maintaining >60% of daily caloric requirements orally. Patients with severe gastro-intestinal toxicity, including severe mucositis, were excluded from randomisation. The primary endpoint of the study was tolerance of route of supplemental nutritional support, defined as <30% of patients needing to change to the alternative route of support for any reason.
Results: In total 38 patients were enrolled onto the study, including 9 patients undertaking myeloablative and 29 patients RIC HPCT. Only 19 patients (50%) required nutritional support post-HPCT. Of these 19 patients, only 9 (47%) were able to be randomized between EN (n=5) and PN (n=4), with 10 patients excluded from the study due to presence of severe gastrointestinal toxicity in7, markedly deranged liver function in1, and withdrawal of consent in 2. The 5 patients randomized to EN met on average 74% of their goal nutrition and 100% required changing to PN due to (gastro-intestinal) intolerance. The 4 patients receiving PN met on average 91% of requirements, with none requiring change to EN.
Summary / Conclusion: For patients undertaking myeloablative or RIC HPCT, supplemental feeding with EN commencing at failure to maintain adequate oral nutritional intake is not feasible, due to the presence of significant gastrointestinal toxicity in these patients at this time. Further research is needed to investigate whether prophylactic nasogastric tube placement would improve the feasibility and tolerance of EN in this patient population.

P347

COMBINED CMV REPLICATION AND CHRONIC GVHD GENERATE A SIGNIFICANT SYNERGY OF ANTI-LEUKEMIC EFFECT AFTER ALLOGENEIC HSCT IN ACUTE MYELOID LEUKEMIA

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Background: Cytomegalovirus (CMV) reactivation is regarded as an important cause of death after allogeneic hematopoietic stem cell transplantation (alloHSCT). Recently, the hypothesis that the early CMV replication is an independent factor for reducing the leukemic relapse risk has been suggested by Elmaagacli *et al*.
Aims: The purpose of this study was to elucidate the effect of CMV replication on leukemic relapse risk after alloHSCT in AML patients and to evaluate the association of CMV replication with another impact factors for relapse risk.
Methods: 78 AML patients who underwent alloHSCT between August 2006 and December 2012 were analyzed retrospectively. All patients were regularly monitored by CMV qualitative PCR. If qualitative PCR was positive, then viral load was determined by quantitative PCR.
Results: The median age was 35 years and the ratio of male to female was 41 to 37. Disease statuses just before alloHSCT were the first complete remission (CR1) of 61 patients (78%), over than CR1 of 5 patients (6%) and non-CR of 12 patients (15%). In 60 of 78 patients (77%), first CMV replication as detected by CMV-specific qualitative PCR developed at a median of 37 days (range, 11-653) after alloHSCT without relapse. 11 of 60 patients (18%) with CMV replication relapsed at median 124 days (range, 43-714) after alloHSCT. Univariate analysis identified several factors for reducing the 5-year cumulative incidence of relapse (CIR); CMV viremia (negative vs. positive; 56% vs. 24%, P=0.001), disease status at alloHSCT (the first Complete remission (CR1) vs. over than CR1 vs. non-CR; 26% vs. 60% vs. 56%, P=0.026) and chronic graft-versus-host disease (GVHD) (negative vs. positive; 43% vs. 13%, P=0.003). Chronic GVHD is a well known factor for preventing relapse. In our data, CMV replication also offers prolonged leukemia-free survival (LFS) and overall survival (OS) as well as chronic GVHD (LFS 5years after HSCT with vs. without CMV; 48% vs. 20%, P=0.002; OS 5years after HSCT with vs. without CMV; 50% vs. 19%, P=0.005). Of note, in patients with higher than 75,000 copies/mL of CMV-PCR titer, CMV replication had no influence on LFS. As the patients were divided into 4 subgroups according to the existence of CMV replication and chronic GVHD (cGVHD), the patients with both CMV replication and cGVHD showed the lowest 5-year CIR (CMV(-)/cGVHD(-) vs. CMV(-)/cGVHD(+) vs. CMV(+)/cGVHD(-) vs. CMV(+)/cGVHD(+); 61% vs. 33% vs. 62% vs. 11%, P<0.001). The patients with both CMV replication and chronic GVHD had the best outcome on leukemia free survival (P<0.001) and overall survival (P<0.001).
Summary / Conclusion: In this study, CMV replication was a prognostic factor for leukemia-free survival in AML. Furthermore, when CMV replication was combined with chronic GVHD, relapse rate after allogeneic HSCT was most significantly decreased in AML patients. Therefore, we suggest that the immunologic understanding of graft-versus-leukemia effect in this setting is critically required.

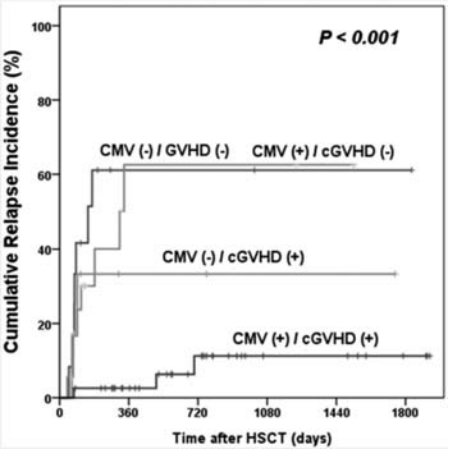

Figure 1.

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HUMAN HERPESVIRUS-6 REACTIVATION AND HHV-6 ENCEPHALITIS AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION: A MULTICENTER, PROSPECTIVE STUDY.

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Background: The epidemiology of human herpesvirus (HHV)-6 encephalitis after allogeneic hematopoietic cell transplantation (HCT) and its relationship with HHV-6 reactivation have not been sufficiently characterized.
Aims: To clarify the 1) morbidity of HHV-6 encephalitis, and 2) the effect of HHV-6 reactivation on the development of HHV-6 encephalitis after allogeneic HCT.
Methods: This prospective, multicenter study including 230 allogeneic HCT recipients investigated the epidemiology of HHV-6 reactivation and HHV-6 encephalitis. Plasma HHV-6 DNA load was prospectively evaluated twice weekly until 70 days after HCT. the relationship between plasma HHV-6 load and development of HHV-6 encephalitis using data on prospectively monitored HHV-6 load.
Results: Cumulative incidence (CI) of positive HHV-6 DNA and high-level HHV-6 reactivation (plasma HHV-6 DNA $\geq 10^4$ copies/ml) at day 70 after HCT was 72.2% and 37.0%, respectively. Multivariate analysis identified myeloablative conditioning (hazard ratio [HR], 1.9; P=0.004), umbilical cord blood transplantation (UCBT) (HR, 2.0; P=0.003), and male sex (HR, 1.6; P=0.04) as risk fac-

tors for displaying high-level HHV-6 reactivation. HHV-6 encephalitis occurred in 7 patients, and CI at day 70 was 3.0%. None of the144 patients without high-level HHV-6 reactivation and 7 of 86 patients (8.1%) with high-level HHV-6 reactivation developed HHV-6 encephalitis (P=0.0009). The prevalence of HHV-6 encephalitis was significantly higher in patients receiving UCBT than in patients with other sources (CI at day 70, 7.9% vs. 1.2%, P=0.008). In each of 7 patients with HHV-6 encephalitis, CNS symptoms developed concomitant with peak plasma HHV-6 DNA (range, 21,656 – 433,639 copies/mL).
Summary / Conclusion: High levels of plasma HHV-6 DNA are associated with higher risk for HHV-6 encephalitis. UCBT is a significant risk factor for HHV-6 encephalitis. HHV-6 encephalitis should be considered if CNS dysfunction develops concomitant to high-level plasma HHV-6 DNA after allogeneic HCT.

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CENTRAL NERVOUS SYSTEM (CNS) RELAPSE IN AML PATIENTS WITH CNS DISEASE UNDERGOING ALLOGENIC STEM CELL TRANSPLANTATION- RISK FACTORS AND PROGNOSIS

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Background: Approximately 2-4% of patients with acute myeloid leukemia (AML) have involvement of the central nervous system involvement (CNS). High presenting white blood count (WBC), peripheral blast (PB)%, elevated LDH, FAB AML subgroups M4 & M5, younger age (<50 years), extra-medullary (EM) involvement, and cytogenetic abnormalities such as inv (16) and 11q23 abnormalities appear to be risk factors. Guidelines with regards to optimal diagnostic and therapeutic strategies for these patients are limited.
Aims: To describe clinical and pathological features of patients with AML diagnosed to have CNS involvement prior to proceeding with allo-SCT. To determine the efficacy of current treatment strategies in preventing post-transplant CNS relapse.
Methods: After due IRB approval, 351 patients of all age groups that underwent allo-SCT for AML at the Mayo Clinic from 1985 to 2012 were reviewed. Patients who were diagnosed to have CNS disease pre transplant were identified. All patients with AML at our institution have a lumbar puncture (LP) performed prior to allo-SCT. Clinical, pathological, prognostic and transplant related data were abstracted retrospectively. Transplant conditioning regimens and graft versus host disease (GVHD) prophylaxis were according to institutional standards.

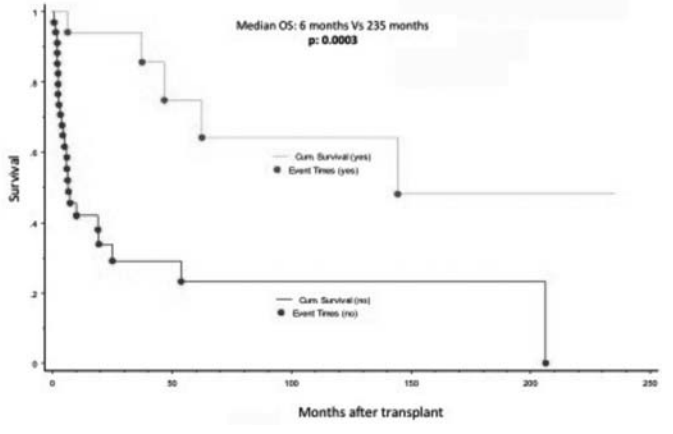

Figure 1. Survival based on chronic GVHD in patients with AML with CNS involvement.

Results: Forty-four (13%) of 351 AML patients had CNS disease detected prior to allo-SCT. Of these 17 (39%) were detected at AML diagnosis due to clinical signs/symptoms, 11 (25%) were detected to have CNS involvement at the time of AML relapse and 16 (36%) were asymptomatic; diagnosed on routine pre transplant L.P. Twenty six (59%) were males, with a median age of 40 years (range; 4-71 years). Eleven (25%) patients received IT-chemotherapy alone (Methotrexate or Ara-C), 3 (7%) received systemic chemotherapy alone and 26 (59%) received IT with systemic chemotherapy (high dose ARA-C). Twenty-two (50%) received cranio-spinal radiation. Twenty (45%) had concurrent EM disease. Eleven (25%) patients underwent allo-SCT in CR1, 14 (32%) in CR2, while 14 (32%) had residual disease. Donor sources were; 26 (59%) matched sibling donors, 17 (39%) matched unrelated donors, and 1 haploidentical transplant. Thirty seven (84%) were peripheral blood grafts, while the remainder were bone marrow grafts. 37 (84%) had total body irradiation (TBI) based conditioning (34- Cytoxan/TBI, and 3 with TBI/Thiotepa/Cytoxan/ATG), 1 patient received Cytoxan and Busulfan while 3 received fludarabine and melphalan. Sixteen (36%) had AML M4/M5, 7 (16%) patients had a core binding factor AML, while 3(7%) had MLL gene rearrangements. At diagnosis median WBC was 71.3x10^9/L, PB blasts % 55, Hemoglobin 9.8 gm/dL and platelet count 76.3x10^3/mL. Five (11%) patients had post-transplant CNS relapse (1 with monocytic leukemia) in spite of CNS directed therapy. Three patients had received IT alone, while 2 received IT and HIDAC. Four (80%) underwent a TBI based myeloablative allo-SCT. With the exception for a higher presenting WBC (median; 124x10^9/L), there were no other clinical, pathological or cytogenetic differences noted. Two (40%) patients had additional EM disease pre-SCT and 1 had EM relapse post SCT. Median OS for these 5 patients was 7 months, compared to 39 months for the patients without CNS relapse. Fifteen (38%) of 39 patients without CNS relapse had chronic GVHD compared to only one patient with CNS relapse. Patients with chronic GHVD had a median survival of 235 months compared to 6 months in those without chronic GVHD (P=0.0003) (Figure 1).
Summary / Conclusion: Routine pre transplant L.P helps identifying a significant number of patients with asymptomatic CNS involvement, resulting in better CNS directed therapies. In spite of this, approximately 10% of patients do have a post-transplant CNS relapse. Major prognostic factors for this phenomenon include higher WBC at presentation and the lack of chronic GVHD.

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COMPARISON OF MESENCHYMAL STEM CELLS AND MESENCHYMAL STEM CELLS COMBINED WITH CORD BLOOD FOR TREATMENT OF ENGRAFT FAILURE FOLLOWING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION: A PROSPECTIVE PI

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Background: Engraft failure after autologoushematopoieticstem cell transplantation (auto-HSCT) is a formidable complication which occurs in 2.0-9.5% of patients and is associated with considerable morbidity and mortality related to infections and hemorrhagic complications. To investigate the efficacy of mesenchymal stem cells (MSCs) alone or combined with cord blood (CB) to engraft failure in auto-HSCT, this multicenter randomized trial was conducted.
Aims: In this study, the efficacy of MSCs alone or combined with CB to engraft failure in auto-HSCTwas evaluated.
Methods: Sixteen patients were enrolled in this multicenter randomized trial.The median age was 40 years with a range of 14 to 60 years. Primary diseases included acute myelogenous leukemia AML(n=10), acute lymphoblastic leukemia (n=1), multiple myeloma (n=4) and Non-Hodgkin's lymphoma (n=1). These patients were randomized into alone MSCs(n=8) or MSCs combined with CB treatments. MSCs obtained from ex-vivo-expanded MSCs derived from HLA-mismatch bone marrow donor, and CB from unrelated CB. In MSCs group, MSCs were administered at a median dose of 1×10^6/kg once with an interval of two weeks, and 2 doses were a cycle of MSCs. If patient was not responsive to the treatment within 28 days after a cycle of MSCs, CB would be used. In MSCs combined with CB group(CB group), MSCs were administered as abovemention single-unitCB was administered withthe first application of MSCs. If patient was not responsive to the treatment within 28 days after treatment, MSCs would continue to administer for 2 doses.
Results: With 56 days after treatments, 5/8 and 8/8 obtained the hematopoietic reconstruction, respectively, in MSCs and CB groups(P=0.20). Of the 3 patients who did not obtain the hematopoietic reconstruction in MSCs group, 2 patients obtained the hematopoietic reconstruction, one was ineffective after CB treatment, who died of leukemia relapse at 103 days post-transplants. Chimerism analysis from peripheral blood samples showed that CB genetic markers were not detected at 15 and 30 days, respectively, after CB infusion.None of patients experienced GVHD. The median time obtaining the hematopoietic reconstruction was 30 (range 11-43) and 22 (range 17-48) days, respectively, in MSCs and CB groups(P=0.516). With a median follow up of 240 (range 103-350) days after transplantation, 13 were alive and 3 patients died of the relapse of primary diseases.
Summary / Conclusion: Our data indicate that MSCs alone are effective to engraft failure and CB can facilitate the effect of MSCs to engraft failure.

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ABCG2 OVEREXPRESSION NEGATIVELY AFFECT OUTCOME OF ALLOGENEIC STEM CELL TRANSPLANT IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Long term survival of acute myeloid leukemia (AML) patients

remains dismal, with a cure rate of 20-40%. So, allogeneic stem cell transplantation (SCT) is considered the recommended therapy. Over-expression of MDR-related protein ABCG2 is associated with higher relapse risk in AML patients achieving remission with chemotherapy. However, little is known on the impact of ABCG2 in patients undergoing allogeneic SCT.
Aims: In the present work we retrospectively analyzed 135 patients with AML who underwent allogeneic HSCT at our Institution between 2002 and 2011, to evaluate the effect of ABCG2 expression on long term outcome of transplantation.
Methods: Median age at SCT was 48 (range: 17-70) years. Sixty eight patients (50%) received grafts from a sibling donor, 67 (50%) from a matched unrelated donors (MUD). In 88 patients (65%) stem cells source was peripheral blood (PB), while bone marrow (BM) stem cells were used in 47 cases. Status at SCT was complete remission (CR) in 91 patients, while 44 patients were transplanted with relapsed or refractory disease.
Results: Fifty-seven of 135 patients (42%) over-expressed ABCG2, while 78 cases (58%) did not. Two-years progression-free survival (PFS) from transplant was 55% for the entire population. PFS was not associated with patient's age, donor type, stem cell source or CD34+ quantity. The only factors positively affecting PFS were status at transplant (CR vs active disease, P<0.0001) and ABCG2 status (negative vs positive, P=0.02). Among the 57 ABCG2+ patients, 31 relapsed (53%), compared with 25 relapses in 78 ABCG2- cases (35%). As a consequence, both 1-year and 2-years PFS were higher in the ABCG2- patients (70% and 67%, respectively) than in the ABCG2+ patients (51% and 46%, respectively) (Figure 1). The difference in relapse rate between the two cohorts was significant in the 91 patients transplanted in CR (13 relapses in 36 ABCG2+ vs 8 relapses in 55 ABCG2-, P=0.03) while was similar in the 44 cases who underwent SCT with active leukemia (18/21 in ABCG2+ vs 17/23 in ABCG2-, P=0.55).
Summary / Conclusion: Our data suggest that overexpression of ABCG2 is associated with a worse outcome in patients undergoing SCT for AML, mainly for a higher risk of relapse, especially in those transplanted in CR. This finding could suggest for a stricter follow-up and prompt intervention (e.g. DLI) in ABCG2+ cases

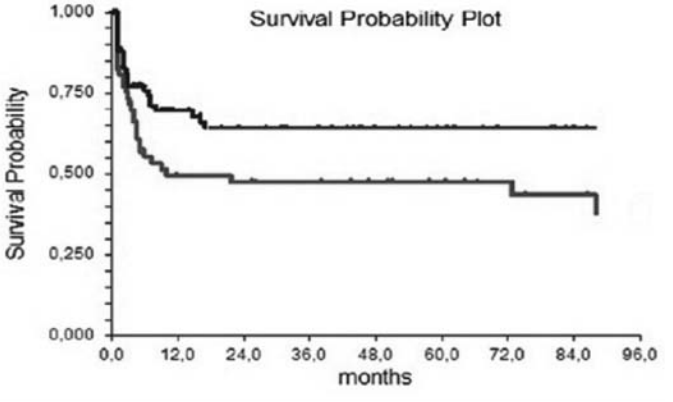

Figure 1.

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"PLERIXAFOR ON DEMAND" IN ASSOCIATION WITH CHEMOTHERAPY AND G-CSF HALVES RATE OF MOBILIZATION FAILURE IN LYMPHOMA AND IN MULTIPLE MYELOMA: PRELIMINARY RESULTS OF A MULTICENTER PROSPECTIVE STUDY

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Background: Failure in PBSC harvest interests 7-10% of Multiple Myeloma (MM) cases and 20-30% of Lymphoma cases. "On demand" use of Plerixafor (PLX) in association with "G-CSF" has recently been reported. However, no prospective data are available on "PLX on demand" in association with "chemotherapy and G-CSF". We have previously published the development of an algorithm for the use of PLX "on demand" in association with High CTX and G-CSF (Milone G, Blood Transfusion 2012).
Aims: We present preliminary results of a phase II, prospective study in which PLX was employed "on demand", according to our algorithm, in patients mobilized using High Dose CTX (4 gr/sqm) or DHAP and G-CSF.
Methods: Seventy-three patients were registered, 68 were evaluated for treatment efficacy; 5 patients did not receive PLX in spite of having criteria for its use. Evaluated patients had a mean age of 54 y., 41 were male and 27 female, 52 were affected with MM and 16 with Lymphoma, 53 were mobilized using HD-CTX and 15 using DHAP. PLX was administered if, on day +13 after chemotherapy, CD34+ cell count in PB was below $10x10^6$/l, or if CD34+ count was between 10 and $20x10^6$/L and first apheresis yielded a CD34+ $<1x10^6$/kg.
Results: Overall success rate of CD34+ mobilization (defined as a CD34+ count in PB > $20x10^6$/L) was 95.5%. Mobilization failure was registered in only 3.9% of MM and in 6.3% of Lymphoma. Overall success rate in harvesting a CD 34+ $>2x10^6$/kg was 94% (MM: 96.1%; Lymphoma: 86.6%). Negative predictive value of the algorithm used was 100%, in fact, 61/61 patients were correctly predicted to reach a successful mobilization and harvest without requiring PLX. Five patients were predicted to not reach successful mobilization. However, due to drug shortage, these patients did not receive PLX. Only one of the five (20%) reached successful mobilization, thus confirming the high positive predictive value of the algorithm (80%). Seven patients were predicted as not reaching successful mobilization according to algorithm and were treated with PLX, 4/7 (57%) had a successful harvest. Overall the rate of PLX indication according to the algorithm was 16.4% (MM 6.5%, Lymphoma 30%).
Summary / Conclusion: Compared to chemotherapy + G-CSF, on demand use of PLX in association with chemotherapy and G-CSF is able to halve the mobilization and harvest failure rate: failure of harvest in MM patients is reduced from 7-10% to 3.5% and in Lymphoma patients from 20-25% to 13.4%.

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A LEBANESE-ITALIAN COLLABORATIVE INITIATIVE ON BONE MARROW TRANSPLANTATION IN PEDIATRIC PATIENTS WITH B-THALASSEMIA MAJOR

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Background: Bone marrow transplantation (BMT) is the only available curative modality for β-Thalassemia Major (β–TM) with best results obtained in younger regularly transfused and chelated patients. We hereby report treatment outcomes of 36 Lebanese children who received BMT in the Mediterranean Institute of Hematology (IME) centers in Italy as part of an Italian - Lebanese collaboration.
Aims: This study aims at assessing treatment outcomes and complications in 36 Lebanese children with β–TM who underwent BMT in Italy. It also addresses the favorable impact of an international collaborative work in facilitating cure for thalassemia.
Methods: 36 children with β–TM receiving transfusion and chelation at the Chronic Care Center, Lebanon underwent BMT from HLA compatible donors in IME centers, Italy supported by the Italian government. Percutaneous liver biopsies were performed on all patients before BMT and children were assigned a Pesaro risk category. Conditioning regimen consisted of busulfan, cyclophosphamide±Thiotepa and ATG. GVHD prophylaxis included cyclosporine A, methotrexate and prednisolone. Engraftment was evaluated by fluorescence in situ hybridization. Estimates of overall survival and event-free survival were calculated by the Kaplan–Meier method. Transplant related mortality (TRM) and other complications were calculated as cumulative incidence. Analyses were performed using SPSS software version 15.0.
Results: 36 HCV PCR negative children with β –TM (M/F 1: I), median age 8.5 years, median follow 6.20 years, underwent BMT from HLA identical donors. 25%, 52% and 23% had Pesaro risk class1, 2 and3, respectively. The donor was an immediate family relative in 95% and unrelated in 5%. Mean injected CD34+ cells, total nucleated cells and CD3+ cells/recipient were $10.7x10^6$ /Kg, $8.78x10^8$/Kg and $6.4x10^7$ /Kg. Absolute neutrophil count $>0.5x10^9$ and platelet count >20 x 10^9 were reached in all patients within a mean of +19.25±4.2 and +21.6 ±3.8 days. 35/36 children (97.3%) had complete engraftment in +20 to + 60 days while 1/36 (2.7%) had partial engraftment but continued to be transfusion independent with a mean Hb of 9g/dcl for +1155 days. 32/36 (89%) children are alive and transfusion independent. 4/36 (11%) died: 1 with multi organ failure, 2 with grade 4 acute GVHD and 1 with fulminant interstitial pneumonitis. 8/36 (22%) had grade 2 - 4 acute GVHD of which 75% resolved on treatment while 25% (all grade 4) were fatal. 9/32 (28%) surviving children had chronic GVHD completely resolved on treatment. Other transplant related complications included CMV reactivation, sepsis, EBV and candida infections, hemorrhagic cystitis, transient cyclosporine related renal and neurotoxicity, cerebral toxoplasmosis and tuberculosis all completely resolved on treatment. Iron overload data on 27 children at + 3.1 year median follow up showed that 9/27 (33.3%) had significant iron overload defined as SF >2500 ng/ml, or LIC >15 mg Fe/g dw, or T2* <20 msec.
Summary / Conclusion: This Italian–Lebanese collaborative study on BMT of children with β –TM reveals an excellent treatment outcome with low TRM and transient and manageable associated complications. It also underscores the importance of post BMT iron overload monitoring and treatment. This scientific work demonstrates the effectiveness of international collaboration in facilitat-

ing cure for thalassemia in developing countries as Lebanon and prompts initiating larger scale collaborations in the Mediterranean regions for achieving cure for other inherited hemoglobin disorders. Given the genetic similarity among Mediterranean populations, establishing a regional BM donor database is warranted

P354

VITAMIN D DEFICIENCY INCREASES THE RISK OF COMPLICATIONS IN ALLOGENEIC STEM CELL TRANSPLANTATIONS

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Background: Patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) have an increased incidence of vitamin D deficiency which can be explained by prolonged illness, hospitalization and nutritional problems. Vitamin D has in later years been shown to have great impact on the immune system and to play a role in the development of autoimmune diseases. Vitamin D receptor is expressed on all immune cells, most pronounced on T lymphocytes, macrophages and dendritic cells, and activation of the receptor leads to a suppression of the immune response. Because of this, vitamin D deficiency has been suggested to increase the risk of development of graft-versus-host disease (GvHD) following HSCT.
Aims: To evaluate a possible association between vitamin D and development of chronic GvHD.
Methods: We have retrospectively investigated vitamin D levels in serum from the time of transplantation in 169 patients undergoing HSCT at Karolinska University Hospital between 2005 and 2010.
Frozen serum from a biobank was analyzed for 25-OH-cholecalciferol (in this paper referred to as vitamin D) at the Laboratory for Clinical Chemistry, Karolinska University Hospital. Normal levels for the method are 75-250 nmol/L. Levels below 50 nmol/L are considered insufficient, and below 25 nmol/l are deficient. The serum levels of vitamin D were then correlated to incidence of extensive chronic GvHD.
Results: A majority of the patients, 65%, had vitamin D insufficiency (<50 nmol/l) , and 12% had deficiency (<25 nmol/L). The median level was 42 nmol/l, range 10-118. Lower levels of vitamin D at transplantation correlated significantly with an increased incidence of extensive chronic GvHD, grade moderate to severe according to NIH criteria, P<0.05, with a relative risk of2,2 for a cut-off at 60 nmol/L. There was also a significant correlation between low levels of vitamin D and increased mortality, P<0.05.
Summary / Conclusion: To conclude, we found a high incidence of vitamin D insufficiency and deficiency in patients planned for HSCT, and these low levels are associated with an increased incidence of extensive chronic GvHD and increased mortality. It is possible that early monitoring of vitamin D levels and substitution could increase outcome in HSCT.

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NON TBI MYELOABLATIVE CONDITIONING REGIMEN FOR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PEDIATRIC ALL.

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Background: Total body irradiation (TBI) – based preparative regimens have been considered the gold standard for allogeneic HSCT in children with ALL; however, there are emerging concerns about the long-term sequeal of TBI in childhood. Substituting i.v. for oral busulfan (Bu) reduces variability in drug exposure, potentially improving the safety and efficacy of the treatment.
Aims: We retrospectively evaluated 60 ALL patients (male 41 /female 19) transplanted in our institution, between January 2005 to November 2012, who received allogeneic HSCT, using a myeloablative i.v. Bu-based conditioning.
Methods: The median age of the patients was 8,4 years (0,6 – 19,9 years). Thirty-six (36) patients were in CR1, 17 in CR2, 5 in CR3 and 2 in advanced disease. Donors were: HLA-matched siblings (n=18), matched unrelated (n=38), 1-Ag mismatched related donors (n=2) and haploidentical (n=2). Thirty-nine (39) patients received bone marrow, 16 peripheral blood stem cells and 5 cord blood. Busulfan was administrated as a 2hs, infusion every 6hs over 4 days (16 doses) in combination with Cyclophosphamide and VP-16 in 44 patients. Graft versus host disease (GVHD) prophylaxis consisted of CSA+MTX in patients receiving blood or marrow stem cells and CSA only in those who received cord blood. Anti-thymocyte globulin was added to those who were transplanted from unrelated donor.
Results: All patients but two achieved sustained engraftment. Median time to ANC>500, and platelets>20.000 was 19 days (14-30 days) and 21 days (12-50 days) respectively. One patient died on day 10 and another patient relapsed on day 22; both were not evaluated for engraftment. There were 14 cases of mild veno-occlusive disease and 12 cases of hemorrhagic cystitis. Grade II-IV acute and chronic GVHD occurred in 28/60 and 9/60 patients, respectively. At median follow-up of 50,7 months (4,5-112,9 months) 40 patients are alive/disease free and one is alive with leukemia. Ten patients relapsed and died and 10 died of transplant-related causes. The overall survival (OS) rate, relapse rate and TRM were 69%, 23% and 10%, respectively.
Summary / Conclusion: Our results are comparable to those reported with TBI- based preparative regimens and suggest that i.v. Bu may be a reliable alternative to TBI in the setting of HSCT for ALL in children.

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CIDOFOVIR AS SECOND LINE THERAPY IN CMV REACTIVATION: CLINICAL AND BIOLOGICAL CORRELATES.

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Background: One of most important causes of morbidity and mortality following allogeneic haematopoietic stem cell transplantation (HSCT) is Cytomegalovirus (CMV), in particular in patients that don’t achieve a rapid CMV clearance. It has been shown that the risk of CMV reactivation following HSCT can be assessed by measuring the CMV-specific T cell response.
Aims: We report cidofovir (CDF) activity in ganciclovir (GCV) resistant patients. We also evaluated $CD8^+$ CMV-specific T cell responses in order to identify predictors of ganciclovir response.
Methods: Between 2004 and 2010, 156 patients following HSCT were treated for CMV reactivation. Of these, 55 were treated with CDF because of GCV resistant CMV infection (n=40), CMV disease (n=11), or pre-engraftment CMV infection (n=4). Patients received 5mg/kg CDF weekly with dose adjustment for renal impairment. Failure of treatment with GCV was defined as CMV PCR positivity after 2 weeks’ treatment. 62 (40%) patients received stem cells from HLA identical sibling transplants, 86 (55%) from matched unrelated donors and 8 (5%) from cord blood units. 63 patients (40%) received myeloablative conditioning, 93 (60%) received reduced intensity conditioning. 126 (81%) patients underwent T-cell depletion with alemtuzumab. Data was collected and analyzed retrospectively. The CMV specific $CD8^+$ T cell response was retrospectively studied in a group of GCV-responsive (n=11) and GCV-resistant patients who received CDF (n=7), using HLA:peptide tetramers specific to CMV peptide-MHC complexes of pp65 and IE-1.
Results: We looked at responses in patients who received at least 12 days’ prior GCV prior to CDF for persistent viremia (n=35), of whom 80% (n=28) responded to CDF. 11 patients were commenced on CDF for CMV disease, of whom 55% (n=6) responded to CDF. No patients died from a cause related to treatment, 18 patients had renal toxicity (only 1 required dialysis). These events led to treatment discontinuation but they had no impact on the CMV reactivation outcome. Renal function improved after CDF discontinuation. 70 patients experienced recurrent CMV reactivation. In this group, 36 patients were treated with CDF. 20/36 patients received CDF for all reactivations, also in this group of patients CDF was well tolereted and 17/20 required dose reduction for reduction in GFR. The median time to eradicate CMV viremia was 15 days (median of 3 administrations). Patients treated with GCV had significantly higher tetramer responses compared to CDF treated patients (P=0.0013 by Mann Whitney test). Looking at day +50 (P=0.012), +100 (P=0.0175) and + 150 (P=0.0426) post HSCT this difference remains significant. Total lymphocyte count, $CD3^+$ and $CD8^+$ T cell counts were not different between the 2 groups. Cidofovir-treated patients were able to clear CMV viremia despite a low absolute $CD8^+$ CMV-specific T-cell response. The CMV viremia was cleared in 4/7 patients whose tetramer responses were measured despite very low absolute tetramer responses measured.
Summary / Conclusion: CDF has a good safety profile and should be considered as second-line therapy in patients who have not responded to GCV. Furthermore it appears to be an important in management of CMV infection in patients who have failed to develop an antigen specific CD8+ T cell response.

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IS IMATINIB MAINTENANCE REQUIRED FOR PATIENTS WITH RELAPSE CHRONIC MYELOID LEUKEMIA POST-TRANSPLANTATION OBTAINING CMR? A PILOT RETROSPECTIVE INVESTIGATION

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Background: Imatinib is capable of inducing durable molecular responses in relapsechronic myelogenous leukemia (CML) after allogeneic hematopoietic stem cell transplantation (allo-HSCT), but it is indefinite whether imatinib therapy is required to maintain this response for patients obtaining complete molecular remission (CMR).We retrospectively reviewed 37 patients with relapse

CML post-transplants who were treated with imatinib (n=20) or donor lymphocyte infusion (DLI) (n=17).
Aims: To compare the efficacy between imatinib and DLI to the relapse CML post-transplants, andevaluate the results of ceasing imatinib in the patients who had achieved CMRand complete donor chimerism via imatinib treatment.
Methods: Once leukemia relapse was diagnosed, immunosuppressants were tapered or discontinued if the patient's condition was acceptable. Those who were in molecular or cytogenetic relapse tapered or discontinued immunosuppressants as a front-line therapy. If these patients were not responsive to this treatment after one month, DLI- or imatinib-based treatments were administrated. Those who were in molecular, cytogenetic or chronic phase (CP) relapse received DLI or imatinib monotherapy as a front-line therapy, and the patients in advanced phase received DLI or imatinib combined with chemotherapy as a front-line therapy. In the DLI-based treatments, patients received DLI once every four weeks until patients obtained complete cytogenetic remission (CCR) or developed graft versus host disease (GVHD). In the imatinib-based treatments,when the patients had recovered donor complete chimerism and had achieved CMR as defined by negative quantitative RQ-PCR at three consecutive time points within a period of 3 months, the patients continued or ceased imatinib therapy according to their willingness.
Results: The rate of CMR was 85% and 76.47%, respectively, in the imatinib and DLI group (P=0.509). The treatment-related mortality was0% and 29.4%, respectively, in the imatinib and DLI groups (P=0.019). Fifteen of the 17 patients obtaining CMR voluntarily ceased imatinib, and did not experience relapse. Eight-year overall survival (OS) was 85%±8% and 40.3±12.1% (P=0.017), 8-year disease-free survival (DFS) was 85%±8% and 40.3±12.1% (P=0.011), respectively, in the imatinib and DLI groups.
Summary / Conclusion: Imatinib therapy resulted in higher OS and DFS than that of DLI in relapse CML post-transplants. Imatinib maintenance might not be required for patients with relapse CML post-transplants after they achieved full donor chimerism and CMR.

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CHARACTERISTICS AND RISK FACTORS FOR HOSPITAL READMISSION IN PATIENTS RECEIVING HEMATOPOIETIC CELL TRANSPLANTATION
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Background: Patients who received hematopoietic cell transplantation (HCT) (autologous [auto-HCT] or allogeneic [allo-HCT]) are often readmitted after hospital discharge. However, these hospitalization episodes have been little studied.
Aims: We conducted a retrospective study to identify the risk factors for readmission in recipients of HCT after 90 days of discharge, and its impact on the outcome of the procedure.
Methods: All consecutive patients receiving HCT in our center between March 2009 and December 2012 were reviewed. Only patients discharged from the first admission were included in the study. Readmission was defined as hospitalization for > 24 hours either in the emergency room or hospitalization unit within the first 90-days after discharge. Base line characteristics were compared using Chi-squared test. Univariate and multivariate analysis (MVA) of risk factors for readmission were performed using logistic regression.
Results: A total of 152 patients (93% of the 163 transplanted patients) were discharged during the study period (85 auto-HCT and 67 allo-HCT). Median age at HCT was 55 years (range 17-70) and median follow-up for survivors was 15 months (range 3-48). Patients were mainly transplanted for acute leukemia (AL) (n= 43, 28%) and NHL (n= 41, 27%) in complete remission (n=84, 55%). Sixty-five patients (43%) were readmitted within 90 days (18 [27%] auto-HCT and 47 [73%] allo-HCT) at a median of 15 days (range 1-89) [1 day (range 1-9) for auto-HCT, and 7 days (range 1-127) for allo-HCT] after first discharge. The most common complications for readmission were fever (n=41, 63%), gastrointestinal disorders (n= 11, 17%) and renal abnormalities (n=5, 8%). Median duration time of second hospitalization was 5 days (range 1-127) (2 days for auto-HCT and 7 days for allo-HCT). Among the 65 readmitted patients, 27 (41%) had a microbiological documented infection, including bacterial or viral respiratory infections (n=17, 26%), urinary tract infections (n= 6, 9%) and others (n=4, 6%). Among patients receiving allo-HCT, 15 patients (32%) were diagnosed with acute GVHD during readmission and seven (14%) had CMV infection. ICU admission was required in 7 patients (11%) during readmission, 5% of all patients who had received an HCT. Eight patients (12%) (7 allo-HCT) died during second admission and 57 (88%) were discharged. Among them, 22 patients (38%) had a third hospitalization at a median of 21 days (range 3-87), mainly because of fever (n=10, 42%). Risk factors for readmission in the MVA for the whole cohort were allo-HCT (vs auto-HCT) (HR 8.5 [95%CI 4.2-16.6], P<0.001), diagnosis of AL (HR 2.1 [95%CI 1.1-3.8], P=0.02) and HCT Comorbidity Index (HCT-CI) >2 (HR 1.7 [95%CI 1.1-2.9], P=0.03). For the allo-HCT patients, risk factors for readmission were male gender (HR 2 [95%CI 1.1-3.9], P=0.03), diagnosis of AL (HR 2.3 [95%CI 1.2-4.3], P=0.01) and HCT-CI >2 (HR 2 [95%CI 1.1-3.8], P=0.02). Overall survival at 2 years for the whole cohort and the allo-HCT patients were 71% (95%CI 76-66) and 66% (95%CI 59-73). NRM at 2-years for the allo-HCT patients were 29% (95%CI 22-36). Ninety-day readmission was not significantly associated with lower OS or NRM.
Summary / Conclusion: Hospitalization within 90 days after HCT discharge is frequent, especially after allo-HCT. Patients with acute leukemia and comorbidities seem to have higher risk for readmission.

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UNRELATED CORD BLOOD TRANSPLANTATION FOR CENTRAL NERVOUS SYSTEM RELAPSE IN HIGH RISK ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: Few clinical studies have investigated the role of unrelated cord blood transplantation (CBT) for central nervous system (CNS) relapse of acute lymphoblastic leukemia (ALL) patients with high risk factors.
Aims: The aim of this report was to identify the potential benefits of unrelated CBT in high risk ALL with CNS relapse who had been treated on CNS directed prophylaxis and relapse strategy treatment in the current era.
Methods: Total of 16 childhood (n=10) and adult (n=6) ALLpatients with CNS relapse who underwent unrelated CBT enrolled in our study between 2001 and 2010, and all of the patients had features associated with poor outcomes, such as high WBC at diagnosis, ph+ chromosome, or a history of bone marrow relapse. All transplants were performed with myeloablative conditioning therapy (BU/CY2 or TBI/CY) plus highly CNS-active agents (carmustine or high-dose cytarabine).
Results: All patients achieved neutrophil engraftment and platelet engraftment. A total of 12 patients (75.0%) developed pre-engraftment syndrome (PES) at a median of 8.5 days, and 5 patients developed acute graft-vs-host disease (GVHD) at a median of 19 days. The median follow-up after CBT was 33.6 months. Six patients died at a median of 154 days (range 85- 327) after CBT. In 4 patients, the causes of death were transplant-related: acute GVHD of gastrointestinal (n=1), refractory autoimmune hemolytic anemia (AIHA) and pulmonary infection (n=1), and viral encephalitis (n=2). Two patient died due to disease relapse on day 109 and day 536 after transplantation. No patients received CNS prophylaxis after transplantation and no patients experienced a CNS relapse. The probability of overall survival at 8 years was 69.4%.
Summary / Conclusion: Our experience suggests that unrelated CBT appears to be an effective treatment option for CNS relapse of childhood and adult ALL patients associated with poor outcome features.

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LENOGRASTIM FROM DAY +7 AND PEGFILGRASTIM ARE EQUALLY EFFECTIVE IN REDUCING TIME TO NEUTROPHIL ENGRAFTMENT, ANTIBIOTIC USE AND INPATIENT STAY IN PATIENTS WITH MYELOMA UNDERGOING AUTOLOGOUS TRANSPLANT
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Background: High dose chemotherapy and Autologous peripheral blood stem cell transplantation (APBSCT) following induction therapy is the standard of care for patients with myeloma with good performance status. Busy haematology centres require safe and effective strategies to hasten neutrophil engraftment, decrease inpatient stay and ease financial burden. GCSF has been proven helpful in this regard. We describe our experience with the use of daily lenograstim from day +7 autograft versus one off pegfilgrastim.
Aims: To identify whether once daily lenograstim given at day + 7 following stem cell infusion is as efficacious as one dose of pegfilgrastim at day +1 with regards to neutrophil engraftment, inpatient stay, days of antibiotic and survival outcomes as compared no G-CSF use.
Methods: Our retrospective study included 113 patients (41 female; 72 male) with a median age at transplantation of 61 years (range 38-72) with myeloma between January 2006 and December 2012. APBSCT was carried out by patients receiving high dose melphalan on day -1 and thawed stem cell reinfusion on day 0. First cohort (35%) was of patients not receiving any G-CSF, second cohort (19%) received pegfilgrastim and third cohort (46%) received lenograstim. Statistical analysis was carried out using Graphpad Prism 4 for windows and SPSS 19 for windows. Sixty five patients had IgG, 22 IgA, 19 light chain and 4 non secretory myeloma. At transplant 7% patients were in complete remission, 21% in partial remission and 72% in very good partial remission.
Results: Median time for neutrophil engraftment in the pegfilgrastim and lenograstim cohorts was 12 days and 12.74 days (Mann Whitney test; p value: 0.1078). Median inpatient stay for the pegfilgrastim and lenograstim cohort was 16 days (Mann Whitney test; p value: 0.2112). Median days of broad spectrum intravenous antibiotic use in the pegfilgrastim and lenograstim cohort was 4.51 days and 3.90 days (Mann Whitney test; P value: 0.4150). Time for neutrophil engraftment, length of inpatient stay and antibiotics use was significantly more in the no G-CSF cohort. There was no difference in the progression free sur-

vival or overall survival between the three cohorts (Log Rank test; p value: 0.455).
Summary / Conclusion: Our data suggest that the use of daily lenograstim from day +7 is an effective strategy in patients with myeloma undergoing APBSCT. It is equally effective as pegfilgrastim in reducing the time to neutrophil engraftment, duration of inpatient stay and antibiotic use. Both daily lenograstim from day+7 and pegfilgrastim are superior to no G-CSF use.
Our data confirms daily lenograstim from day +7 is a cost effective alternative strategy to pegfilgrastim in patients undergoing APBSCT making lenograstim a valuable commodity in the time pressured and cost aware tertiary haematology centres.

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TWO DIFFERENT PLERIXAFOR STEM CELL MOBILIZATION STRATEGIES. A MULTICENTER EXPERIENCE.
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Background: Autologous stem-cell transplantation (ASCT) has became a widely applied treatment for hematologic malignancies. The vast majority of ASCT are performed with the support of peripheral blood stem cells (PBSC). A significant proportion of patients with non-Hodgkin's lymphoma (NHL), Hodgkin's disease (HD) or multiple myeloma (MM) are hard-to-mobilize with conventional mobilization regimens. Depending on disease 5-25% of patients considered for high-dose therapy failed to mobilize or have a suboptimal mobilization. Plerixafor is a novel drug used to improve mobilization of blood stem-cells.
Aims: We present our experience in patients who failed a previous mobilization attempt and compare then with patients who plerixafor was added if poor first mobilization was demonstrated.
Methods: We retrospectively collected data from 5 spanish centres on 54 patients affected by NHL, HD, MM or amyloidosis while receiving plerixafor in mobilization. From January 2008 to March 2012 44 patients received plerixafor associated G-CSF in a second attempt at mobilization when they have already failed a first mobilization. From march 2012 to February 2013 10 patients preemptively received plerixafor who seem mobilize poorly: (1) blood CD34+ cells/μL <10 on the programmed day to initiate aphaeresis after G-CSF or (2) <1x10^6/kg after the first aphaeresis process. All patients provided written informed consent.
Results: Plerixafor in second mobilizations: 44 patients received plerixafor in this setting. The median age was 61y (25-70). 26 had NHL, 13 MM, 3 HD and 2 amyloidosis. 43 received standard plerixafor dosage, 1 patient received adjusted dosage by renal insufficiency, and all of patients received G-CSF with a median dose of 10 μg/kg/d (10-20). Median number of plerixafor injections was 2 (1-4) and median CD34+ cells count after the first plerixafor injection was 14/μL (7-113). 37 (84%) of them initiated aphaeresis. A median of 2.23x10^6/kg (0-10.71) CD34+ cells were collected with a median of 1 (1-4) aphaeresis. >2x10^6/kg CD34+ cells were achieved in 28 (63%) patients (61.53% in NHL, 66.6% in HD and 84.61% in MM). Pre-emptive use of plerixafor was administered to 10 patients (5 had NHL, 4 MM and 1 HD) with a median age of 68y (40-69). The reason to use plerixafor pre-emptively where low blood CD34+ counts in 9 patients an poor yield collection in 1. All received standard plerixafor dosage before 4-day G-CSF therapy with a median dose of 10 μg/kg/d (10-20). Median number of plerixafor injections was 2 (1-4). Median CD34+ cells count the programmed day to initiate aphaeresis was 7/μL (0.84–11.39). Thus 8 (80%) of the 9 patients began aphaeresis due to delay the start of harvest to the first plerixafor administration when the peripheral CD34+ count has increased to a median of 13.42/μL (6.23-127.5). A median of 2.58x10^6/kg (0.6-4.81) CD34+ cells were collected with a median of 1 (1-4) aphaeresis. >2 x10^6/kg CD34+ cells were achieved in 7 (72%) patients.
Summary / Conclusion: Results from this study showed that plerixafor is a safe and active mobilising agent in the two strategy arms with 63% efficacy using it in remobilization and 72% as pre-emptive strategy. Although pre-emptive use may not be able to completely eliminate the need for a second attempt to mobilize, this strategy seems more efficient and should be considered in patients judged as poor mobilizers using it to optimize patient outcome and reduce of other hospital resources.

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LOW PRE-TREATMENT MIR-181A-1 AND MIR-181A-2 EXPRESSION ASSOCIATE WITH RELAPSE IN INTERMEDIATE RISK ACUTE MYELOID LEUKEMIA (AML) AFTER REDUCED-INTENSITY CONDITIONING (RIC) ALLOGENEIC TRANSPLANTATION
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Background: AML patients (pts) with higher pre-treatment *miR-181a* expression have better outcomes following standard chemotherapy (Marcucci *et al.* NEJM 2008; Schwind *et al.* JCO 2010; Li *et al.* Blood 2012). Clinical attempts to increase *miR-181a* expression in AML patients to improve outcomes have been initiated. Mature *miR-181a* originates from two precursor molecules (*miR-181a-1* & *miR-181a-2*) which may impact on AML biology & outcome differently. Allogeneic hematopoietic cell transplantation (HCT) represents a post-remission therapy offering potential cure for AML pts. RIC, with a therapeutic approach mainly based on immunological graft-versus-leukemia effects, is increasingly used in AML pts undergoing HCT.
Aims: In this analysis we concentrated on the clinical characteristics & prognostic impact associated with *miR-181a-1* & *miR-181a-2* pre-treatment expression in AML pts treated with RIC-HCT.
Methods: *miR-181a-1* & *miR-181a-2* expression were assessed by real-time PCR and normalized to an internal control (*ABL*) in pre-treatment bone marrow (BM). The respective median expression defined high & low *miR-181a-1* & *miR-181a-2* expressers in 39 consecutive AML pts (median age, 62 [range 27 – 67] years) with intermediate cytogenetic risk (according to the Medical Research Council [MRC] Classification), who received RIC-HCT at the University of Leipzig & who had adequate material available. The preparative regimen for all pts was Fludarabine 30 mg/m^2 from day -4 to -2 followed by 200cGy total body irradiation at day 0. Donors were human leucocyte antigen (HLA)-matched related (n=5), matched unrelated (n=29) and mismatched unrelated (≥1 antigen; n=5) to the recipients. Acute graft-versus-host disease (GvHD) grade 2-4 was observed in 12 pts & limited/extensive chronic GvHD in 24 pts. Median follow-up was 6.5 years for pts alive (n=21). Informed consent for the study was obtained from all pts.
Results: *miR-181a-1* & *miR-181a-2* expression correlated well (R^2=.83), but 31% (n=12) of pts had a discordant *miR-181a-1* & *miR-181a-2* expresser status. At diagnosis low *miR-181a-1* associated with higher white blood count (P=.03), while low *miR-181a-2* associated with normal cytogenetics (CN; P=.03) & with higher % BM blasts by trend (P=.08). In univariate analysis pts with both low *miR-181a-1* & low *miR-181a-2* had a higher probability of relapse (P=.008, Gray's test; Figure A). Low *miR-181a-1* & low *miR-181a-2* expression also associated with shorter overall survival (OS) by trend (P=.06, Log-Rank test; Figure B). When limiting our analysis to CN-AML pts (n=28) low *miR-181a-1* & *miR-181a-2* also associated with a higher probability of relapse (P=.007). In multivariable analysis low *miR-181a-1* & low *miR-181a-2* associated with a higher probability of relapse (P=.01) & shorter OS (P=.01) in all intermediated risk pts.
Summary / Conclusion: In conclusion, expression of *miR-181a-1* & *miR-181a-2* is not fully concordant & associates with different clinical characteristics at AML diagnosis, indicating a distinct impact on disease biology. However, low expression of both seems to impact outcome after RIC-HCT. Low pretreatment *miR-181a-1* & *miR-181a-2* expression associated with a higher probability of relapse & shorter OS in AML pts with intermediate cytogenetic risk. Increasing *miR-181a-1* and/or *miR-181a-2* levels either pharmacologically (eg Hickey *et al* Blood 2013) or by synthetic miR-replacement therapy may improve outcomes of AML pts receiving RIC-HCT.

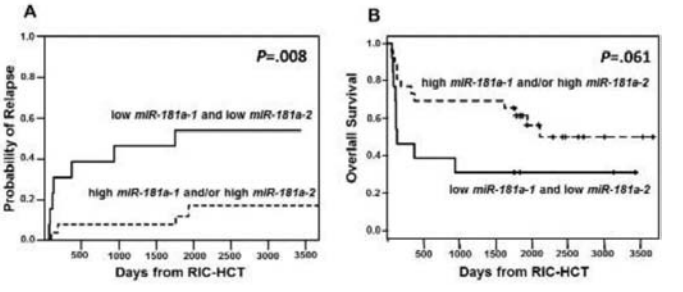

Figure 1.

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INTRABONE CORD BLOOD TRANSPLANT (IB-CBT) IN HAEMATOLOGICAL MALIGNANCIES: A CLINICAL AND BIOLOGICAL STUDY ON EARLY HEMATOPOIETIC RECONSTITUTION.

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Background: Intrabone route of CB-HSC administration is a safe and efficient way to overcome the main impediment to the extensive use of CB transplant in adults, i.e. the insufficient amount of HSC in CB units. The "Intrabone effect" is speculatively elicited by the hypoxic bone marrow niche microenvironment, which primes CB-HSCs prior to set off the repopulation of the whole hematopoietic compartment.

Aims: Here we report on a phase II prospective study approved by the local EC and registered at http://clinicaltrials.gov/, with the primary endpoint of engraftment rate. From the biological side, we investigated hypoxia-induced genes whose expression can be correlate with engraftment.

Methods: 23 patients (median age 36 yrs, mean weight 63 kg -52 to 93-) were enrolled: 17 were AML, 4 ALL, 1 MM and 1 CML. Phase at transplant was mainly advanced stage (10 primary refractory or resistant relapse AL, 6 beyond first and 5 first cariotypic response; 1 progression for MM and 1 CB for CML). 3 patients had previously undergone allotransplant and 4 autotransplant. CB units were matched 4/6, except for three cases (5/6). Infusion on both iliac crests was performed in operating room with monitored care sedation. Conditioning regimens were Busulfan- or TBI- based; GVHD prophylaxis was Ciclosporin-A, Micophenolate Mofetil 15 mg/kg/bid and ATG-F (15-30 mg/kg).To follow-up hematopoietic reconstitution, peripheral blood was examined daily and bone marrow aspirates were performed at day 10, 20 and 30 after transplant. The samples were used to immune-magnetically separate CD34+ cells under hypoxia condition and to assess the mRNA level of target genes by Real Time PCR.

Results: Hematologic recovery: the cumulative incidence of ANC recovery (>0.5x10^9/L) was 82±9% at day+60; the cumulative incidence of plt recovery (>20 or 50x10^9/L) was 82±9% and 70±10% at day+90, respectively. In multivariate analysis, the number of infused CD34 cells was a prognostic factor for haematologic recovery. Importantly, haematological recovery was highly correlated with post-transplant day 10 mRNA level of c-mpl (TPO receptor) even when the number of infused CD34 cells were included in the multivariate analysis. Two patients failed to engraft and were IB infused with a second CB unit. One year OS and NRM were 66±11% and 20±9%, respectively. aGVHD was 39% with only one patient at grade III. Three limited and one extensive cGVHD were recorded. All but two patients achieved complete response. CMV reactivation occurred in 13/23 pts.

Summary / Conclusion: from the clinical point of view, we confirm, in a prospective study, that the intrabone route of CB transplant is feasible and leads to a remarkably fast platelet recovery. On the biological side, we show that the c-mpl/thrombopoietin receptor system contributes to the "intrabone effect" on hematologic recovery. These data provide hints about the strategy to improve early seeding and expansion of CB in the hypoxic HSC niche.

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DONOR AND RECIPIENT STR ANALYSIS BEFORE ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION: POSSIBLE CORRELATION WITH POST-TRANSPLANT OUTCOME

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Background: The chimerism analysis after allogeneic haematopoietic stem cell transplantation (HSCT) is a useful tool to monitor the engraftment of donor cells and to early predict graft failure or disease relapse. Among several available methods to perform chimerism study, multiplex fluorescent short tandem repeat (STR) analysis represent a sensitive, accurate and reproducible technique. It provide a high rate of discrimination between donor and recipient cells since that fluorescent signal is proportional to the cells number, obtaining a quantitative assessment of chimerism.

Aims: Evaluation of a possible correlation between basal donor/recipient allelic discrepancies and post-transplant outcome in terms of GvHD development, neutrophil engraftment, relapse, DFS and OS.

Methods: We enrolled 111 patients (pts) affected by onco-haematological diseases, consecutively submitted to HSCT at our division between February 2005 and December 2012. Pts were 67 males and 44 females with a median age of 51 years (range 14-70). Underlying diseases were: 58 AML, 10 MDS, 16 ALL, 8 CLL, 6 MM, 2 HL, 9 NHL, 1 IMF, 1 MPD. Myeloablative conditioning regimen was used in 26 pts while a reduced conditioning regimen was used for the other 85 pts. Graft was obtained from sibling donor and unrelated donor in 65 and 46 pts, respectively. GvHD prophylaxis was performed with cyclosporine (CSA) and methotrexate in 61 pts, CSA and mycophenolate mofetil in 43 pts and CSA in 7 pts. Donor and recipient allelic status was performed using a multiplex PCR amplification of ten STR loci: Amelogenin alleles X/Y, D3S1358, FGA, D8S1179, D18S51, D13S317, vWA, D21S11, D5S818, D7S820. We evaluated the donor/recipient (D/R) match or mismatch for each locus and the following variables: GvHD development and onset time, time to neutrophil engraftment, relapse, DFS and OS. Statistical analysis was performed by Kaplan Meier analysis using IBM SPSS Statistics 20 Core System.

Results: Pts with D/R mismatch for D8S1179 locus achieved neutrophil engraftment (ANC>1x10^9/L) at a median time of 18 days (CI 95% 17.202-18.798) compared with 21 days (CI 95% 17.328-24.672) of pts with D/R match for the same locus (P=0.007) (Figure 1A).

Pts with D/R mismatch for D3S1358 locus developed acute GvHD at a median time of 20 days (CI 95% 15.781-24.219) compared with 29 days (CI 95% 15.421-42.579) of pts with D/R match for the same locus (P=0.031) (Figure 1B).

Pts with D/R mismatch for D3S1358 locus showed an OS of 16 months (CI 95% 11.016-20.984) compared with 41 months (CI 95% 25.420-56.580) of pts with D/R match for the same locus (P=0.025) (Figure 1C).

Pts with D/R mismatch for D8S1179 locus showed an OS of 16 months (CI 95% 8.528-23.472) compared with 56 months (CI 95% 11.254-78.057) of pts with D/R match for the same locus (P=0.012) (Figure 1D).

No relationships were found with relapse, DFS neither for the other loci.

Summary / Conclusion: Our results highlight that pts who presented a D/R mismatch for D8S1179 locus showed an earlier neutrophil engraftment but a worse OS compared with the others. Moreover, for D3S1358 locus, D/R mismatch was associated with an earlier onset of acute GvHD after HSCT and with a shorter OS than the D/R match ones. STRs are highly polymorphic di-, tri- and tetra-nucleotide repeat non-coding sequences, which are interspersed throughout the genome. Whether or not discrepancies between donor and recipient basal allelic status could be considered useful in predicting post-transplant outcome will require validation in a large sample of pts.

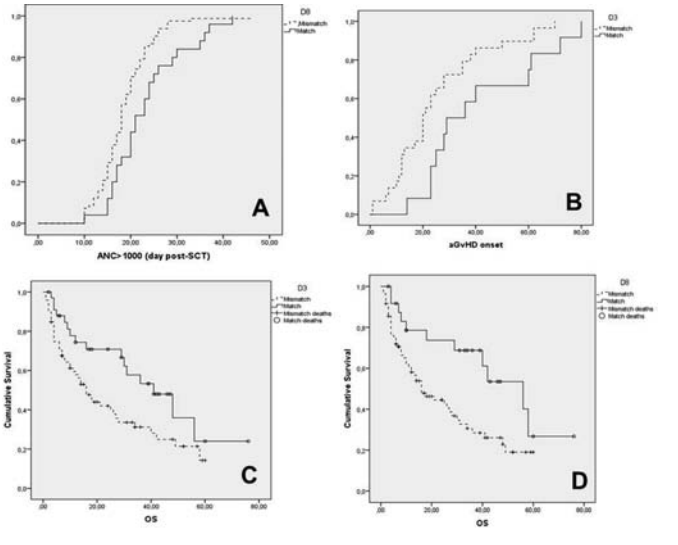

Figure 1.

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COMPARISON BETWEEN LONG-TERM AND SHORT-TERM ADMINISTRATION OF ITRACONAZOLE FOR PRIMARY ANTIFUNGAL PROPHYLAXIS IN ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION: A MULTICENTER, RANDOMIZED TRIAL

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Background: Although antifungal prophylaxis has been demonstrated effective for prevention of IFI after allogeneic haematopoietic stem cell transplantation (allo-HSCT), optimal agents and duration of prophylaxis remain a matter of discussion.
Aims: To investigate the effect of prophylaxis duration for IFI after transplantation, this multicenter, randomized, open-label study was conducted to compare the efficacy and safety between long-term and short-term administration of itraconazole for primary antifungal prophylaxis in recipients of allo-HSCT.
Methods: A total of 128 patients without a history of IFI were enrolled in this study. The primary antifungal prophylaxis was initiated when WBC count < 1.0×10^9/L or on day 0 (the day of allo-HSCT). Itraconazole was administered intravenously with a loading dose of 400 mg/d for 2 days followed by 200 mg/d and switched to oral solution of itraconazole on day +14 or when WBC count > 1.0×10^9/L until 30 days after transplantation in short-term arm or 90 days in long-term arm. The trough serum concentrations of itraconazole and its active metabolite hydroxyitraconazole were measured on days 3 and 8 after the beginning of administration, and then once every week during the period of prophylaxis. The primary endpoint was the incidence of IFI within 90 days after transplantation.
Results: Of the 128 patients enrolled in study, data of 121 cases were used to determine the primary endpoint in the intent-to-treat population (59 for long-term and 62 for short-term). The baseline demographic and transplanted characteristics were similar in the two arms. Within 90 days post-transplantation, 8 patients developed IFI including 1 probable and 7 possible cases. No significant difference in the incidence of IFI between long-term and short-term arm was shown by analysis of the intent-to-treat (6.78% vs. 6.45%, 95%CI of difference: 0.24% to 0.42%), indicating the noninferiority of long-term administration against short-term administration. The incidence of breakthrough IFI during the period of prophylaxis in patients with long-term and short-term administration were 6.78% and 0%, respectively. Between 30 days to 90 days after transplants, the incidence of IFI was not significant different in the two arms (0% in long-term arm vs. 6.45% in short-term arm,P=0.11 by analysis of the intent-to-treat; 0% in long-term arm vs. 6.67% in short-term arm, P= 0.12 by analysis of the per protocol set). After 2 days of treatment with intravenous itraconazole, the mean trough serum concentration of itraconazole was 520.72±483.26ng/mL and maintained greater than 500ng/ml throughout administration. Eleven patients were withdrawn from prophylaxis because of adverse events. The incidence of withdrawal in patients with long-term and short-term prophylaxis was 6.78% and 0%, respectively (P=0.054). The incidences of drug-related adverse events in the two arms were similar (18.6% in long-term arm vs. 12.9% in short-term arm, P=0.386).
Summary / Conclusion: Itraconazole with 90 days administration was as effective as that with 30 days administration in preventing fungal infection after transplantation. Prolonged-term prophylaxis might not increase side effect of itraconazole. The pharmacokinetics of itraconazole in the recipients of allo-HSCT is coincident with that in non-recipients of allo-HSCT.
This trial was registered at www.clinicaltrials.gov as NCT01160952.

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BENDAMUSTINE, ETOPOSIDE, CYTARABINE AND MELPHALAN (BEEAM) FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION INDUCE LONG-LASTING COMPLETE REMISSIONS IN A HIGH PROPORTION OF RESISTANT/RELAPSED LYMPHOMA P

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Background: We previously demonstrated (Visani *et al*, Blood 2011) the safety of a new conditioning regimen with bendamustine, etoposide, cytarabine, and melphalan (BeEAM) prior to autologous stem cell transplant (ASCT) in resistant/relapsed lymphoma patients (EUDRACTnumber2008-002736-15). Furthermore, this regimen showed significant anti-lymphoma activity (80% CR), specially in patients with chemosensitive disease and non-Hodgkin lymphoma (NHL). However, median follow-up for surviving patients was short (18 months) at the time of publication. Therefore, it was not possible to draw final conclusion on the efficacy of the BeEAM regimen.
Aims: We evaluated the efficacy of the BeEAM regimen in terms of disease-free (DFS) and overall survival (OS) after a median follow-up of 32 months.
Methods: Forty-three patients (median age 47 years, range 18-70) with resistant/relapsed NHL (28) or Hodgkin lymphoma (HL, 15) were consecutively enrolled in the study. Twenty-one patients had primary refractory disease, whereas 22 had relapsed disease, 5 of whom where in second or subsequent relapse, at the time of enrolment. At transplant, 14/43 patients (34%) were in CR, 22/43 (50%) were in partial response (PR) and 7/43 (16%) were either resistant or in progression. At the time of publication, after a median follow-up of 18 months, 35/43 patients (81%) were in CR, and only 2 patients with HL died. Disease type (NHL versus HL) and disease status at transplant (chemosensitive versus chemoresistant) were the only statistically significant variables influencing PFS (P=0.01;P=0.007). Disease status at transplant (chemosensitive versus chemoresistant) had a significant impact also on OS (P=0.004).
Results: we updated the follow-up at 32 months after transplant. Thirty-one out of 43 patients are still in CR (72%), as documented by both PET and CT scan. Two patients with HL were refractory and rapidly died, whereas 10/43 patients (23%) relapsed after a median time of 7.5 months (range:3-35) from transplant. Five patients died (3 NHL, 2 HL), whereas 5 patients are still alive after relapse. Median PFS and OS were still not reached. Interestingly, disease type at transplant is no longer influencing PFS (P=0.4), and still does not influence OS (P=0.3). On the other hand, disease status at transplant (chemosensitive vs chemoresistant) is still a strong predictor of both PFS and OS (P=0.03 and P=0.04, respectively). At present, one patient developed myelodisplasia after transplant. No other late effects were observed up to now.
Summary / Conclusion: The new BeEAM regimen confirms its safety, even after 32 months of follow-up from transplant. Furthermore, these data confirm the high efficacy of this regimen in heavily pretreated lymphoma patients. Interestingly, the statistical difference between NHL and HL patients in terms of both PFS and OS was not confirmed in the long run.
Acknowledgements: supported in part by AIL Pesaro Onlus.

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A CONDITIONING PLATFORM WITH FLUDARABINE, BUSULFAN AND ATG CONDUCTS TO IDENTICAL PROMISING RESULTS IN PATIENTS UNDERGOING ALLOGENEIC TRANSPLANTATION FROM BOTH MATCHED AND MISMATCHED UNRELATED DONOR

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Background: Reduced-toxicity conditioning (RTC) regimen containing fludarabine (FLU), intravenous busulfan (Bx) and 5 mg/kg total dose of rabbit antithymocyte globulin (ATG) (FBx-ATG) showed low incidences of graft-versus-host disease (GVHD) and non-relapse mortality (NRM) after allogeneic stem cell transplantation (Allo-SCT) from HLA-matched related or unrelated donors (MUD). It is not known if this FBx-ATG RTC could provide such a safety used

in the setting of one-locus-HLA mismatched unrelated donor (MMUD).
Aims: The objective was to evaluate the mortality and toxicity of the FBx-ATG RTC in the setting of Allo-SCT from MMUD in a comparison with patients transplanted from MUD.
Methods: We retrospectively selected patients younger than 65 years, transplanted in our institution from 2008 and 2012 for hematological malignancies, from MUD or MMUD peripheral blood stem cells and prepared with FBx-ATG RTC (FLU 30 mg/m²/d over 5 days, Bx 3.2 mg/kg/d over2, 3 or 4 days, ATG 2.5 mg/kg/d over 2 days). GVHD prophylaxis consisted of Cyclosporine A (CsA) alone or in association with mycophenolate mofetil (MMF). We then compared outcome of MUD (n=88) vs. MMUD (n=45) patients. Primary end point was the incidence of NRM. GVHD incidences and survivals were analyzed as secondary end points.
Results: Median age was 54 years [range: 19-64] and patients characteristics were without difference between MUD and MMUD patients. Sixty patients (45%) were transplanted for AML or MDS. Remaining patients were transplanted for lymphoma and myeloma. According to institutional guidelines and treatment protocols, MMUD patients received more frequently CsA + MMF as GVHD prophylaxis (89%) than MUD patients (16%) (P<0.001). Repartition of HCT-CI score adapted from Sorror *et al*. and disease risk index adapted from Armand *et al*. were similar in the 2 donor groups. Median follow up was 25 months [range: 5-73].
Early NRM at day 100 was low in MUD (6%) patients as well as in MMUD (7%) patients. One-year NRM was similar in the 2 donor groups (MUD: 17% vs. MMUD: 18%, P=0.533). There was no difference in the cumulative incidence of grade II-IV acute GVHD (MUD: 31% vs. MMUD: 36%, P=0.745) but MMUD patients developed more grade III-IV acute GVHD (MUD: 6% vs. MMUD: 16%, P=0.020). There was no difference in the cumulative incidences of overall chronic GVHD (MUD: 35% vs. MMUD: 29%, P=0.644) and extensive chronic GVHD (MUD: 21% vs. MMUD: 27%, P=0.540). Progression-free survival (MUD: 59% vs. MMUD: 55%, P=0.334) and overall survival (MUD: 61% vs. MMUD: 61%, P=0.748) were not different between MUD and MMUD patients. With a median follow up of 25 months, 42 of the 88 MUD patients (47%) and 20 of the MMUD patients 45 (44%) were free of both disease progression and immunosuppressive treatment (P=0.597).
Summary / Conclusion: We confirm that FBx-ATG RTC regimens provides low incidence of NRM in patients younger than 65 years transplanted from MUD, and equally from MMUD. This kind of RTC could safely extend the use of Allo-SCT to patients for whom only an MMUD is identified.

Table 1.

	All patients (n=133)	MUD (n=88)	MMUD (n=45)	p
	% (95CI)	% (95CI)	% (95CI)	
Day-100 Acute GVHD				
Grade II-IV	32 (24-40)	31 (21-40)	36 (21-50)	0.745
Grade III-IV	9 (4-14)	6 (1-11)	16 (5-26)	0.020
2-year Chronic GVHD				
Overall	33 (25-41)	35 (25-45)	29 (15-44)	0.644
Extensive	22 (15-29)	21 (12-29)	27 (13-41)	0.540
NRM				
Day-100	6 (2-10)	6 (1-11)	7 (0-14)	0.533
1-year	18 (11-24)	17 (9-25)	18 (7-30)	
Progression-free Survival				
2-year	57 (49-67)	59 (49-71)	55 (42-72)	0.334
Overall Survival				
2-year	61 (52-70)	61 (51-73)	61 (47-78)	0.748

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MOLECULAR MONITORING AND STEPWISE PREEMPTIVE THERAPY FOR EPSTEIN-BARR VIRUS VIREMIA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Epstein-Barr virus (EBV)-associated diseases progress rapidly and are severe complications after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Despite various therapeutic options being developed, the optimal preemptive therapy forEBV-associated diseases remains under discussion. We developed a stepwise preemptive therapy (antiviral agents andreduction of immunosuppressants [RI] followed by rituximab) for EBV viremia based on duration of EBV-DNA positive and changes of viral loads.
Aims: To explore the efficacy of stepwise preemptive therapyand the optimal preemptive therapy forEBV-associated diseases after allo-HSCT.
Methods: The blood EBV-DNA loads were regularly monitored by quantitativereal-time polymerase chain reaction in251 recipients undergoing allo-HSCT. Stepwise preemptive therapy was administeredin patients experiencingblood EBV-DNA positive (more than 500 copies/ml) twice consecutively. Administration of antiviral agents and RI were first taken. If EBV-DNA was continuously positive four times with a rising trend, rituximab was administered weekly until EBV-DNA was negative or for a total of 4 times. The patients who were diagnosed as EBV-associated diseases orappeared the signs and symptomsof EBV-associated diseases within three days after preemptive therapy were withdrawn from the study of preemptive therapy.
Results: Seventy-two patientsdeveloped EBV viremia and 35 developed EBV-associated diseases, including 22 post-transplant lymphoproliferative diseases (PTLD)and 13 EBV-associated other diseases (8 EBVfever, 1 pneumonia, 1 encephalitis, 1 myelitis, 1 encephalitis with lung involvement as well as 1 encephalitis with lung and liverinvolvement). Univariate analysis revealed that antithymocyte globulin, HLA-mismatched, unrelated donor and intensified conditioning constituted the risk factors of EBV viremia and EBV-associated diseases, as none of the grafts were T-cell-depleted.The 3-year cumulative incidence of EBV viremia and EBV-associated diseases were 5.0%±2.8%, 13.3%±4.2%, 50.0%±10.2%, 63.1%±8.3%, 63.1%±10.0% (EBV viremia) and 3.5%±2.4%, 2.9%±2.0%, 28.3%±9.3%, 25.9%±6.2%, 46.4%±10.8% (EBV-associated diseases) for patients with 0, 1,2, 3 and 4 major risk factors, respectively (P<0.001, P<0.001). The blood EBV-DNA loads exceeded the thresholdfor 0 to 17 (median 7) days before the clinical manifestations of EBV-associated diseases emerged.Of the 64 patientsreceiving the first-step preemption,24(37.5%) cases achieved complete response (CR)and 40 showed no response, including 25 progressing to EBV-associated diseases duringthe first-step preemption. Fourteen (93.3%) achieved CR and one progressed toPTLD in the15 patients undergoing rituximab preemption. Of the 26 patients progressing to EBV-associated diseases during preemptive therapy,20 obtained CRin the 23 cases with rituximab-basedtreatments. Patients with ineffective preemption had more major risk factors than those with effective preemptive therapy (3.15 ±0.73 versus 2.53±1.06, P=0.007). B-cell reconstitution was significantly delayed for at least 6 months in patients with rituximab preemption. The risk of herpesvirus infection was similar in patients with effective first-step and rituximab preemption (P=0.094).
Summary / Conclusion: The strategy of stepwise preemptive therapy to prevent EBV-associated diseases is worthy further exploring. It is shown that the efficacy of preemptive therapy is associated with the numbers of major risk factors for EBV infections. To avoid over-treatment, RIplus antiviral agents could-begiven priorityto low-riskpatients; whereas considering the rapid and aggressive evolution of EBV viremia toward EBV-associated diseases, more frequent monitoring of blood EBV-DNA and earlier preemptive rituximab should be advocated inpatientsat high risk of EBV-associated diseases.

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BONE MARROW VERSUS PERIPHERAL BLOOD STEM CELL GRAFTS FOR UNRELATED DONOR TRANSPLANTATION WITH MYELOABLATIVE CONDITIONING IN ADULTS WITH HIGH-RISK ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: In the setting of matched sibling donor transplantation, several randomized trials showed that peripheral blood stem cells (PBSC) resulted in faster engraftment but increased the risk of acute and chronic graft-versus-host disease (GVHD), as compared with bone marrow (BM). In addition, some studies showed a decreased rate of relapse and better survival with PBSC, especially in patients with more advanced hematologic malignancies. However, these results may not be applicable to unrelated donor (URD) transplants, given the greater risk of GVHD due to the greater genetic diversity. In the setting of URD transplantation, few data are available comparing PBSC and BM, especially in patients with adult high-risk acute lymphoblastic leukemia (ALL).
Aims: To determine which graft source is superior in patients with adult high-risk ALL, we compared the long-term outcomes of URD transplantation using PBSC and BM.
Methods: To maximize the homogeneity of the patient population, only patients who received a uniform strategy of pre-transplant chemotherapy, myeloablative conditioning (total body irradiation [≥12 Gy]-based), and an identical GVHD prophylaxis (tacrolimus plus methotrexate) were included in this analysis. Between 2000 and 2009, 106 patients with high-risk ALL underwent myeloablative conditioning URD transplantation with PBSC (n=38) or BM (n=68) grafts. The main presenting clinical and biological features between the two groups

were comparable. The median follow-up of survivors was 60 months (range, 38 to 81 months) for PBSC transplants and 102 months (range, 49 to 147 months) for BM transplants.
Results: PBSC transplants showed faster time to recovery of neutrophil (12 days vs. 14 days; P<0.001) and platelet (12 days vs. 21 days; P<0.001) counts than BM transplants. No difference was observed in the cumulative incidence of acute GVHD between the two groups (76.3% for PBSC transplants vs. 63.2% for BM transplants), but PBSC transplants showed higher incidence of chronic GVHD than BM transplants (68.4% vs. 41.2%, P=0.009). At 5 years, PBSC transplants showed comparable outcomes to MAC transplants in terms of relapse risk (26.8% vs. 35.1%), nonrelapse mortality (19.8% vs. 26.6%), disease-free survival (57.9% vs. 48.5%), and overall survival (57.9% vs. 50.0%). In multivariate analysis, graft source had no impact on transplantation outcomes. Regardless of graft source, transplants without chronic GVHD had higher relapse risk (53.2% vs. 14.4%; HR, 4.88; 95% CI, 2.04-11.66; P<0.001) and poorer disease-free survival (41.5% vs. 70.4%; HR, 2.62; 95% CI, 1.38-4.98; P=0.003) and overall survival (43.9% vs. 70.4%; HR, 2.20; 95% CI, 1.14-4.23; P=0.018). In addition, transplants in CR1 had lower relapse risk (62.8% vs. 22.8%; HR, 3.51; 95% CI, 1.62-7.58; P=0.001) and better disease-free survival (60.2% vs. 21.7%; HR, 3.02; 95% CI, 1.57-5.80; P=0.001) and overall survival (61.4% vs. 21.7%; HR, 2.92; 95% CI, 1.49-5.73; P=0.002).
Summary / Conclusion: Our data suggest that long-term outcomes are similar between PBSC and BM in the setting of URD transplantation in adults with high-risk ALL.

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LONG-TERM OUTCOMES OF REDUCED-INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION FOR ADULT HIGH-RISK ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The role of reduced-intensity conditioning (RIC) allogeneic stem cell transplantation (SCT) in adult acute lymphoblastic leukemia (ALL) remains unclear because of the small sample size, short follow-up duration, various regimens for conditioning and graft-versus-host disease (GVHD) prophylaxis, and the heterogeneity of the criteria used to select patients for transplantation. Previously, we conducted a phase 2 trial of RIC-SCT in adults with high-risk ALL in complete remission (CR). Our interim analysis data showed the potential role of RIC-SCT (Leukemia. 2009;23:1763-1770).
Aims: We report on the updated results of RIC-SCT by analyzing 60 consecutive adult high-risk ALL transplants in CR1 (n=52; 86.7%) or CR2 (n=8; 13.3%) with a sufficient median follow-up duration of 67 months (range, 32 to 148 months).
Methods: The indications for RIC-SCT were advanced age (≥50 years; n=26; 43.3%) and the presence of organ dysfunction or active infections (n=34; 56.7%). All patients were given a uniform strategy of pre-transplant chemotherapy and an identical RIC regimen consisting of fludarabine (150 mg/m^2) and melphalan (140 mg/m^2). GVHD prophylaxis was attempted by administering calcineurin inhibitors (cyclosporine for all sibling donor transplants and tacrolimus for all unrelated donor transplants) plus methotrexate (10 mg/m^2 on days1,3, 6, and 11). If residual leukemia was detected in the absence of GVHD at 3 months after transplantation, calcineurin inhibitors were rapidly discontinued.
Results: Neutrophil recovery occurred in all patients at a median of 12 days (range, 9 to 20 days), and platelet recovery occurred at a median of 13 days (range, 7 to 23 days) after transplantation. Twenty-nine patients developed grade II to IV acute GVHD (22 grade II, 3 grade III, 4 grade IV). The cumulative incidence of acute GVHD at 5 years was 48.3±6.3%. Of the 56 patients who survived for at least 100 days with sustained engraftment after transplantation, 34 developed chronic GVHD (15 limited, 19 extensive), resulting in a 5-year cumulative incidence of 56.7±6.4%. After a median follow-up of 67 months, the cumulative incidence of relapse and nonrelapse mortality at 5 years were 34.2±6.7% and 21.2±5.8%, respectively, and the 5-year disease-free survival and overall survival rates were 50.8±6.6% and 54.5±6.5%, respectively. In multivariate analysis, transplants without chronic GVHD had a higher relapse risk (68.5% vs. 14.4%; HR, 9.04; 95% CI, 2.93-27.83; P<0.001) and poorer disease-free survival (27.3% vs. 71.5%; HR, 7.62; 95% CI, 3.08-18.85; P<0.001). In addition, transplants in CR1 showed better disease-free survival (54.7% vs. 25.0%; HR, 3.96; 95% CI, 1.27-12.40; P=0.018) and tended to show a lower relapse risk (29.0% vs. 66.7%; HR, 2.79; 95% CI, 0.97-8.03; P=0.057).
Summary / Conclusion: RIC can be considered as a reasonable choice for providing a sufficient long-term graft-versus-leukemia effect for adult high-risk ALL patients ineligible for myeloablative conditioning.

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REDUCED TOXICITY CONDITIONING AND ALLOGENEIC STEM CELL TRANSPLANTATION IN ADULTS USING FLUDARABINE, BCNU, MELPHALAN, AND ANTITHYMOCYTE GLOBULIN (FBM-A): OUTCOMES DEPEND ON DISEASE RISK INDEX

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Background: Allogeneic hematopoietic cell transplantation (HCT) is the only curative option for many individuals with high-risk or refractory hematologic malignancies. The therapeutic benefit of allogeneic HCT ultimately derives from a graft-versus-malignancy effect that is based on immunologic disparity between recipient and donor. Until recently, allogeneic HCT was considered suitable only for younger and generally healthier patients due to increased regimen related toxicity seen with ablative doses of chemotherapy and/or radiation. Over the past decade reduced intensity conditioning (RIC) regimens have become standard of care for older and comorbid patients with high-risk hematologic malignancies who require HCT. However the optimum regimen has yet to be defined, and may differ for patients with varying pre-transplant risk factors. In 2008, Marks *et al*. reported results in 133 patients transplanted between 1998 and 2003 who were conditioned with a reduced toxicity regimen of fludarabine, BCNU (Carmustine), and Melphalan (FBM). Given that patients in that study were ineligible for standard ablative conditioning, older (median age 55.6), and often had active disease at transplant, the long-term results were very favorable (5-year EFS of 42%). We incorporated this regimen for treatment of hematologic malignancies, added rabbit antithymocyte globulin (Genzyme), and report results here.
Aims: 1. Confirm the safety, tolerability, and efficacy of the FBM regimen in patients with hematologic malignancies. 2. Assess the impact of patient characteristics, comorbidities, and CIBMTR disease risk assessment on HCT outcomes. 3. Determine the impact or rabbit antithymocyte globulin (Genzyme) on the incidence of acute and chronic GVHD.
Methods: We report results in 100 consecutive adults age 18-69 who received RIC with fludarabine, BCNU, melphalan, and rabbit antithymocyte globulin (FBM-A). Patients had high risk myeloid (AML, n=41; MDS, n=23, MPD, n=14, systemic mastocytosis, n=1) or lymphoid (ALL, n=8; NHL, n=8, Hodgkin lymphoma, n=4, CLL, n=1) malignancy. Median age was 55 (range 19 – 69), and most patients were ineligible for standard myeloablative conditioning due to age, organ dysfunction, or prior autologous HCT (45 had an HCT-CI score of >/=3). Donors were unrelated in 74, of which 34 were mismatched at one or 2 HLA loci; grafts were PBSC in 98 patients, and bone marrow and cord blood in 1 patient each. Outcomes were stratified using disease risk index (DRI) (Armand et. al. 2012).
Results: Overall survival (OS), event-free survival (EFS), non-relapse mortality (NRM), and relapse at 2 years were 68%, 66%, 20%, and 18%, respectively. Using the DRI, 68 patients were classified as low (n=1) or intermediate risk (n=67; DRI Low/Int), and 32 as high (n=28) or very high risk (n=4; DRI High/VH). With a median follow-up of surviving patients of 18 months, the Kaplan-Meier estimate of OS at 2 years for patients in the Low/Int risk group is 79%, compared to 61% in the High/VH group (P=.098). Two-year cumulative incidence estimates of relapse and NRM in the Low/Int group are 9.9% and 17%, respectively, vs. 25% and 21% in the High/VH group (P=0.057 and 0.69, respectively). The incidence of acute GVHD (grades III-IV) at 6 months was 9.8%, and the incidence of NIH-defined moderate-to-severe chronic GVHD was 22% at 2 years. Outcomes were not influenced by age, HCT-CI score, donor type, donor gender, or presence of mismatch.
Summary / Conclusion: The FBM-A regimen is safe, well-tolerated, and effective in patients up to age 69 with a wide array of hematologic malignancies. FBM-A may be most suitable for patients with low/intermediate risk disease by the DRI who have otherwise incurable hematologic malignancies. Despite advanced age and often high comorbidity scores, the 2-year OS was 79% for the low/int risk group and incidence of severe chronic GVHD was small. Curative strategies for very advanced or higher-risk hematologic malignancies will require additional pre- or post-transplant approaches to reduce disease burden or eradicate minimal residual disease. The FBM-A regimen offers a safe and effective platform to investigate such approaches.

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PREVALENCE OF METABOLIC SYNDROME AND METABOLIC PROFILE IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: In a previous paper (Airaghi L, J Endocrinol Invest 2011,34:6), we retrospectively observed an increased prevalence of metabolic syndrome (MS) in long term survivors after hematopoietic stem cell transplantation

(HSCT). In comparison to patients (pts) with spontaneous MS, we observed a difference in the clinical features, with hyperleptinemia rather than hyperinsulinemia being the key finding in HSCT recipients with MS

Aims: In pts referred for HSCT, we monitored prospectively the appearance of MS features and the evolution of laboratory parameters possibly involved in its pathogenesis. Here we present data from baseline pre-HSCT evaluation compared with those of 29 spontaneous MS, 29 post-HSCT MS pts and 29 healthy subjects derived from the above-mentioned retrospective study.

Methods: From May 2011 to February 2013 sixty-eight consecutive pts undergoing HSCT (M/F 35/33, median age 47.5, range 20-67 yrs; autologous 34, allogeneic 34) were enrolled in the study. For each patient, clinical history, physical examination, routine laboratory tests, endocrine profile, adipokines and TNF values were recorded. The comparison according to above criteria was performed univariately.

Results: A diagnosis of MS was established in 15 pre-HSCT cases; 10 had hypertension, 13 obesity, 4 hyperglycemia, 8 low HDL-cholesterol and 9 high triglycerides; 7 were candidate to autologous and 8 to allogeneic HSCT. Irrespective of having MS, 9 pts had double dyslipidemia. Pts pre-HSCT had significantly higher leptin (median 11.9 vs 7 ng/mL, P>0.01) and lower adiponectin (12.4 vs 18 mcg/mL, P<0.05) levels, in comparison with healthy subjects. Leptin levels in pre-HSCT pts were comparable with those of spontaneous MS (11.9 vs 15.3 ng/mL, P=n.s.) and significantly lower than those of HSCT survivors (11.9 vs 22 ng/mL, P<0.01). In pts pre-HSCT, adiponectin levels were similar to those of post-HSCT MS (12.4 vs 16 mcg/mL, P=n.s.), significantly lower than those of healthy subjects (12.4 vs 18 mcg/mL, P<0.05) and significantly higher than those of spontaneous MS (12.4 vs 9.4 mcg/mL, P<0.05). No difference among the separate groups was recorded in resistin levels. Insulin blood levels were comparable (11.5 vs 26 vs 26.5 mcU/mL, P=n.s.) in pre-HSCT pts, post-HSCT MS and spontaneous MS, whereas healthy subjects had significantly lower values in comparison with the three groups (10.1 mcU/mL, P<0.01). TNF levels were similar in pre-HSCT and in spontaneous MS (1.3 vs 2.8 pg/mL, P=n.s.), but significantly higher in post-HSCT MS (1.3 vs 11 pg/mL, P<0.05); fibrinogen was also significantly higher in post-HSCT MS than in pre-HSCT pts (402 vs 305 mg/dL, P<0.01).

Summary / Conclusion: Our data show a rather high prevalence of MS in pts referred to undergo HSCT, with a high prevalence of double dyslipidemia. Pts undergoing HSCT seem to comprise a self-standing population, characterized by high leptin, high insulin and low adiponectin levels, analogous to those of spontaneous MS. As spontaneous MS pts, they further differ from post-HSCT MS group, since the latter shows even higher leptin levels, with an increase in fibrinogen and TNF indicating a chronic inflammatory status. Our results point out to leptin rather than insulin resistance as a key pathogenetic clue in the development of post-HSCT MS, unlike to spontaneous MS. Therefore, the development of MS in HSCT recipients may be viewed as a multi-step process where conventional chemotherapy and conditioning regimens exert an additive effect in inducing a progressive increase in leptinemia, finally leading to full blown-MS.

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PREDICTION OF MOBILIZATION FAILURE FOR PRE-EMPTIVE PLERIXAFOR ADMINISTRATION: THE PAMPLONA PROTOCOL

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Background: The role of Plerixafor in routine peripheral blood progenitor cells (PBPC) mobilization in Myeloma or Lymphoma patients remains to be established.

Aims: In our institution we have developed a mobilization guideline (The Pamplona Protocol) to assess the safety and efficacy of Plerixafor + G-CSF in patients predicted to be poor mobilizers (PM).

Methods: The Pamplona protocol classifies patients according to PM risk factors including: a) previous mobilization failure; b) advanced disease; c) prior extensive radiotherapy; d) prior Hyper-CVAD, Fludarabine, Lenalidomide or alkylating agents; e) extensive BM involvement; f) >2 lines of chemotherapy; and g) age > 65. Patients with 3 or more risk factors are classified as PM and a PB CD34+ cell count is taken on day 4 of mobilization. All other patients have a CD34+ cell count on day 5 immediately before the apheresis procedure.
Plerixafor is administered at 6pm subcutaneously (0.24 mg/Kg) to: a) patients with a circulating CD34+ cell count < 10/uL (on either day 4 or 5) and b) patients with a first apheresis yield <1.0x10^6 CD34+/kg. G-CSF continues at the same dose (5-10 ug/Kg) and an apheresis is scheduled for the following morning.

Results: Since October 2009, a total of 146 patients were managed according to the Pamplona Protocol. 11 out of the 13 patients (85%) who were classified as PM before mobilization (9%) had a circulating CD34+ cell count <10/uL on day 4 and received Plerixafor as planned, demonstrating the high positive predictive value of the protocol. In addition, 9 patients (6%) failed to mobilize on day 5 despite having a median of 0.77 risk factors and also received Plerixafor. The diagnosis for the 20 patients that received Plerixafor was MM (n=7), NHL (n=11), HL and Amyloidosis (n=1 each). We observed a median of 3 fold increase in the number of CD34 circulating cells. The median CD34+ cell dose harvested was 2.33x10^6/Kg (range 0.32-5.69). There were no differences between the CD34+ cell dose in patients receiving Plerixafor on day 4 (median 2.3x10^6/Kg) and day 5 (median 2.5x10^6/Kg). The median number of apheresis was 1 (1-3). Only one patient failed to mobilize sufficient CD34+ cells. G-CSF and Plerixafor treatment was well tolerated. Adverse events were grade 1 in severity and were mostly gastrointestinal.

Summary / Conclusion: The Pamplona Protocol was able to predict PM before G-CSF administration allowing for early Plerixafor administration. Despite a 13.7% of PM in our series, 145/146 (99%) patients were able to harvest sufficient CD34+ cells for autologous SCT.

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DOES ALLOGENEIC STEM CELL TRANSPLANTATION OVERCOME THE ADVERSE RISK OF FLT3 POSITIVIVITY IN CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKAEMIA?

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Background: Cytogenetic abnormalities in acute myeloid leukaemia (AML) guide risk stratification regarding the use of haemopoietic stem cell transplantation (SCT). However, many AML patients have no karyotypic abnormality detectable: Cytogenetically normal AML (CN-AML). The presence of internal tandem duplication in the FMS-like tyrosine kinase 3 gene(FLT3), which is present in 23-25% of acute myeloid leukaemias with normal karyotype, has been associated with a poor outcome in CN-AML. Growing evidence suggests that SCT may improve outcome versus chemotherapy alone. We performed a retrospective analysis in our institution of all patients who underwent SCT for CN-AML between 2003 and 2011 in whom FLT3 status was available.

Aims: To examine the outcome post SCT of patients with CN-AML in whom FLT3 status was known in our institution.

Methods: 76 patients with CN-AML were identified from our transplant database. We excluded those in whom FLT3 status was not available. Baseline characteristics, disease status, stem cell source and survival were noted.

Results: FLT3 status was available in 52/76 patients. Of these, 35/52 (67%) tested negative for FLT3 and 17/52 (33%) tested positive. Median follow up was 28 months (1 month-96 months) in the overall group. Overall survival (OS) was 57% in the FLT3 negative group and 71% in the FLT3 positive group. 88% of the FLT3 positive group were transplanted in first complete remission (CR1) compared to 60% of those in the FLT3 negative group.

Summary / Conclusion: Patients with CN-AML who were FLT3 positive had a superior survival to both the FLT3 negative group and our institution's overall survival for intermediate risk acute myeloid leukaemia (59%) notwithstanding some differences in groups, most notably the proportion of patients in CR1 prior to SCT. This survival benefit, whilst not statistically significant given small numbers, is consistent with other reports supporting the suggestion that patients with CN-AML who are FLT3 positive can benefit from SCT in CR1.

Table 1.

	FLT 3 negative, n=35	FLT 3 positive, n=17
Age (years), median (range)	44 (22-64)	50 (20-64)
Male: female	20:15	7:10
SCT in CR1	21 (60%)	15 (88%)
SCT in CR2	13 (37%)	1 (6%)
SCT other	1 (3%)	1 (6%)
Stem cell source: BM:PBSC	16:19	10:7
Sibling donor: unrelated donor	20:15	10:7
Myeloablative conditioning	19	7
Reduced intensity conditioning	16	10
Follow up, median (range) *censored at death	29.8 months (1-97)	20.3 months (2-96)
Relapse rate	10 (29%)	6 (35%)
Death (all causes)	15 (43%)	5 (29%)
Overall survival (%)	57	71

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NK ALLOREACTIVE DONOR IS ASSOCIATED WITH REDUCED RELAPSE IN REFRACTORY AML BUT HIGH NONRELPASE MORTALITY IN PATIENTS WITH APLASTIC ANEMIA FOLLOWING T-REPLETE HAPLOIDENTICAL PBSCT WITH POST TRANSPLANT

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Background: NK cell alloreactivity has been shown to positively influence the outcome of high risk acute leukemia after T deplete Haploidentical PBSCT. However its impact on non-malignant disorders has not been studied particu-

larly in T replete Haploidentical PBSCT.
Aims: We conducted a pilot study on Haploidentical HCT with posttransplant cyclophosphamide (PTCY) and PBSC graft. The donors selected were either mother or NIMA mismatched siblings irrespective of NK cell alloreactivity. Ten antigen HLA typing, KIR genotyping and NK ligand typing were carried out on all patients and donors from stored samples. NK alloreactivity was determined based on missing self theory.
Methods: The conditioning protocol was developed based on FHCRC regimen of Fludarabine and low-dose Cyclophosphamide pre-transplant with escalating dose Melphalan 70-140 mg/m^2 replacing 2 Gy TBI. PTCY was administered 72hrs after infusion of the graft at 50 mg/kg twice at 24 hrs interval followed by Cyclosporine and MMF. The ones with AML received high dose AraC and mitoxantrone from day-14 to -12, along with Alemtuzumab 0.2 mg/kg/day. Alemtuzumab at above doses was administered from D -8 to D-4 in patients with SAA. This was replaced with Antithymocyte Globulin for the last 2 patients
Results: 12 patients (median age-16, 5-43) underwent Haplo-HCT; 7 patients with refractory AML (Cohort A) had a median BM blast count of 50% (20-80%) having failed at least two lines of treatment. Five patients had severe aplastic anemia (Cohort B). There was no difference between the two groups in age, gender, number of HLA disparities and donor gender. Patients in Cohort B were more heavily transfused (median 40 vs 20 units, P=0.005). The conditioning was tolerated without any major non-hematological toxicity in both the cohorts. The median CD34 was 4.40 x 106/kg (range 3.05-11.06) and CD3 was 517x10^6/kg (range 80-735) in CohortA whereas CD34 was 6.4x10^6(range5.0-10.0) and CD3 was 165x10^6/kg (range 40- 500) in cohort B (P=ns). All patients engrafted with neutrophils > 500/µL on day +13 (range12-17) and platelet count > 20,000/µL on day +12 and +18 in cohorts B and A (range 8-22) with > 95% donor chimerism on day +30 with morphological CR in cohort A and 100% donor chimerism in cohort B. Only one patient of Cohort A developed de-novo GVHD grade 2. Three relapsed between days 100-150 and two of them achieved a CR following a second transplant from the same donor. One patient in CR died of multi-drug resistant gram-negative bacterial sepsis. Three patients remained in CR. 4/5 patients of Cohort B engrafted promptly, but developed unexpected alloreactivity. Two developed early HLH, one with GVHD and both succumbed to the complications. Other two developed acute lung injury immediately post transplant and periengraftment. The fifth patient is well and alive at 180 days postransplant. Unlike others he had received multiagent IST as part of the conditioning. 8/12 patients amongst the entire group had NK alloreactive donor. In Cohort A, 3/7 patients received graft from a NK alloreactive donor and none relapsed. All 4 patients transplanted from donors without NK alloreactivity relapsed. 4/5 patients of Cohort B with SAA transplanted from NK alloreactive donors developed severe alloreactivity which were fatal (P=0.03). KIR haplotype, presence or absence of KIR2DS1or any other activating receptors did not impact the outcome
Summary / Conclusion: In patients with AML, unmanipulated PBSC graft from a NK alloreactive donor with PTCY was associated with strong GVL effect without GVHD. However, NK alloreactivity in heavily transfused SAA patients was associated with severe alloreactivity and early mortality despite prompt engraftment. Our study highlights for the first time that NK alloreactivity might be associated with an adverse outcome in non-malignant diseases in contrast to the beneficial effect demonstrated in malignant diseases

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RITUXIMAB-BASED TREATMENTS FOLLOWED BY ADOPTIVE CELLULAR THERAPIES FOR POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE IN RECIPIENTS OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: The introduction of rituximab has improved the complete remission(CR) rate of EBV-associated post-transplant lymphoproliferative disease(PTLD), thus the recurrence of PTLD becomes the main cause affecting long-term survival, which is closely related with the reestablishment of immune functions. Unfortunately, PTLD happens generally at the early stage of transplants and the reestablishment of fullimmune needs for 3-5 years in the recipients of allo-HSCT.
Aims: In this study, a sequential therapeutic strategy that based on rituximab followed by adoptive cellular therapies (G-CSF mobilized donor lymphocyte infusion (DLI) or EBV-specific cytotoxic T lymphocyte infusion (EBV-CTL)) was evaluated for decreasing relapse of PTLD.
Methods: Twenty-one patients with EBV-PTLD were enrolled in this prospective study. Once PTLD was diagnosed,immunosuppressants would be withdrawn in a stepwise fashion (ie, total dose reduced by 20%/week)if the condition of the patient was acceptable. The rituximab-based treatments (rituximab alone or combined with chemotherapy) were administrated based on PTLD histopathology and the blood cells counts. After CR or 2 cycles of rithuximab-based treatments, DLI or EBV-CTL therapy would be performed in this cohort. The rituximab-based treatmentswould be discontinued once patient obtained CR, and DLI or EBV-CTL infusion would be performed once monthlytill GVHD occurring or for a total of 4 doses after CR.
Results: Of the 21 patients enrolled in this study, the data of 18 cases were used to determine the primary endpoint in the intent-to-treat population and 3 patients died of PTLD progression or other causes within 2 cycles of the rituximab-based treatments, 13 patients obtained CR , 3 obtained PR, 2 were NR and 3 died after 2 cycles of the rituximab-based treatments. The CR rate of 2 cycles of rituximab-based treatments was 61.9%. The 5 patients with no CR all obtained CR after the rituximab-based treatments combined with the adoptive cellular therapies. Of the 15patients receiving the adoptive cellular therapies, 12 patients accepted DLI and 3 EBV-CTL therapy. Fivepatients developed DLI-associated aGVHD(grade II in 4) and 4 experienced cGVHD(limited cGVHD in 3 and extensive cGVHD in 1). One patient experienced the relapse of PTLD. With a median follow-up of 509 days (range,52 to 1642 days) after PTLD, the 2year cumulativeoverall survival and disease-free survivalwere 57.8±13.8% and 48.9±14.7% respectively. Till now, only one patients experienced PTLD relapse.
Summary / Conclusion: Rituximab-based treatmentscombined with the adoptive cellular therapies might elevated the CR rate of EBV-associated PTLD, and the rituximab-based treatments followed by the adoptive cellular therapy might decrease the relapse of PTLD in the recipients of allo-HSCT.

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EARLY-PHASE ELEVATED TUMOR NECROSIS FACTOR ALPHA RECEPTOR-1, SOLUBLE INTERLEUKIN-2 RECEPTOR, AND LEUCINE-RICH ALPHA 2 GLYCOPROTEIN PREDICT POOR PROGNOSIS FOR ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS

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Background: Treatment related mortality (TRM) after allogeneic stem cell transplantation (allo-SCT) remains high. Organ damage in the early phases of transplantation due to preconditioning agents, allo-immune reactions, or infectious disease is associated with subsequent mortality. Reliable biomarkers to reflect destructive inflammation or organ damage and predict prognosis or TRM of allo-SCT patients have yet to be identified. Previously, tumor necrosis factor alpha receptor-1 (TNF alpha RI) and soluble interleukin-2 receptor (sIL-2R) were considered potential indicators of graft-versus-host disease (GVHD), but their specificity for predicting GVHD is insufficient. Leucine-rich alpha 2 glycoprotein (LRG) is a novel biomarker for monitoring disease activity in inflammatory disorders.
Aims: This study investigated whether selected biomarkers predict TRM in patients receiving allo-SCT.
Methods: A total of 37 patients who underwent allo-SCT at Oita University Hospital from January 2009 to July 2012 were enrolled. Plasma TNF alpha RI, sIL-2R, and LRG levels were measured by sandwich ELISA in samples obtained weekly (n=279); median samples per patient, 8. Prognosis was compared between patients who had high (≥median) and low (<median) levels of each biomarker. Written informed consent was obtained from each patient.
Results: The cumulative incidences at day 100 of Grade II to IV and III to IV acute GVHD were 37.8% and 2.7%, respectively. Ten patients died from TRM (infectious diseases, 3; organ failure, 6, bleeding, 1), but no patients died as a direct result of GVHD. Analysis of biomarker kinetics revealed a tendency for plasma TNF alpha RI, sIL-2R and LRG to peak around 2-3 weeks after transplantation. Levels of each biomarker at both the 2nd and 3rd week after transplantation were significantly associated with subsequent mortality. One-year overall survival (OS) was 94.7% and 35.6% in patients with high and low TNF alpha RI, respectively (P<0.01); 82.0% and 49.4% in patients with low and high sIL2R, respectively (P=0.03); and 77.1% and 55.6% in patients with low and high LRG, respectively (P=0.17). The 1-year incidence of TRM was 44.4% and 5.3% in patients with high and low TNF alpha RI, respectively (P=0.002); 44.4.0% and 5.6% in patients with high and low sIL2R, respectively (P=0.002); and 38.9% and 10.5% in patients with high and low LRG, respectively (P=0.014). Poor performance status (>2), reduced intensity conditioning (especially melphalan use), and fever in the conditioning period correlated with higher levels of TNF alpha RI, sIL-2R and LRG at weeks 2 and 3.
Summary / Conclusion: Our data demonstrate that elevation of TNF alpha RI, sIL-2R and LRG at the engraftment phase strongly predict subsequent occurrence of TRM. We speculate that elevations of these markers reflect severe organ damage or existence of a vigorous immune reaction.

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A GENETIC VARIANT IN THE CD53 INTRON FUNCTIONALLY PREDICTS TRANSPLANT OUTCOMES AFTER HLA-MATCHED UNRELATED BONE MARROW TRANSPLANTATION

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Background: The CD 53 antigen is a member of the tetraspanin family that is expressed in the leucocytes, endothelial cells, thymus, and lung, and plays roles in the regulation of cell growth and activation, inhibition of apoptosis, and immunity. The single nucleotide variation, rs6679497 (C>G), in the intron of the *CD53* gene is associated with TNFα inducibility (Bos SD: *Eur J Hum Genet*. 2010).
Aims: This study examined the impact of donor and recipient variation in the *CD53* gene on the clinical outcomes of patients undergoing allogeneic T-cell-replete bone marrow transplantation using an HLA-matched unrelated donor.
Methods: The *CD53* variation was retrospectively analyzed in a cohort consisting 322 pairs of patients with hematologic malignancies and their unrelated donors transplanted through the Japan Marrow Donor Program. Next, the functional relevance of the rs6679497 variation was investigated using leucocytes from healthy individuals.
Results: The recipient GG genotype was found to be associated with a lower overall survival (OS) in the univariate analysis (29% *vs*. 53% at 5-y; P=0.01) and multivariate analysis (relative risk=1.97; 95% confidence interval, 1.22-3.18; P=0.01). *In vitro* stimulated leucocytes from healthy individuals possessing the G allele showed a trend toward increased TNFα secretion than those without the G allele. In the allele-specific quantitative PCR with a TaqMan probe for healthy donors with heterozygous genotypes of rs6679497, the ratio of the G allele transcripts *vs*. C allele transcripts was higher than that of DNA amplicons, suggesting that the G allele has higher transcriptional activity than the C allele.
Summary / Conclusion: These findings substantiate the functional relevance of the rs6679497 variation, and indicate that the increased TNFα secretion by individuals with the G allele likely account for their worse OS.

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RELATIONSHIP BETWEEN INFUSION FEVER FOLLOWING PERIPHERAL BLOOD STEM CELL AND PERI-ENGRAFTMENT SYNDROME IN PEADIATRIC PATIENTS UNDERGOING HAPLOIDENTICAL ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Our previous study showed the association between infusion fever following peripheral blood stem cell(infusion fever) and subsequent allogeneic immune reactions in adults patients in haplo-identical transplantation. Similar infusion fever has been observed in some paediatric patients under our haplo-identical transplant setting.
Aims: To explore the relationship of infusion fever with peri-engraftment syndrome and acute graft-versus-host disease (GVHD) in paediatric patients under haploidentical allogeneic hematopoietic stem transplantation (Allo-HSCT).
Methods: Clinical data of 59 peadiatric patients received haploidentical Allo-HSCT from Jan,2012 to Dec,2012 was invetstigated. Infusion fever was defined as unexplained fever>38°C not assocaited with infection within 24 hours following the infusion of allogeneic peripheral harvest. Peri-engraftment syndrome was defined as febrile reaction almost with skin rash and/or other immune reactions within 5 days of engraftment. The patients were classified into 2 groups as infusion fever group and none-fever group.
Results: Paediatric patients (median age,12 years;range,2-17 years) received myeloablative conditioning as previous reports from Peking University Institute of Hematology. Primary diseases included acute myeloid leukemia (n=23), acute lymphoblastic leukemia (n=23), chronic myeloid leukemia (n=3) and other diseases (n=10). 22 (37.3%) out of all the patients had infusion fever. The median times to neutrophil recovery and platelet recovery were 12 (10-19) days and 16 (7-83) days. Both of neutrophil and platelet reconstitution were were similar between 2 groups.32(54.2%) out of all the patients had peri-engraftment syndrome. The median of peri-engraftment syndrome was 11 (7-20) days. Infusion fever group developed significantly high incidence of peri-engraftment syndrome compared to none-fever group.The incidence rate of peri-engrafment syndrome in infusion fever group and none-fever group were 90.9% and 32.4%, respectively (P=0.000). The incidences rate of grades I–IV,II-IV and III-IV aGVHD among the whole patients were 44.1% (26/59), 30.5%(18/59) and 8.5%(5/59),respectively. The incidence rates of grade I-IV, II-IV and III-IV aGVHD were similar in fever group and none-fever group were 45.5%(10/22) versus 43.2%(16/37),36.4%(8/22) versus 27.0%(10/37) and 13.6%(3/22) versus 7.4% (2/27), respectively (P>0.05).The day-100 treatment related mortality (TRM) and survival were not significantly different in infusion fever group and none-fever group.
Summary / Conclusion: Infusion fever, as a new clinical feature belongs to haploidentical allogeneic stem transplant, was associated with the occurrence of peri-engraftment syndrome in paediatric patients.Our results suggested the potential role of infusion fever as predictor of early allogeneic immune reaction.

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BISPHOSPHONATES DO NOT DELAY ENGRAFTMENT AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH NEWLY DIAGNOSED MYELOMA

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Background: Several factors have been found to affect the engraftment after autologous stem cell transplantation (ASCT) in multiple myeloma (MM), including e.g. use of blood stem cells, amount of infused CD34+ cells, prior use of myelotoxic agents and use of haematopoietic growth factor. Recently, bisphosphonates were shown to delay haematological recovery after ASCT in mice when compared to untreated controls (Lymperi *et al*. 2011). To the best of our knowledge, this issue has not been studied in the clinical setting.
Aims: The aim of this study was to evaluate the impact of bisphosphonates on haematological recovery after ASCT in patients with MM.
Methods: 221 myeloma patients transplanted 1993-2010, whose data were prospectively collected in our institutional ASCT registry, were analyzed. There were 176 patients who had received bisphosphonate treatment before ASCT and 45 who had not.
Results: There were no difference in the recovery times of blood neutrophil and platelet counts between these two groups: blood neutrophils reached the count of $1.0x10^9$/L on days 11 and 11, and platelet the count of $20x10^9$/l on days 14 and 13, respectively (Table 1). Similarly, no differences in engraftment times were seen in patients who had received either clodronate, pamidronate or zoledronate. The patients were also divided into two other groups, those who had received Thal-Dex (n=40, 74 % bisphosphonate users) or VAD (n=147, 77% bisphophonate users). It appeared that the Thal-Dex group had a significant delay in their engraftment by a median of three days (Table 1).
Summary / Conclusion: In opposite to the bisphosphonate effect on engraftment after ASCT in mice, these drugs seem not to have a similar effect in myeloma in man. Regarding the impact of the initial treatment on engraftment post-ASCT, controversial results have been published (Ghobriel *et al*. 2003, Breitkreutz *et al*. 2007). In our study, a delayed engraftment in patients previously treated with Thal-Dex was observed.

Table 1.

	All	Bp	W/o bp	VAD	Thal-Dex
Number of patients	221	175	46	147	40
CD34+ (E6/kg)	5,02	4,85	5,79	5,38	4,45
G-CSF (n)	214	170	44	147	37
VAD (n)	147	113	33	147	7
Thal-Dex (n)	40	31	8	-	40
Neutrophils ≥ 1,0 x10^9 (days post-transplant)	11	11	11	11	12
Platelets ≥ 20 x 10^9 (days post-transplant)	13	14	13	13	15
Both neutrophils and platelets (days post-transplant)	14	14	13	13	16

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STENOTROPHOMONAS MALTOPHILIA INFECTION IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION — RISK FACTORS AND THERAPEUTIC STRATEGIES

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Background: *Stenotrophomonas maltophilia* (*S. maltophilia*) is aglucose non-fermentative Gram-negative bacillus, which can be isolated from humectant environments. *S. maltophilia* has multi-antibiotic resistance because of the natural possession of metallo-β-Lactamase. *S. maltophilia* infection is rare, but can develop a fatal bloodstream infection or hemorrhagic pneumonia. There are some reports about *S. maltophilia* infection in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-SCT).
Aims: To clarify the risk factors and the effective therapeutic strategies for *S. maltophilia* infection in allo-SCT.
Methods: We retrospectively analyzed medical records of 259 patients under-

went allo-SCT between January 2004 and December 2011 in Hokkaido University hospital, Sapporo, Japan. We also analyzed the patients with hematologic diseases who did not be underwent allo-SCT at the same period.
Results: *S. maltophilia* were detected by culture-based identification methods in 39 patients (15%), and 25 patients of those were developed *S. maltophilia* infection. Of all 52 colonization events, *S. maltophilia* infections occurred in 33 events (63%): sepsis (n=20), pneumonia (n=8), were developed as pneumonia, multiple infection (n=5). We compared with 32 non-allo-SCT patients with *S. maltophilia* at the same period. Neutropenia was more severe in allo-SCT patients compared to non-allo-SCT patients at the time of detection of *S. maltophilia*, and consequently, the rate of onset of *S. maltophilia* infection was significantly higher in allo-SCT patients (63% vs 29%, P <0.01). Moreover, fatal cases from *S. maltophilia* infection were observed in only allo-SCT patients. Next, we compared the period detected *S. maltophilia* during allo-SCT. In the period from the initiation of conditioning therapy to neutrophil engraftment, the rate of infection onset of was higher, neutropenia was more severe, and max CRP values were more elevated compared to other periods. Especially, the onset of infection in early periods from SCT was suggested as a risk factor of *S. maltophilia* related mortality (Figure 1). In such the period of neutropenia, we frequently transfuse granulocytes from healthy donors. Indeed, the efficacy of Granulocyte transfusion (GTX) was observed in several cases of *S. maltophilia* infection. According to the analysis of 4 fatal cases from *S. maltophilia* infection, 3 cases were underwent cord blood transplantation (CBT), and in all cases, only one antibiotic with sensitivity was administered after the identification of *S. maltophilia*. Additionally, none of fatal cases removed central venous catheter (CVC), but only changed to new CVC after the onset of infection. We examined the difference of the efficacy between "CVC removal" and "CVC change". Compared to "CVC removal", the efficacy of "CVC change" was limited, especially in the period of neutropenia, the efficacy of "CVC change" was only 17%.
Summary / Conclusion: *S. maltophilia* colonization in allo-SCT was higher risk for infection development among hematologic treatments, and onset of infection in early periods from SCT (especially CBT) was the risk for *S. maltophilia* related mortality. For the treatment of *S. maltophilia* infection, 1. Removal of CVC, 2. Administration of multiple antibiotics sensitive to *S. maltophilia*, 3. GTX may be considered.

Variable	Prerengraftment		Other		Pvalue
Infection events / Total events	11 / 14 (79%)		22 / 38 (58%)		.21
Type of infection	Sepsis	5	Sepsis	15	
	Pneumonia	3	Pneumonia	5	
	Sepsis and Pneumonia	2	Sepsis and Pneumonia	1	
	Sepsis and Cellulitis	1	Sepsis and Cellulitis	1	
Neutrophils at the time of infection (median, range)	0 (0 – 2754)		1660 (0 – 9048)		<.01
Max CRP (mg/dl) (median, range)	16.00 (1.42 – 36.81)		6.68 (1.00 – 28.41)		.03
Presence of CVC	14 / 14 (100%)		35 / 39 (90%)		.56
S.maltophilia related mortality	3 / 10 (30%)		1 / 15 (7%)	Pvalue .05	.36
Onset of infection (from Day0)					
~ day7	3 / 7 (43%)				
day8 ~	0 / 3 (0%)				n.s.

Figure 1.

Signalling, transcription and apoptosis

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CALM LINKS CYTOKINE SIGNALS TO HEMATOPOIETIC CELL GROWTH AND SURVIVAL BY REGULATING INTRACELLULAR TRAFFICKING OF RECEPTOR TYROSINE KINASES

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Background: Clathrin Assembly Lymphoid Myeloid leukemia protein (CALM) was originally cloned at the brakepoint of t(10;11)(p13;q14-21) translocation as CALM/AF10 fusion protein, which is observed in 8-10% of T-ALL and a smaller percentage of AML. CALM is ubiquitously but highly expressed in hematopoietic and neuronal systems. CALM is implicated in the formation of clathrin-coated vesicles and plays an essential role in clathrin-dependent endocytosis (CDE), which mediates the entry of growth factor receptors and nutrients into the cells. However, the physiological role of CALM has yet to be elucidated.
Aims: The present experiments were designed to clarify the role of CALM in hematopoiesis, especially how CALM can regulate cytokine signaling events and biological responses in hematopoietic cells**.**
Results: To elucidate the physiological role of CALM, we established CALM-deficient ($^{-/-}$) mice through gene targeting. Although CALM$^{-/-}$ mice were obtained at the expected Mendelian ratios, they were growth retarded with about 35% weight compared with wild-type mice. Also, more than 90% of CALM$^{-/-}$ mice died during the weaning period. CALM$^{-/-}$ mice exhibited severe anemia (mean of RBC count 268x10^{10}/L; Hb 3.7 g/dl) due to the impairment of erythroid maturation. As for this reason, we found that CDE of transferrin receptor was impaired in CALM$^{-/-}$ erythroid cells, which leads to the iron deficiency in these cells. To further clarify the role of CALM in hematopoiesis, we isolated Lin^{-}Sca-1^{+}c-Kit^{+} hematopoietic stem/progenitor cells (LSKs) from the bone marrow of wild-type and CALM$^{-/-}$ mice. When we cultured these cells with SCF, FLT3 ligand, and TPO, the growth of CALM$^{-/-}$ LSKs was severely impaired, which was partly cancelled by the addition of IL-3. This result suggests that SCF- and/or FLT3-mediated signaling might be perturbed by CALM deficiency. However, an apparent difference was not observed in SCF-induced phosphorylation of c-Kit between CALM$^{-/-}$ and wild-type LSKs. Also, CALM-deficiency didn't influence the internalization of SCF/c-Kit complex in LSKs. In contrast, SCF-induced phosphorylation of Akt and extracellular regulated kinase (ERK)1/2 was enhanced and prolonged in CALM$^{-/-}$ LSKs as compared with wild-type LSKs, while CALM-deficiency did not affect IL-3-induced JAK/STAT signaling. These results suggest that appropriately regulated c-Kit signaling is required for the normal growth of LSKs. To further elucidate the role of CALM in the clathrin-mediated intracellular trafficking of c-Kit, we isolated and immortalized murine embryonic fibroblasts (MEFs) from CALM$^{-/-}$ and wild-type mice. Then, we introduced c-Kit into these MEFs. In response to the stimulation with SCF, c-Kit was found to be incorporated into the cytoplasm and then located around the nucleus in wild-type MEFs by confocal microscopy. In contrast, loss of CALM resulted in the predominant accumulation of c-Kit in the enlarged early endosomes, which was canceled by the retroviral transduction of wild-type CALM. These results indicate that the trafficking of c-Kit from the early to the late endosome or the endosomal recycling compartment is disrupted by CALM deficiency.
Summary / Conclusion: Our results raise the possibility that CALM might be a critical regulator of the intracellular trafficking of cytokine receptors in hematopoietic cells, thereby controlling normal and abnormal hematopoiesis.

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SMAC MIMETICS ENABLE INTACT APOPTOSIS SIGNALING IN FORMER APOPTOSIS RESISTANT PRIMARY ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

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Background: Although multiagent chemotherapy regimens have led to improvement of remission induction and long-term survival, patients with high risk ALL or relapse do not respond well to current treatments and still have a poor prognosis. Defects in apoptosis signaling pathways e.g. overexpression of "Inhibitor of Apoptosis" (IAP) proteins might be one reason for treatment failure and relapse of acute leukemia since they are associated with poor prognosis. Therefore, new small molecules targeting IAP proteins, so called SMAC mimetics, present a suitable new strategy for therapeutic intervention.We already showed that neutralizing IAP proteins by small molecule IAP inhibitors is an effective approach to sensitize childhood acute leukemia cells for death

receptor- or chemotherapy-induced apoptosis.
Aims: This study identifies the capability of SMAC mimetics to induce cell death in acute leukemia cell lines and primary acute lymphoblastic leukemia cells and demonstrates the activated signaling pathways.
Results: Here, we report that small molecule SMAC mimetics alone induce apoptosis at nanomolar concentrations in acute leukemia cells. Cell lines which are sensitive for apoptosis induction by SMAC mimetics show rapid degradation of cIAPs, activation of NF-kappaB and secretion of TNFalpha, leading to an autocrine, apoptosis-inducing TNFalpha-loop. Further analysis of signaling pathways reveals that IAP inhibition causes TNFalpha-dependent loss of mitochondrial membrane potential, caspase activation and apoptosis. Whereas the inhibition of RIP-Kinases has no effect on apoptosis-induction upon SMAC mimetic treatment, knockdown of RIP1 results in significant reduction of SMAC mimetic-induced loss of mitochondrial membrane potential, caspase activation and apoptosis. Thereby, the availability of RIP1 in the TNFalpha-receptor complex plays a decisive role for subsequent apoptosis signaling. Furthermore, we tested small molecule SMAC mimetics on a variety of 36 primary ALL samples isolated from ALL bearing mice of established patient derived NOD/SCID/huALL xenograft leukemias. Treatment with SMAC mimetics at nanomolar concentrations clearly induces cell death also in the vast majority of primary ALL samples (25/36). Since cell death induction by SMAC mimetics in primary ALL cells could also be inhibited by the soluble TNF-alpha receptor Etanercept we assume a TNFalpha-loop upon treatment with SMAC mimetics in primary ALL cells too. We previously described that rapid engraftment of ALL cells transplanted onto NOD/SCID mice analyzed as weeks from transplantation to onset of leukemia related morbidity in the recipients (short Time To Leukemia, TTL^{short}) is associated with deficient apoptosis signaling in the cells and is indicative for early patient relapse. Importantly, primary xenograft ALL samples with a TTL^{short}/early relapse phenotype showed increased cell death upon treatment with SMAC mimetic BV6 and activation of the constitutive deficient apoptosis signaling pathway, demonstrating that SMAC mimetics enable intact apoptosis signaling in former apoptosis resistant primary ALL cells. Thereby, intact apoptosis signaling was functionally analyzed assessing mitochondrial cytochrome c release and activation of the effector caspase-3.
Summary / Conclusion: Thus, induction of apoptosis by the new generation of small molecule SMAC mimetics provides a promising novel strategy for targeted therapy of high risk acute lymphoblastic leukemia.

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IDENTIFICATION OF A NOVEL CANDIDATE GENE CAUSING AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME (ALPS)-III-LIKE DISEASE
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Background: Patients suffering from the Autoimmune Lymphoproliferative Syndrome (ALPS) typically harbour germline or somatic mutations in genes involved in the CD95 death receptor signalling pathway. In ALPS type I and II patients, disease causing mutations of CD95, CD95 ligand or Caspase 10, respectively, have been identified. For 20-30% of patients, collectively classified as ALPS-type III cases, the genetic cause is still unknown.
Due to defective apoptotic signalling via the CD95 death receptor mainly T-lymphocytes are inefficiently cleared. ALPS is thus characterized by lymphadenopathy, hepatosplenomegaly, autoimmune cytopenias and an elevated number of double negative T-cells (DNT; $CD3^+$, $TCR\alpha/\beta^+$ $CD4^-CD8^-$).
Aims: The objective of this study was to employ next-generation-sequencing to search for novel gene candidates underlying ALPS-type III. The list of potential candidates should be narrowed down using an in-house developed bioinformatic analysis pipeline for patient-based gene prioritization based on protein-protein interaction networks. Resulting candidates should be validated and their impact on CD95 signaling studied.
Methods: Peripheral blood samples were collected from three patients diagnosed with ALPS based on clinical phenotype and accumulation of DNT cells. Their DNA was analyzed for germline and somatically acquired CD95, CD95L and Caspase-10 mutations by PCR/Sanger sequencing. Apoptosis was checked employing flow cytometry. Whole exome sequencing was carried out for all three patients and their parents. Single nucleotide variations (SNVs) were called, annotated using NGS-SNP and mapped to a protein-protein-interaction network using STRING. A random walk algorithm was employed to identify functional modules and gene candidates. SNVs were verified by PCR and Sanger sequencing. Candidates' protein expression and impact on CD95L expression was tested by FACS and ELISA.
Results: 216,948 sequence nucleotide variations (SNVs) were called from three ALPS type III patient samples. 59,957 SNVs remained after removing variations also present in the healthy relatives.4,749 of these SNVs were homozygous and affected exonic regions. Application of the newly developed interaction analysis pipeline reduced this number to 29 candidate genes. One of the three patients harboured a homozygous mutation in the gene for the Interleukin 12 receptor beta 1 (IL12RB1), a subunit of the IL12 receptor. The mutation led to a premature stop at codon 212 (exon7, R212stop). IL-12RB1 is a protein comprising 662 amino acids. 17 exons encode a peptide leader sequence (exon 1), extracellular domain (exons 2-13), transmembrane domain (exon 14) and an intracellular domain (exons 15-17). The mutation led to a complete loss of IL12RB1 expression on the cell surface of the patient cells and downmodulation of CD95L expression. In this patient IL12RB1 was the only identified gene with an impact on CD95 signaling.
Summary / Conclusion: IL12RB1 was identified as a candidate gene underlying an ALPS type III case. Our and previous studies confirm that IL12RB1 affects apoptotic signalling via the CD95 receptor, a prerequisite for an ALPS causing gene. Loss of IL12RB1 has also been shown to predispose for mycobacterial infections. However, the patient analyzed here had a distinct ALPS phenotype (lymphadenopathy, splenomegaly, hepatomegaly, various autoimmune symptoms, elevated DNT cell numbers) and never suffered from mycobacterial infections. Further investigation of IL12RB1 deficient patients may reveal more cases with ALPS-like symptoms.

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ONCOGENIC PHOSPHORYLATION OF NIPA IS MEDIATED BY ERK2
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Background: Regulated oscillation of protein expression is an essential mechanism of cell cycle control. We previously reported cloning of NIPA (Nuclear Interaction Partner of ALK) in complex with constitutively active oncogenic fusions of the tyrosine kinase ALK, contributing to the development of lymphomas and sarcomas. Subsequently we characterized NIPA as a F-Box protein that defines an oscillating ubiquitin E3-ligase. The SCF^{NIPA} complex targets nuclear cyclin B1 for ubiquitination in interphase while phosphorylation of NIPA in late G2 phase and mitosis inactivates the complex to allow for accumulation of cyclin B1, a critical event for proper G2/M transition. Thus, SCF^{NIPA} executes an important G2/M checkpoint control.
Aims: Within our stuies, we aim to identify the kinases involved in G_2/M transistion as well as the kinases involves in pathologic signalling via oncogenes like NPM-ALK.
Results: We recently specified a sequential NIPA phosphorylation at G2/M, where initial Ser 354 and 359 phosphorylation is most crucial for SCF^{NIPA} inactivation by dissociating the SCF^{NIPA} complex. Using *in vitro* kinase assays we identified both ERK1 and ERK2 to phosphorylate NIPA with high efficiency. By combining cell cycle synchronization with stable expression of shRNAs targeting either ERK1 or ERK2, we show that ERK 2 but not ERK1 mediates NIPA inactivation at G2/M. ERK2 knockdown led to a delay at the G2/M transition, a phenotype also observed in cell expressing a phosphodeficient mutant of NIPA. NIPA has been shown to be constitutively phosphorylated in NPM-ALK expressing cells and the serine kinase responsible for this S354 phosphorylation remains unknown. We thus asked whether ERK2 is also involved in the oncogenic, cell cycle independent phosphorylation of NIPA in NPM-ALK positive cells. Therefore we treated HEK 293T cells coexpressing NPM-ALK and NIPA with different MEK/ERK inhibitors. NPM-ALK induced phosphorylation of NIPA could be completely abrogated by pharmacological inhibition of the MEK/ERK pathway. To further substantiate these data in human lymphoma cells we analyzed the effects of ERK inhibition in a human T-cell lymphoma cell line (KARPAS 299). Indeed, treatment of KARPAS 299 with U0126 abolished the phosphorylation of NIPA at S354.
Summary / Conclusion: These results indicate an involvement of ERK-kinases not only in cell cycle dependent NIPA phosphorylation but also in oncogene mediated cell cycle independent phosphorylation of NIPA. Since checkpoint proteins such as NIPA are constitutively inactivated in tumor cells ERK2 might represent an interesting target to reconstitute important cell cycle checkpoint control in malignant cells.

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TYROSINE PHOSPHATASE SHP-1 MODULATES LFA-1-MEDIATED ADHESION IN PRIMARY T CELLS
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Background: The potential of transplantation is often restricted by T cell-mediated alloresponses that in patients cause graft rejection or graft versus host disease. Besides the engagement of the T cell receptor, the adhesive interaction between potentially alloreactive T cells and antigen presenting cells of allogeneic origin is essential for alloresponses to occur. So far, the molecular processes underlying signaling events between T cell receptor activation and

cellular adhesion are not fully understood. The vast majority of published studies was done with various tumor-derived cell lines, thus producing controversial results depending on the cell line used.
Aims: To overcome these drawbacks, we used *in vitro* generated and expanded polyclonal primary T cells to study the lymphocyte function-associated antigen 1 (LFA-1)-mediated adhesion.
Methods: Primary T cells based on the priming of naïve T cells from B10.A mice (responder) with denditic cells from C57BL/6 mice (allogeneic stimulator) or from B10.A mice (syngeneic stimulator) were examined by using biochemical and cellular techniques such as protein tyrosine phosphatase activity assays, adhesion assays or immunofluorescence microscopy.
Results: We identified a novel role of the protein tyrosine phosphatase SHP-1 in the regulation of LFA-1-mediated adhesion: SHP-1 activity is significantly reduced upon alloactivation, in turn resulting in an enhanced ability of alloactivated T cells to adhere to MHC-mismatched tissues. In addition, SHP-1 impairs the adhesion-associated signaling cascade SLP-76 → ADAP → LFA-1 by modulating the tyrosine phosphorylation of ADAP.
Summary / Conclusion: The novel and decisive role of SHP-1 in the regulation of LFA-1-mediated adhesion may be of importance for a better understanding of T cell-mediated alloresponses, and provides evidence to the development of new immunosuppressive pharmaceutical agents.

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ERYTHROPOIETIN IMPROVES BRAIN DEVELOPMENT IN SHORT-TERM HY0POXIA IN RAT EMBRYO CULTURES

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Background: Erythropoietin (EPO) is a primary regulator of erythropoiesis and is fundamentally produced by the kidney in adults and by hepatocytes in the fetus in response to hypoxia. Besides its established function in erythropoiesis, EPO is currently also appreciated for its neuroprotective effects.
It has been shown that both VEGF and EPO exert neuroprotective effects in both *in vitro* and *in vivo* experimental models, probably through the common oxygen-sensing pathway stimulated by hypoxia. However, to date, the exact molecular mechanisms by which EPO might mediate its actions in the central nervous system, and the possible implications for VEGF in this regard are not well understood.
Aims: The aim of the present study was to investigate the effects of EPO on hypoxia-induced growth retardation and embryo brain development during the organogenesis period and to determine whether VEGF has an effect on the action mechanism of EPO.
Methods: Two sets of experiments were carried out. First, the culture of rat embryos with different gassing concentrations was evaluated, and second, the culture of rat embryos in rat serum with increasing concentrations of EPO was studied; VEGFR expressions were measured in all rat embryo brains.
The five pregnant rats were humanely killed by ether overdose at 9.5 days of gestation, and the embryos (approximately 10 embryos from each pregnant rat) were removed from the mother by the explantation procedure described by New (1978). Using a dissection microscope, the decidual mass was split to expose the conceptus, which was gently teased free and immediately immersed in Hank's balanced salt solution. Four embryos were placed in each culture bottle of 4 ml volume, and the bottles were placed on a roller incubator at 37^0C. WRS was used in the control groups. Fifty embryos were used in total: 10 for 0 h controls, 10 for controls in which EPO (100 U/mL) was administrated in control cultures without hypoxia, 10 for 24 h hypoxia, and 20 for EPO supplementation (50 and 100 U/mL). The control group embryos were cultured in WRS and WRS+EPO (100 U/mL). Different gas concentrations were tested to obtain hypoxia. At the of the procedures, all embryos were gassed for 1 min in the culture bottles using a mixture of 5% O_2, 5% CO_2, and 90% N_2 and then incubated at 37^0C in an incubator. During incubation, the culture bottles were continuously rotated at 30 rpm and re-gassed after 24 hours. Embryos were gassed for 1 min with 20% O_2, 5% CO_2, and 75% N_2 in the control (only WRS) and WRS+EPO groups, and with 5% O_2, 5% CO_2, and 90% N_2 in the hypoxia group after 24 h. Embryos were gassed for 1 min with 40% O_2, 5% CO_2, and 55% N_2 in all groups at hour 44, 4 hours before the morphological evaluation. The experimental groups were cultured in hypoxic medium, 50 and 100 U/mL EPO per bottle after hypoxia, for 24h. In order to assess the effect of EPO on total embryonic growth and brain development, the embryos were cultured in different concentrations of EPO (50-100 U/mL) to produce the appropriate concentration required for the embryos to develop.After 48-h culture, the embryos from each group were harvested to be analyzed according to a morphological scoring system and also genetically to measure brain VEGFR expression.
Results: The results showed that the embryos had severe growth retardation in the hypoxia and in the 50U/mL EPO in WRS groups when compared with control embryos cultured in only WRS and in the presence of 100U/mL EPO. Compared with the control groups (normal and with EPO), the experimental groups displayed higher morphological scores and somite numbers.
The mean brain development of embryos was also significantly lower in the hypoxia and 50U/mL EPO groups when compared to the controls and the 100 U/mL EPO group. Development of the neural tube was also similar in the control groups and 100 U/mL EPO group, but developmental retardations were found in the other EPO and hypoxia groups ($P<0.05$). The mean brain development of embryos was also significantly lower in the hypoxia and 50U/mL EPO groups when compared to the controls and the 100 U/mL EPO group.
Development of the neural tube was also similar in the control groups and 100 U/mL EPO group, but developmental retardations were found in the other EPO and hypoxia groups ($P<0.05$).RT-PCR showed that all three VEGF (VEGFR-3 included) receptors expressed mRNAs. The levels of VEGFR-1, R-2, and R-3 expression were significantly elevated in the 100U/mL EPO group compared to the hypoxia group ($P<0.05$)
Summary / Conclusion: In conclusion, our results suggest that EPO may contribute to early developmental processes including the proliferation, differentiation and maturation of specific neuronal populations via specific VEGF receptors in the developing rat brain. EPO treatment can be used to prevent intrauterine brain injury, especially under conditions of hypoxia. Further studies should be performed on the use of EPO in in-utero hypoxia and neural tube closure defects in clinical practice.

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DISULFIRAM/COPPER COMPLEX COULD ELIMINATE RAJI CELLS IN VITRO AND VIVO THROUGH ACTIVATION OF OXIDATIVE STRESS AND INHIBITION OF NRF2 AND NF-KB

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Background: Disulfiram (DS), an antialcoholism drug, demonstrates strong antitumor activity in a copper (Cu)-dependent manner. Our previous study showed that it was highly cytotoxic in doxorubicin resistant leukemia cells and enhanced cytotoxicity of doxorubicin. DS/Cu induces reactive oxidative stress (ROS) which activates stress related signaling pathway (c-Jun-amino-terminal kinase, JNK). Cancer cells possess higher ROS activity and some antiapoptotic factors. Therefore cancer cells may be effectively targeted by simultaneously inducing ROS and inhibiting antiapoptotic factors.
Aims: To determine the cytotoxicity of DS/Cu complex on human Burkitt's lymphoma cell line Raji *in vitro* and *in vivo* with mechanism being explored.
Methods: Cytotoxicity of DS/Cu complex on Raji cells was detected using MTT assay *in vitro*. Subcutaneously lymphoma animal model was established using nude mice to explore the potentiality of DS/Cu complex to eliminate Raji cells *in vivo*. Annexin-V/PI and DCFH-DA were employed for apoptosis and intracellular ROS level analysis by flow cytometric analysis. Western blotting was used to determine the change of anti-oxidative transcription factor Nrf2 (NF-E2-related factor 2), JNK as well as p65 expression. Statistical analysis was carried out with a one-way ANOVA followed by Dunnett's test to assess statistical significance (*$P<0.05$).
Results: MTT assay showed that with a low concentration (1 μM) of Cu^{2+}, DS exerted cytotoxicity to Raji cells with IC_{50} of 0.085±0.015 μM. *in vivo* experiment using subcutaneously lymphoma animal model also demonstrated the cytotoxicity of DS/Cu complex to Raji cells. Annexin-V/PI staining assay showed that the DS/Cu induced apoptosis was time-dependent. The apoptotic proportion of Raji cells increased from 18.89±5.86% to 81.03±7.91% when exposed to DS (3.3 μM) and Cu (1 μM) for6, 12 and 24h. Nrf2 is a key antioxidant factor. Western blot indicated that anti-oxidative transcription factor Nrf2 nuclear translocation changed in a time-dependent manner after cells being treated by DS/Cu. Nrf2 expression increased when Raji cells were treated for less than 12h and decreased after 18h or 24h treatment. ROS levels were closely related to Nrf2. Flow cytometric analysis showed that DS/Cu induced ROS generation. Western blot manifested that DS/Cu complex induced phosphorylation of JNK expression and inhibited P65 activity. N-acetyl-L-cysteine (NAC), an antioxidant, can partially attenuate DS/Cu complex-induced apoptosis, restore Nrf2 nuclear translocation, reactivate P65 activity and block JNK activation.
Summary / Conclusion: DS/Cu complex could induce apoptosis in Raji cells both *in vitro* and *in vivo*. Generation of ROS might be the core step in DS/Cu induced apoptosis. Moreover, ROS-related activation of JNK pathway as well as inhibition of NF-κB and Nrf2 may also contribute to the induced apoptosis.

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THE EXPRESSION OF TOLL-LIKE RECEPTORS IN PATIENTS WITH ACUTE MYELOID LEUKEMIAS.

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Background: Toll-like receptors (TLRs) play an important role in the host defense against microorganisms. TLRs are mainly expressed in human immune related cells such as monocytes, neutrophils, macrophages, dendrit-

ic cells, T cells, B cells and NK cells. Theireffect is connected with secretion of cytokines and chemokines which recruit immune cells death that limits microbe expanding. The expression or up-regulation of TLRs has been demonstrated in some tumors and tumor cell lines but the role of TLRs in pathogenesis and development of acute leukemias remains unclear.

Aims: The aim of this study was to evaluate the expression of TLR2, TLR4 and TLR9 and their significance as prognostic factors in patients with acute myeloid leukemias.

Methods: 103 patients with newly diagnosed acute myeloid leukemia (AML) were evaluated (47 females and 56 males). The median age of patients was 51 years. The diagnosis was established according to the WHO criteria for AML. There were 57 patients (55%) with acute myeloid leukemias: minimally differentiated, without maturation and with maturation (M0,M1 and M2 acc. FAB classification) and 46 patients (45%) with acute mielomonocytic and monoblastic leukemia (M4 and M5 acc.FAB classification). The healthy control group included 20 age-matched individuals (9 females and 11 males). Bone marrow samples were taken before induction therapy. Using quantitative reverse transcriptase PCR, the mRNA expression of genes TLR2, TLR4 and TLR9 was measured. The relative quantitation was indicated by cycle threshold (Ct) values. The Ct value of the target genes was normalized (ΔCt) to the Ct value of the GUS gene of the samples. The results were statistically analysed using 'STATISTICA 8,0'. Statistical analysis was performed by means of Mann-Whitney's U-test and P<0,05 indicated a significant difference.

Results: 60 patients (58%) with AML achieved complete remission (CR) after induction therapy, 7 patients (7%) achieved partial remission (PR) and 36 patients (35%) had no response. TLR2, TLR4 and TLR9 mRNA were expressed in all samples. The mRNA expression of TLR2 and TLR4 was significant higher in patients with NR after induction therapy than in patients with CR and PR. Moreover we observed that mRNA expression of TLR2 and TLR4 were significantly higher in patients with mielomonocytic and monoblastic acute leukemia. In comparison to control groupTLR2 and TLR4 mRNA expression was higher in AML patients than in healthy individuals although there was no statistically significant difference (ΔCt TLR2 0,9±0,85 vs 0,82±0,87 and ΔCt TLR4 0,29±0,32 vs 0,33±0,23). The results are shown in Tables 1 and 2.

Summary / Conclusion: Our results demonstrate that increased TLRs expression is related with reduced efficacy of induction chemotherapy in AML patients. We postulate that TLRs could be an independent prognostic factor for response rate after induction therapy in patients with acute myeloid leukemias. This observation should be validated by larger study.

Table 1. Correlation between mRNA expression of TLRs and response to induction therapy.

	CR+PR n=67	NR n=36	p
ΔCt TLR2	0,78±0,82	1,31±0,87	<0,01
ΔCt TLR4	0,28±0,29	0,35±0,35	<0,01
ΔCt TLR9	0,003±0,002	0,003±0,003	ns

n=number of patients ns=not significant

Table 2. Correlation between mRNA expression of TLRs andtype of AML.

	M0-M2 n=57	M4-M5 n=46	p
ΔCt TLR2	0,66±0,51	1,14±1,05	<0,01
ΔCt TLR4	0,19±0,18	0,40±0,39	<0,01
ΔCt TLR9	0,003±0,003	0,002±0,001	ns

n=number of patients ns=not significant

Red blood cells and iron; physiology and disease (anemia) - Clinical

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THE SAFETY PROFILE OF DEFERASIROX REMAINS CONSISTENT AS NON-TRANSFUSION-DEPENDENT THALASSEMIA PATIENTS APPROACH THE TARGET LIVER IRON CONCENTRATION OF <3 MG FE/G DW FOR INTERRUPTING CHELATION

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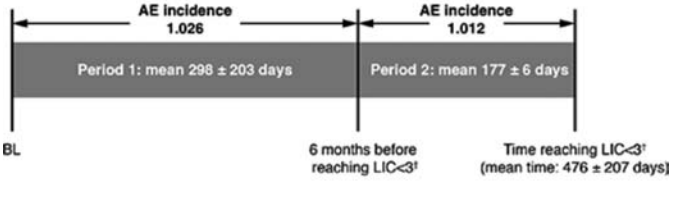
Background: Non-transfusion-dependent thalassemia (NTDT) patients can develop clinically worrisome iron overload, putting them at risk of iron-related complications. The THALASSA 1-year extension study showed continued efficacy and safety in patients receiving deferasirox up to 2 years with more patients achieving the chelation stopping target of liver iron concentration <3mg Fe/g dw (LIC<3) compared with the core study. Given the potential concern with overchelation, it is important to determine the safety profile of deferasirox as LIC approaches3, a level at which it is recommended to interrupt chelation and monitor LIC for recurrent iron overload.

Aims: To characterize the safety profile of deferasirox as patients approach their chelation stopping target of LIC<3.

Methods: Study design/inclusion-exclusion criteria have previously been described (Taher *et al. Blood* 2012). Patients randomized to deferasirox during the core (starting dose 5 or 10 mg/kg/day) and those receiving placebo had the option to enter a pre-planned, 1-year, open-label extension. Deferasirox starting doses in the extension were based on LIC at the end of core and prior chelation response. Patient number reaching LIC<3 was one of the study endpoints. Treatment was interrupted in patients with LIC<3. Safety profile was assessed between baseline and 6 months before reaching LIC<3 (Period 1), and in the 6 months before reaching LIC<3 (Period 2). Given the difference in exposure between Periods 1 and 2 an exposure-adjusted adverse event (AE) characterization was carried out.

Results: 24/166 (14.5%) patients achieved LIC<3 during study (18 received deferasirox in core + extension; 6 switched from placebo to deferasirox in the extension). Baseline characteristics (12 β-thalassemia intermedia, 6 α-thalassemia, 6 HbE/β-thalassemia) were similar to the overall population. At baseline, mean ±SD LIC was 8.1 ±3.2 mg Fe/g dw and median serum ferritin (SF) was 825 ng/mL (range 393–2169), both lower than for the overall population (LIC: 14.5±8.8 mg Fe/g dw; SF: 992 ng/mL [304–6419]). Mean ±SD LIC at the end of Period 1 and 2 was 5.2 ±2.9 (range 3.2–16.8) and 2.2±0.5 (range 1.2–2.9)mg Fe/g dw, respectively (average deferasirox dose 9.7 mg/kg/day). Median SF at the end of Period 2 (or nearest assessment) was 363 ng/mL (range 74–773). Exposure-adjusted AE incidence regardless of drug causality did not differ between Periods 1 and 2 (Figure 1).



*Exposure-adjusted incidence = number of patients with new/worsened AE during period/ [total number of days patient was on treatment summed for all patients/365.25 days]
†LIC assessed by MRI-Ferriscan

Figure 1. Exposure-adjusted incidence* of AEs.

AE type regardless of drug causality reported in these 24 patients did not differ from that in the overall population. Only 1 AE of special interest was reported (blood creatinine increase in Period 1). Creatinine, creatinine clearance and ALT near the time of LIC<3 assessment were no different to those at the previous LIC assessment. 3 patients reported >33% increase in creatinine from baseline and >ULN on 2 consecutive visits (1 patient in Period 1 where creati-

nine returned to baseline levels on dose interruption but subsequently elevated in Period 2 and the remainder of the study; 2 patients during Period 2 [near the time that LIC<3 was reached] where increases were reversible and baseline levels reached at study end).
Summary / Conclusion: In NTDT patients receiving deferasirox, exposure-adjusted AE incidence did not differ from baseline to 6 months before reaching LIC<3 compared to the immediate 6 months prior to reaching LIC<3. These results indicate the safety profile of deferasirox remains constant as patients approach their chelation stopping target of LIC<3 mg Fe/g dw, indicating that with appropriate monitoring and dose adjustments this target may be reached without increased risk of overchelation.

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PROGNOSTIC CMR PARAMETERS FOR HEART FAILURE AND ARRHYTHMIAS IN A LARGE COHORT OF WELL TREATED THALSSEMIA MAJOR PATIENTS

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Background: Cardiac complications are the main cause of death in thalassemia major (TM) patients. Cardiovascular Magnetic Resonance (CMR) plays a key role in the management of TM, allowing to assess cardiac iron burden, biventricular dimension and function, atrial dimensions, and myocardial fibrosis.
Aims: The aim of this study was to determine the predictive value of CMR parameters for heart failure and arrhythmias in TM.
Methods: We followed prospectively 537 white TM patients enrolled in the MIOT network. Fifty patients were excluded from the analysis because a cardiac complication was present at the time of the first CMR. The prognostic variables analyzed were retrieved from the MIOT database. All variables showing an association with the outcome at the univariate Cox proportional hazards model were placed in the multivariate model and were ruled out only if they did not significantly improve the adjustment of the model.

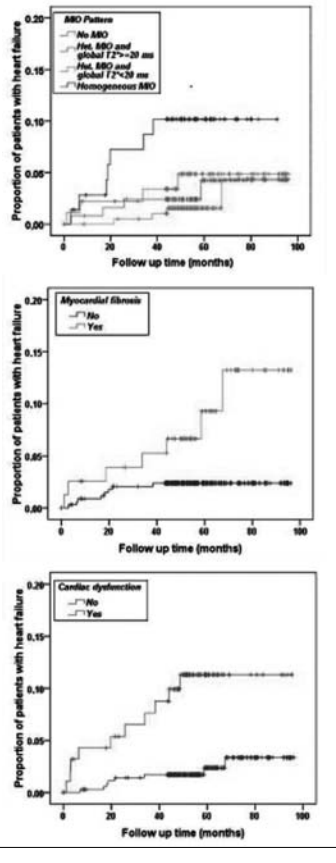

Figure 1.

Results: At baseline the mean age of the patients was 29.5±9.0 years, 222 were males and the mean serum ferritin level were 1742.49±1592.72 ng/l. The mean follow-up time was 58±18 months. After the first CMR scan only the 37.8% of the patients did not change the chelation regimen or the frequency/dosage of the chelators. We recorded 19 episodes of heart failure, diagnosed by clinicians based on symptoms, signs and instrumental findings (electrocardiogram, echocardiography and CMR) according to the current guidelines. Male sex, heart iron,ventricular dysfunction, ventricular dilation, atrial dilation, and myocardial fibrosis were significant univariate prognosticators. In the multivariate analysis the independent predictive factors were an homogeneous pattern of myocardial iron overload (compared to no MIO) (HR=5.81, 95%CI=1.42-23.74, P=0.014), global heart T2*<10 ms (compared with >20 ms) (HR=6.19, 95%CI=1.95-19.67, P=0.003), myocardial fibrosis (HR=4.76, 95%CI=1.69-13.46, P=0.003) and ventricular dysfunction (HR=3.21, 95%CI=1.13-9.12, P=0.029) (Kaplan–Meier survival curves in Figure 1). Arrhythmias occurred in 19 patients and all were supraventicular hyperhyperkinetic. Male sex, atrial dilatation and ventricular dysfunction were significant univariate prognosticators. In the multivariate analysis the independent predictive factors were male sex (HR=3.17, 95%CI=1.02-9.87, P=0.047) and atrial dilation (HR=3.07, 95%CI=1.14-8.23, P=0.026).
Serum ferritin and liver iron were not predictive factors for heart failure or arrhythmias.
Summary / Conclusion: We detected few cardiac events thanks to a CMR-guided, patient-specific adjustment of the chelation therapy. Severe and homogeneous myocardial iron overload, myocardial fibrosis and ventricular dysfunction identify patients at high risk of heart failure. Heart T2* doesn't have any power in predicting arrhythmias while male sex and atrial dilation are independent prognosticators.

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IMPACT OF MONITORING WITH LIVER MRI-R2 ON LIVER IRON BURDEN AND DEVELOPMENT OF ENDOCRINOPATHIES: A RETROSPECTIVE AUDIT

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Background: Monitoring of body iron stores continue to be a challenge in patients with transfusion-dependent β-thalassaemia major. Liver MRI-R2 is a noninvasive scanning method which using standard clinical MRI instruments and analysed telemedically. Published data suggest a strong correlation (r=0.98) between liver iron concentration measured by biopsy and that derived from the R2 scan (St Pierre, Blood, 2005).
Aims: Having recently adopted the use of MRI-R2 in liver iron monitoring, a major tertiary thalassaemia unit in London evaluates the relationship between liver iron concentration measured by MRI-R2 and serum ferritin, compliance to chelation, chelation regimen and new endocrinopathies developed between 2008-2012.
Methods: Clinical records of the 124 patients who had at least two MRI-R2 scans during the study period were reviewed to obtain demographic and clinical data. Patients were risk-stratified based on their liver iron concentration – low risk (<7 mg/g dry weight, target according to UK Thalassaemia Society guidelines), moderate risk (7-15 mg/g dry weight and risk of endocrine complications) and high risk (>15 mg/g dry weight and risk of cardiac damage) (Olivieri, Blood, 1997). Healthcare providers responsible for the patients were asked to rank the patients' compliance to chelation therapy as optimal or sub-optimal.
Results: The study group had a mean age of 32 (range 5-57) with a mean time of 2.2 years (range 8 months - 3.9 years) between the scans. 1 patient died within the study period due to sepsis unrelated to thalassaemia or iron overload. *Ferritin:* There was a weakly positive linear correlation between liver iron concentration measured by MRI-R2 and serum ferritin within three months of the scan (n=319, r^2=0.447). *Continuous monitoring:* Mean liver iron concentration decreased from 11.36±1.06 mg/g dw at initial scan to 6.10±0.55 mg/g dw at final scan (n=121, P<0.001). The proportion of patients in moderate and high risk categories nearly halved, from 48.7% to 27.3%. A continuous decrease was also shown in patients with 3 successive scans (n=56, P<0.05), where the number of patients in the high risk category decreased steadily from 28.6% to 23.2% to 12.5%. *Compliance:* 14.9% of patients were rated by their healthcare provider as sub-optimally compliant to chelation therapy. Patients who were rated as optimally compliant to therapy had better control of their liver iron concentration than those who were suboptimally compliant (P<0.001). In both optimally compliant and suboptimally compliant groups, liver iron concentration decreased from initial to final scan (P<0.01). *Chelation:* At final scan, 17.4% patients were on desferoxamine, 47.9% on desferasirox, 14.0% on deferiprone and 19.8% on combination therapy of desferroxamine and deferiprone. The chelation regime made no difference to the mean initial or final liver iron concentration (P>0.05). *Endocrinopathies:* 35 patients developed new endocrinopathies within the study period. There was no significant difference in the mean liver iron concentration in the group which developed endocrinopathies and those which did not (P=0.695). The relative risk of devel-

oping new endocrinopathies at moderate and high risk levels of liver iron compared to low risk levels was found to be insignificant at 1.04.
Summary / Conclusion: A significant improvement in liver iron concentration was demonstrated with the use of sequential MRI-R2 scans for monitoring iron overload. This is attributed to both better titration of chelation regimen and to encouraging compliance, as liver iron concentration improved even in the suboptimal compliance group. Our results emphasise the importance of optimising compliance to chelation rather than favouring any particular regime. As short-term monitoring of liver iron concentration does not predict development of further endocrine complications, we suggest the early adoption of regular MRI-R2 monitoring, routine screening for endocrinopathies regardless of scan results and re-evaluation of current targets for liver iron concentration.

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MYOCARDIAL FIBROSIS BY CMR LGE IN A LARGE COHRT OF PEDIATRIC THALASSEMIA MAJOR PATIENTS

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Background: Cardiovascular Magnetic Resonance (CMR) by late gadolinium enhancement (LGE) allows to detect myocardial fibrosis. Myocardial fibrosis was shown to be a relative common finding in large cohort of Italian thalassemia major (TM) patients mainly related to HCV infection, but specific studies involving only pediatric patients are not available.
Aims: Our aim was to investigate the prevalence and clinical-instrumental correlates of myocardial fibrosis in pediatric TM patients.
Methods: We studied retrospectively 76 pediatric patients with TM (44 boys, 4.2 -17.9 years old, mean age 13.6±3.4 years) enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) Network. All patients were well transfused and chelated since the early childhood. LGE images were acquired to detect myocardial fibrosis. Myocardial iron overload (MIO) was measured by T2* multislice multiecho technique. Biventricular function parameters were quantitatively evaluated by cine images.
Results: Myocardial fibrosis was detected in 12 (15.8%) patients. In all patients the location of the fibrosis was epi-mesocardial, with no ischemic pattern. The youngest patient showing myocardial fibrosis had 13 years of age.
Table 1 shows the comparison between patients with and without myocardial fibrosis. A significant higher MIO was detected in patients with myocardial fibrosis. The left atrial area, all the left ventricular (LV) indexed volumes, the LV mass index, and the right ventricular (RV) stroke volume index were significantly higher in the fibrosis group than in the no-fibrosis group.
Summary / Conclusion: In pediatric TM patients myocardial fibrosis is not a rare finding to keep in mind in the cardiological management. When appropriate treatment has been administered since early childhood, CMR LGE can be postponed until 13 years of age. By the natural history of this large cohort of pediatric patients where HCV infection has been appropriately prevented, myocardial fibrosis seem to be associated with MIO and high cardiac output.

Table 1.

	Fibrosis group (N=12)	No-fibrosis group (N=64)	P
Sex (M/F)	10/2	34/30	0.062
Age (years)	15.4 ± 1.8	13.3 ± 3.5	0.073
HCV antibodies	0	3 (4.8%)	0.437
Global Heart T2* (ms)	20.9 ± 13.9	30.6 ± 9.7	0.022
N. of seg. with abnormal T2*	9.0 ± 7.0	3.8 ± 5.2	0.030
Left atrial area (cm^2)	18.3 ± 3.1	15.9 ± 3.9	0.050
Right atrial area (cm^2)	16.9 ± 4.3	14.9 ± 3.5	0.169
LV end-diastolic volume index (ml/m^2)	102.9 ± 23.5	87.0 ± 16.3	0.005
LV end-systolic volume index (ml/m^2)	42.0 ± 12.1	35.1 ± 8.9	0.022
LV stroke volume index (ml/m^2)	60.7 ± 12.4	51.8 ± 10.7	0.012
LV mass index (g/m^2)	65.3 ± 11.4	53.8 ± 11.4	0.003
LV ejection fraction (%)	59.2 ± 4.4	59.7 ± 5.9	0.368
RV end-diastolic volume index (ml/m^2)	96.9 ± 25.6	81.6 ± 17.1	0.089
RV end-systolic volume index (ml/m^2)	36.9 ± 13.7	32.3 ± 8.3	0.458
RV stroke volume index (ml/m^2)	61.5 ± 11.6	48.9 ± 14.1	0.005
RV ejection fraction (%)	62.6 ± 4.4	60.2 ± 7.1	0.175

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CMR SURVEY IN A LARGE COHORT OF TI PATIENTS CATEGORIZED IN DIFFERENT TRANSFUSIONAL REGIMENS

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Background: Little is known about cardiac involvement in thalassemia intermedia (TI) using cardiovascular magnetic resonance (CMR). This survey is particularly significant considering the debate on the opportunity to transfuse the TI patients.
Aims: Our aim was to investigate myocardial iron overload (MIO), biventricular parameters, and myocardial fibrosis in a large cohort of TI categorized in different transfusional regimens.
Methods: We studied retrospectively 252 adult TI patients (119 females, 39.5±10.4 years) enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network. MIO was assessed using a multislice multiecho T2* approach. Cine sequences were obtained to quantify biventricular function parameters. Myocardial fibrosis was evaluated by late gadolinium enhancement (LGE) acquisitions.
Results: 188 (74.6%) patients showed no MIO in any segment, fifty-six (22%) patients had an heterogeneous myocardial iron distribution (52 with a global heart T2*<20 ms), and 8 (0.3%) showed an homogeneous MIO. Left ventricular (LV) and right ventricular (RV) dilatations were present in 113 (45%) and in 49 (19%) patients, respectively. LV dysfunction was present in the 18.0% of the cases while RV dysfunction in the 3.63%. High LV mass indexes were present in 22 (8.7%) patients. LGE acquisitions were made in 227 patients and 52 (22.9%) showed myocardial fibrosis. Myocardial fibrosis was associated only with LV dysfunction (P=0.001) and high LV mass indexes (P=0.038). 48 (19.0%) patients were no transfused, 66 (26.2%) were sporadically transfused and 138 (54.8%) were regularly transfused (mean time of the regular transfusional regimen 22.6±13.3 yrs). Among the CMR parameters considered the 3 group were significantly different for the LV volume indexes, the LV mass indexes, the cardiac output and the myocardial fibrosis (Figure 1).
Summary / Conclusion: CMR plays a key role in the management of TI patients. Heart iron was not absent in although the majority of the patients showed an heterogeneous distribution. A consistent number of the TI patients had the stigmata of the high cardiac output state cardiomyopathy and myocardial fibrosis seems to be related to the high cardiac output state. The signs of the high cardiac output state were controlled in the regular transfused patients. Conversely, in our cohort the regular transfused regimen seems to be started too late for preventing myocardial fibrosis.

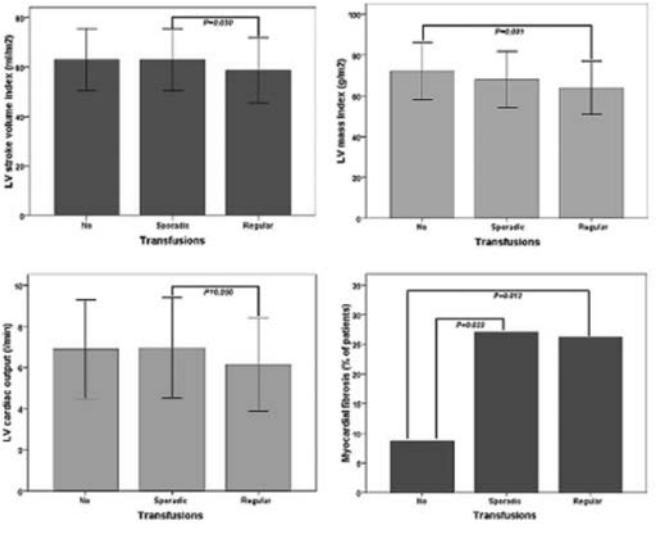

Figure 1.

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EVALUATION OF GLYCAEMIC ABNORMALITIES IN BETA THALASSEMIA MAJOR USING CONTINUOUS GLUCOSE MONITORING SYSTEM AND ORAL GLUCOSE TOLERANCE TEST

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Background: Glycaemic abnormalities have been reported in patients with β thalassemia major (TM). The use of continuous blood glucose monitoring system (CGMS) for early detection of glycaemic abnormalities has not been studied thoroughly in these patients. The aims of this study were to evaluate glycaemic abnormalities, in young adult TM patients using oral glucose tolerance test (OGTT) and 72-h CGMS, and to determine whether glycaemic abnormalities are due to insulin deficiency and /resistance.
Aims: Fourteen TM patients were selected randomly. All patients were investigated using a standard 75 gm oral glucose tolerance test (OGTT) and 72-h CGM by Medtronic system. Fasting serum insulin and ferritin concentrations were also measured. HOMA-B, HOMA-IR and QUICKI index were calculated using basal glucose and insulin levels.
Methods: OGTT fasting and 2hours postprandial as well as CGMS were done in all patients, all patients with abnorml OGTT has repetition for confirmation, CGMS were fixed for three days american college of Diabetes criteria were used to interpret the results, HOMAB,HOMAIR were calculated using equation
Results: Using OGTT, 2 patients had impaired fasting glucose (IFG) only, 2 had both IFG and IGT (glucose <11.1 mmol/L) and 1 had diabetes. In contrast, by CGMS 6 patients had (IGT) only, 3 patients had had both IFG and IGT, and 4 patients were Diabetics with glucose level ≥11.1 mmol/L. The mean values of HOMA and QUICKI in patients with TM were (1.6±0.8) and (0.36±0.03) respectively. Ferritin concentrations were positively correlated with the fasting BG r= 0.69, P<0.01, serum ferritin correlated with the average (r=0.75; P<0.01) and the maximum BG recorded by CGM (r = 0.64, P<0.05). Using OGTT, 2 patients had impaired fasting glucose (IFG) only, 2 had both IFG and IGT (glucose < 11.1 mmol/L) and 1 had diabetes. In contrast, by CGMS 6 patients had (IGT) only, 3 patients hadhad both IFG and IGT, and 4 patients were Diabetics with glucose level ≥11.1 mmol/L. The mean values of HOMA and QUICKI in patients with TM were (1.6± 0.8) and (0.36±0.03) respectively. Ferritin concentrations were positively correlated with the fasting BG r= 0.69, P<0.01, serum ferritin correlated with the average (r=0.75; P<0.01) and the maximum BG recorded by CGM (r = 0.64, P<0.05).
Summary / Conclusion: Our data suggest that CGMS is more sensitive than OGTT in detecting glycaemic abnormalities in young adult TM patients .It seems that defective β-cell function rather than insulin resistance is the most likely explanation for glycaemic abnormalities in these patients

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HEMATOLOGIC DETERMINANTS OF CARDIAC REMODELLING AND CARDIAC INDEX IN SICKLE CELL DISEASE PATIENTS.

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Background: In sickle cell disease (SCD), relationship between cardiac remodeling, cardiac index(CI) and hemoglobin (Hb), red blood cell count (RBC) and fetal hb (HbF) have not been well characterized.
Aims: Our aims were to study biological determinants of cardiac involvement in SCD and to describe cardiac characteristics during SCD in a large cohort of patients
Methods: We interrogated our hospital database including 1780 adult SCD patients to provide new insights on biological determinants of cardiac characteristics in SCD. Inclusion criteria were: homozygous or S-β_0Thalassemia patients having an echocardiography and steady state biological values before hydroxyurea or blood transfusion. Exclusion criteria were patients less than 18 years old and pregnancy. SCD patients were compared with 25 age-matched black healthy subjects. Echocardiography included M-mode, 2D, pulsed Doppler and Left Ventricular tissue Doppler.
Results: 656 SCD patients were included in the analysis with a mean age of 31 years (25; 40).Compared to control, SCD had significant higher left ventricular diastolic diameter indexed to body surface area (LVEDDind), left atria diameter indexed (LADind), Cardiac Index (CI), and lower left ventricular ejection fraction (LVEF). Systolic pulmonary artery pressure (sPAP) was not different between the two last groups. In SCD, LVEF was not correlated with Hb (P=0.74) whereas CI and LVEDDind were (both R:0.25, P<0.0001). Systolic dysfunction (LVEF<50%) was observed in only 3.4%. There was poor relationship between LVEDD and CI (R=0.15, P=0.01). Patients were divided in quartiles of CI and LVEDDind. Patients in the fourth quartiles (Q4) of LVEDDind (median/range : 35 (34; 37)) and of CI (4.7 (4.5; 5.3)) versus the three others quartiles (Q1-3) had significantly lower Hb, HbF and RBC, and higher lactate dehydrogenase, bilirubin and Dense Red Blood Cells (DRBC). Determinants of Q4 LVEDDind and CI using multivariate binomial regression analysis were respectively lower Hb and HbF and lower RBC and HbF. Patients with two alpha-thalassemia gene deletions had a significant lower CI and LVEDD.
Summary / Conclusion: In SS and S-β_0Thalassemia SCD, cardiac remodeling and elevated cardiac output are determined by hematologic variables associated with the "hyper-hemolytic phenotype".

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EFFECTS OF THE ANTI RANK LIGAND DENUSOMAB ON BETA THALASSEMIA MAJOR-INDUCED OSTEOPOROSIS

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Background: Osteoporosis is defined as a metabolic bone disease characterized by low bone mass and microarchitectural deterioration of bony tissue leading to enhanced bone fragility and a consequent increase in fracture risk.Some drugs proved effective to reduce vertebral and non-vertebral fracture risk. Denosumab is a fully human monoclonal antibody to the receptor activator of nuclear factor-κB ligand (RANKL), However the efficacy and safety of Denosumab in BTM-induced osteoporosis has not been tested. This is the first study addressing this issue
Aims: To evaluate the efficacy and safety of anti RANK ligands on the biochemical and radiological parameters of bone mineralization in patients with BTM-induced osteoporosis. Radiological evaluation was done by DEXA scan (using WHO criteria) and biochemical evaluation of bone turnover markers included bone specific alkaline phosphatase and type 1 collagen carboxy telopetide (1CCT).
Methods: we studied 30 patients with BTM-induced osteoporosis as per WHO criteria (T score of less than -1.0 being defined as osteopenic and a T score of less than -2.5 being referred as osteoporotic). 19 males and 11 females aged between 17 and 32 years, with full pubertal development (Tanner's stage 5) at the time of the study(T-scores between -2.5 and -4.0 at the lumbar spine [LS] or total hip [TH]) . Their serum ferritin levels ranged from 500 to 5922 ng/mL (mean= 2686 ng/ml). Every patient underwent DEXA scan as baseline and after 12 months of Denosumab therapy. All patients were evaluated biochemically by checking their serum calcium, phosphorus, bone specific alkaline phosphatase and 1CCT with the use of enzyme-linked immunosorbent assay (ELISA) (Nordic Bioscience Diagnostics A/S) at baseline and 12 months after starting Denosumab. Fasting serum samples were collected before and 1 and 6 months after the injection. Follicle stimulating hormone(FSH) ,Luteinizing hormone (LH) and testosterone (T) in males were measured at baseline and repeated every 3months. Renal function and electrolytes including calcium and phosphorus were measured at baseline and every two months. Circulating parathyroid hormone (PTH) levels were checked at baseline and then every 3months. Patients with renal impairment, hypocalcaemia or hyperparathyroidism were excluded from the study. 60 mg of Denosumab was administered subcutaneously twice yearly for a year. The mean bone mineral density T scores were −2.7 at the lumbar spine, −1.8 at the total hip, and −2.1 at the femoral neck.
Results: Denosumab therapy for a year was associated with a significant increase in bone mineral density of 9.2% (95% CI, 8.2 to 10.1) at the lumbar spine and 6.0% (95% CI, 5.2 to 6.7) at the total hip. Denosumab treatment decreased serum TICCT levels by 56% at 1 month and normalized them in all patients at 1 year. Significant correlations were found between bone mineral density T score before and 1year after Denosumab in vertebral (r=0.752, P<0.001) and both hips (r=0.758 respectively P<0.001). The most common side effects were pain in the extremities (12%) and nausea (10%) of patients. Hypocalcaemia was not reported in any patient.
Summary / Conclusion: Denosumab therapy for a year significantly decreased bone resorption and increased bone mineral density through inhibition of RANKL and is associated with a rapid and sustained reduction in bone turnover markers, a continuous marked increase in bone mineral density at vertebral and hips of patients with BTM. However further studies are required to confirm long-term effects of this therapy.

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REFERENCE RANGES FOR BIVENTRICULAR VOLUMES AND EJECTION FRACTION AND FOR LEFT VENTRICULAR MASS IN ADULT THALASSEMIA INTERMEDIA PATIENTS WITHOUT MYOCARDIAL IRON OVERLOAD

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Background: Thalassemia intermedia (TI) patients were shown to have significantly higher cardiac output and cardiac volumes with respect to thalassemia major (TM) patients. So, to compare biventricular parameters in TI patients with established ranges from TM may be misleading.
Aims: The aim of this study was to establish the ranges for normal biventricular volumes and ejection fraction (EF) and for left ventricular (LV) mass assessed by cardiovascular magnetic resonance (CMR) in TI.
Methods: Among the 294 TI patients with >18 years of age enrolled in the Myocardial Iron Overload (MIOT) network who underwent CMR, we selected 68 patients with no known risk factors or history of cardiac disease, normal electrocardiogram, no myocardial iron overload (all the cardiac segments with a normal T2* value) and no myocardial fibrosis. Biventricular parameters were quantitatively evaluated in a standard way by SSFP cine images using MASS® software. LV and right ventricular (RV) end-diastolic volume (EDV), end-systolic volume (ESV) and stroke volume (SV) were normalized by body surface area (EDVI, ESVI, SVI), as well as the LV mass.
Results: The selected patients had a mean age of 36.5±9.2 and 37 were males. Biventricular volumes indexes were significantly larger in males than in females, with the exception of the RV ESVI. The LV mass was significant higher in males while the LV and the RV EFs were not different between the sexes. No significant differences among TI regularly (N=36), sporadically (N=16) and no transfused (N=16) were found in biventricular parameters, so no division was made on the basis of the transfusional regimen.
The biventricular parameters are detailed in Table 1 with differentiation for sex. Table 1 reports also the cut-off of normality defined as mean - 2 standard deviation (SD) for the volumes and the LV mass and as mean - 1 SD for the EF (considering the high cardiac output state in anemic patients).
Summary / Conclusion: Reference ranges for biventricular volumes and function specific to adult TI patients were defined. These new reference ranges are important for avoiding a misdiagnosis of cardiomyopathy in TI patients.

Table 1.

	Males			Females		
	Mean ± SD	95% CI	Normal value	Mean ± SD	95% CI	Normal value
LV EDVI (ml/m2)	101.5 ± 22.6	93.9-109.0	<147	89.4 ± 15.1	83.8 – 94.9	<120
LV ESVI (ml/m2)	38.1 ± 11.2	34.4 – 41.8	<60	32.2 ± 7.9	29.3 – 35.1	<48
LV SVI (ml/m2)	63.6 ± 13.9	58.9 – 68.2	<91	57.4 ± 12.3	52.9 – 61.9	<82
LV Mass I (g/m2)	69.2 ± 12.6	64.9 – 73.4	<94	57.7 ± 10.9	53.7 – 61.8	<79
LV EF (%)	62.6 ± 5.6	60.7 – 64.4	>57.0	63.8 ± 5.9	61.6 – 66.0	>57.9
RV EDVI (ml/m2)	94.7 ± 19.8	88.1 – 101.3	<134	83.9 ± 16.1	77.3 – 90.6	<116
RV ESVI (ml/m2)	33.6 ± 8.1	30.9 – 36.3	<50	30.5 ± 9.3	27.2 – 33.9	<49
RV SVI (ml/m2)	61.1 ± 14.8	56.2 – 66.1	<91	52.9 ± 13.6	47.9 – 57.9	<80
RV EF (%)	63.9 ± 5.7	62.1 – 65.9	>58.2	63.2 ± 7.9	60.3 – 66.1	>55.3

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CLINICAL PROGRESS IN FOUR CARRIERS OF BETA GLOBIN LOCUS DELETIONS

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Background: The HBB locus contains several globin genes highly and specifically expressed at different human development stages. The ε and γ genes are expressed during embryogenesis and fetal development, respectively. The adult erythropoiesis occurs in the bone marrow. Here, the δ and β genes begin their expression. Located several Kb upstream the locus, the main regulatory element leading to high globin expression is known as LCR (Locus Control Region). Large DNA deletions may affect one or more of these genes, resulting in a thalassemic phenotype at the developmental stage in which the deleted gene is required. When a deletion removes all globin genes in the locus, or even when the deletion is limited to the LCR, a phenotype named εγδβ thalassemia is produced. In this case, globin gene expression in the HBB locus is abolished.
Aims: Here we present the genetic study and clinical progress of four εγδβ thalassemic patients.
Methods: Four non related patients were submitted for genetic screening of thalassemia because they showed a moderately-severe anemia at birth (patient I: Hb 10,1 gr/dl; MCV 80,1 fl; MCH 25 pg; patient II: Hb5,7; MCV 63,5; MCH 20,1; patient III: Hb 7,3; MCV 65,7; MCH 21,4; and patient IV: Hb 6,9; MCV 74,7; MCH 21,8). Patient IV was the only one requiring a sporadic blood transfusion for the treatment of the anemia. The genetic screening was performed by MLPA, with a commercial kit (P102-B2 HBB, MRC-Holland). This technique allows the detection of any large deletion in a genetic region spanning more than 80 Kb, including the HBB locus and the LCR. In order to follow the patients clinical progress it has been compared theirs hematological indices at birth versus the more recent data available (patient I at 7 years old: Hb 11,8; MCV 60,4; MCH 20,3; patient II at3,5 years old: Hb 10,6; MCV 59,8; MCH 18,6; patient III at2,5 years old: Hb 10,6; MCV 59,1; MCH 18,4; and patient IV at 2 years old: Hb 11,2; MCV 53,2 ; MCH 17,9).
Results: MLPA showed that all patients were carriers for large deletions affecting the genetic region studied here. Patients I to III have a deletion removing more than 80Kb, including the LCR and all the globin genes. Patient IV is carrier for a deletion limited to the LCR and ε gene, leaving intact the remaining globin genes in the locus. The clinical progress of all four patients is showed in Figure 1.
Summary / Conclusion: There are 15 previously known deletions causing εγδβ thalassemia. Most of these cases were *de novo* mutations. Parents of our patients I to III showed a normal hematologic profile. Thus, it is plausible that these deletions also were produced in a similar way that those cited above. In contrast, the father of patient IV presents a thalassemic trait, so he could be a carrier of the same deletion. In all our cases, the phenotypic substantially improves over time, possibly occurring in parallel to fetal to adult globin switching. Interestingly, many of the previously known heterozygote carriers for this kind of deletions have a clinical course marked by a variable moderate-severe hemolytic anemia, followed by the hematological picture of normal HbA2 β thalassemia trait in adult life. It is though that, somehow, fetal erythrocyte precursors could be more supceptible to α globin chain excess than adult precursors. However, the molecular basis responsible of both the phenotypic variability of εγδβ thalassemias in the perinatal period and the subsequently remission of the patients have not been explained so far.

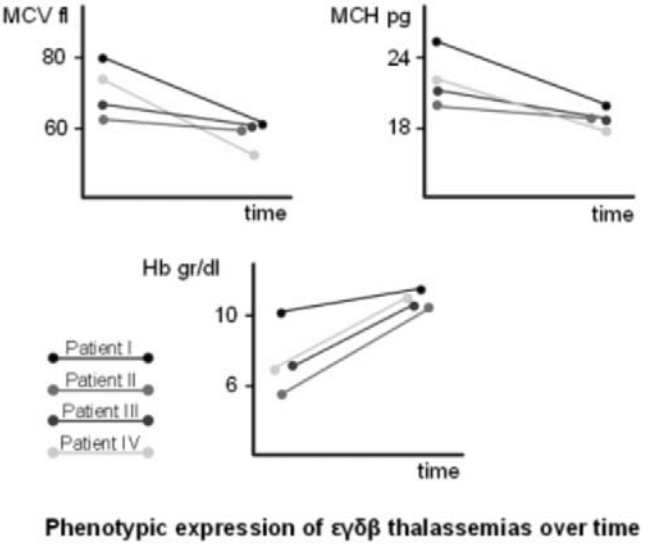

Figure 1.

P401

PITUITARY MRI IN CORRELATION WITH IRON OVERLOAD AND HYPOGONADISM IN YOUNG THALASSEMIA MAJOR PATIENTS

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Background: Iron over load is common for patients with thalassemia major and

other transfusion-dependent anemias. The toxic effects of iron include heart failure, diabetes, and hypogonadism. Hypogonadism is the most common endocrinopathy in thalassemia major, with a prevalence rate of over 50% in multicenter studies. Early recognition of pituitary iron loading is imperative because hypogonadism is only partially reversible by intensive chelation.
Aims: Our aim was to determine MRI signal intensity young beta-thalassemia patients with transfusional iron overload. And to correlate MRI findings with degree of hemosidrosis and gonadal dysfunction.
Methods: We recruited 56 patients divided into 2 groups; group 1 comprised 22 patients with hypogonadism (chronological age range :12.5-19.6 years) and group 2 comprised 34 patients with normal gonadal function (chronological age range :12 -16.6 years). Hypogonadism was defined clinically based on the timing of secondary sexual characteristics or the need for sex hormone replacement therapy. MR imaging of pituitary gland using 1.5 T unit with survey sagittal T1-weighted image and a sagittal T2-weighted image on Fast Field Echo sequence. Signal intensities were measured in consensus by the use of 3 operator-defined regions of interest . Thalassemia patients were subjected to questionnaire about data at diagnosis, age , disease duration ,chelation therapy, blood transfusion with calculation of the mean pretansfusion hemoglobin and transfusion index. Through clinical examination, weight, height standard deviation scores, Pubertal stage assessment, bone age according to Greulich and Pyle method as well as mean serum ferritin in the last 2 years prior to the study and hormonal assay (Growth hormone, Gonadotropin-stimulating hormones both basal and after stimulation). Patients were compared to 40 healthy age and sex matched subjects served as controls.
Results: All studied patients had high seum ferritin (mean=3156.25± 740.38 µg/L) and they show significant reduction in their signal intensity of pituitary MRI compared to controls (<0.001). Moreover there was significant reduction of signal intensity in group 1 compared to group 2 (P<0.005). Negative correlation was found between pituitary signal intensity and mean serum ferritin (r = -0.78, P<0.001). Thalassemic patients with hypogonadism had significantly higher serum ferritin, older age, lower height and weight SDS with lower mean pituitary MRI signal intensity. Moreover thalassemia patients with hypogonadism had significantly lower LH and GH basal levels and after stimulation (P<0.05). A negative moderate correlation between pituitary MRI SI and age (r= -0.48, P=0.002). Best cut off value for MRI signal intensity was 369 with sensitivity of 87.5%, specificity 68 %, and diagnostic accuracy of 73%. o differentiate between patients with and without hypogonadism.
Summary / Conclusion: The use of pituitary MRI signal intensity is a useful noninvasive tool for detecting adenohypophyseal iron overload in patients with transfusional hemosidrosis and for predicting the pituitary gonadal dysfunction

P402

HBH CONSTANT SPRING DISEASE (HBHCS) HAS LOWER SERUM FERRITIN RELATIVE TO LIVER IRON CONCENTRATION (LIC) COMPARED TO DELETIONAL HBH DISEASE: IMPORTANCE OF LIC MEASUREMENT IN HBHCS

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Background: HbH Constant Spring disease (HBHCS) is a non-deletional HbH disease which generally has a more severe phenotype than deletional HbH disease (HBH) due to its greater ineffective erythropoiesis from alpha^{CS} chains accumulation within the erythroid precursors. Therefore, there may be differences in the correlation of serum ferritin (SF) with liver iron concentration (LIC) between HBHCS and HBH.
Aims: This retrospective study aims to investigate the difference in the correlation of SF with LIC between HBHCS and HBH.
Methods: T2* measurement by CMRtools is available to our patients (pts) as the mode of non-invasive liver iron assessment. We included all HBHCS and HBH pts within an adult haematology unit who ever had liver T2* and SF measured within 3 months of the T2*. Beta thalassemia intermedia (TI) pts were also selected for comparisons with the HBHCS pts as TI had been repeatedly shown in published studies to have lower than expected SF to LIC ratio. Pts with active hepatitis B or C and infections at the time of SF measurement were excluded. The pts selected had liver T2* and SF assessments prior to regular iron chelation. Liver T2* was converted to LIC (in mg/g dw) using calibrations previously derived by Garbowski M W *et al* [Reference: Calibration of Improved T2* Method for the Estimation of LIC in Transfusional Iron Overload. Blood (ASH Annual Meeting Abstracts), 2009; 114: 2004.]. Data on baseline characteristics and correlation of SF with LIC were retrospectively collected and compared between HBHCS and other types of thalassemia (HBH and TI). Statistical significance was taken as a two-tailed P-value of <0.05.
Results: 7 HBHCS, 13 HBH and 12 TI were included. No HBHCS and TI pts were transfusion dependent. Four HBH pts required intermittent transfusion for symptomatic anaemia while the rest did not. At the time of T2* HBHCS pts were similar in age (median 53 (18-57) years) as HBH pts (median 56 (41-78) years, P=0.251) but were significantly older than the TI pts (median 28 (18-61), P=0.035). Overall, 53% of pts were males and 25% had splenectomy. There were no significant differences in the proportion of males (P≥0.350) and splenectomy (P ≥0.270) between HBHCS and other types of thalassemia. SF was measured at a median of 17 (0-85) days within T2* assessment and were not significantly different between HBHCS, HBH and TI (P ≥ 0.663). There was a positive correlation between SF and LIC for HBHCS (R^2= 0.8, P=0.007), HBH (R^2= 0.6, P=0.001) and TI (R^2= 0.9, P=0.000). SF of HBHCS pts (median 1345 (904-1874) mcg/L) did not differ significantly from HBH pts (median 1278 (422-6322), P=0.663) and yet had significantly higher LIC (median 19.5 (6.7-30.7) mg/g dw) than HBH patients (median 9.1 (3.5-22.2) mg/g dw, P=0.022). TI pts have similar SF (1487 (299-4616) mcg/L, P =0.933) and LIC (median 18.4 (4.5-34.1) mg/g dw, P=0.966) as HBHCS pts. HBHCS pts had significantly lower SF to LIC ratio (median 65 (56-134) mcgL^{-1}/ mgg^{-1}dw) than HBH pts (median 125 (48-285) mcgL^{-1}/ mgg^{-1}dw, P=0.013). They had similar SF to LIC ratio as the TI pts (median 68 (26-136) mcgL^{-1}/mgg^{-1}dw, P=0.612). The difference in the correlation of SF with LIC between HBHCS and HBH was also reflected by the linear regression graphs which had significantly different slopes (P=0.013, Figure 1). We have shown that SF and LIC correlations are not uniform across all types of HbH disease. HBHCS has lower SF relative to LIC compared to HBH, and may be similar to non-transfusion dependent TI which has been well established to have lower than expected SF to LIC ratio. This may be explained by the greater ineffective erythropoiesis in HBHCS, resulting in more hepcidin suppression, iron absorption and iron release from the reticuloendothelial system, thereby leading to lowering of SF relative to LIC compared to HBH.
Summary / Conclusion: Iron load in HBHCS needs to be monitored differently from the more common HBH. SF may underestimate the degree of iron overload in HBHCS pts who need LIC measurement for more accurate total body iron load assessment, especially for decision-making on the initiation of iron chelation.

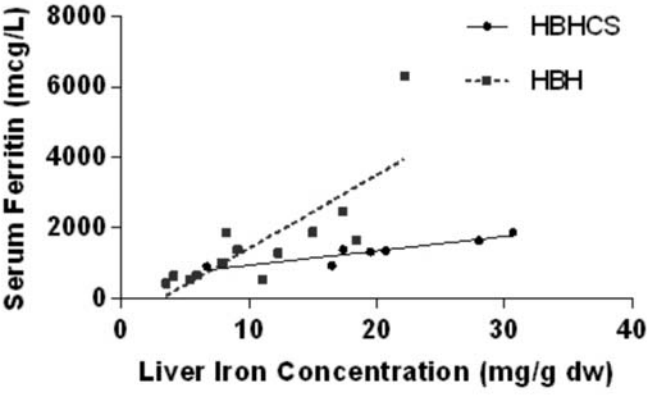

Figure 1. Serum ferritin vs liver iron concentration.

P403

AN AUTOMATIC METHOD FOR MYOCARDIAL T2* CURVE FITTING IN THALASSEMIA PATIENTS WITH SEVERE IRON OVERLOAD

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Background: Myocardial iron overload assessment by multislice multiecho T2* technique is used in the clinical management of thalassemia major (TM) patients. Signal decay curves are extracted from the 16 left ventricular (LV) segments and the fitting of these curves to a mono-exponential model provides the corresponding T2* values. In patients with severe cardiac iron overload, where signal will decay quickly becoming comparable to image noise, manual truncation of signal decay curves excluding later echo times (TEs) is adopted.
Aims: In this study an automatic truncation method avoiding the variability associated with the manual selection of the truncation point is introduced and validated.
Methods: Twenty patients (13 males, age 33±7 years) enrolled in the MIOT Network and diagnosed for severe iron overload (T2*<10 ms) were considered. Using a previously validated software the segmental T2* values were evaluated by the standard methodology (i.e. manual truncation). Images were independently analysed by the developed automated approach. The percentage fit-

ting error *e* was computed as the root mean square error between the signal decay curve and the mono-exponential model normalized to the mean value of the signal. If *e* was >5%, the algorithm cut-off the last TE and performed again the fitting. The procedure was iterated until the error become <5% or the number of TEs become equal to three. To assess the inter-operator variability, the dataset was processed by a second operator.

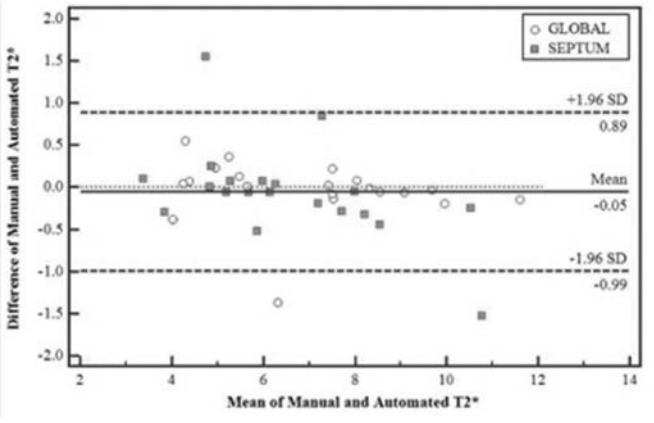

Figure 1.

Results: The Coefficient of Variability (CoV) for inter-observer variability was 6.82±4.01%. The CoV between automated and manual analysis was 6.15±3.92 %, not significantly different from inter-observer variability (P=0.332). No significant difference was detected between mid-septum and global T2* values evaluated with manual and automated procedure (P=0.26 and P=0.91, respectively). The figure shows the Bland-Altmann plots. The mean fitting error was not significantly different in manual and automated analysis (4.10±2.11 vs. 4.52±2.12, P=0.53). In segmental analysis, no significant differences were found between manual and automatic procedure (P>0.01 for all segments).
Summary / Conclusion: Truncation of signal decay curve needed to compensate for low signal in later echoes in patients with severe iron overload can be effectively automatized avoiding operator induced variability.

P404

MOVEMENT ABNORMALITIES IN THE LEFT VENTRICLE OF THALASSEMIA MAJOR PATIENTS

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Background: Movement abnormalities of the left ventricle (LV) have been reported in thalassemia major (TM) patients. Movement abnormalities can be detected through a qualitative analysis of cine MR images. Moreover, MR is the gold standard technique for the evaluation of myocardial iron overload (MIO), biventricular global systolic function and myocardial fibrosis.
Aims: The aim of this study was to investigate the relationships between movement abnormalities and MIO, left ventricular (LV) function and myocardial fibrosis.
Methods: CMR (1.5T) was performed in 1092 TM patients (537 male; 30.6±8.5 years) enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network. Cine SSFP images were acquired in vertical and horizontal long-axis, and in sequential 8-mm short-axis plans (gap 0 mm) from the atrio-ventricular ring to the apex. These sequence were used to evaluate the wall motion and to quantify LV volumes and ejection fraction (EF) by means of the MASS software in a standard way. For MIO assessment, three parallel short-axis views of the LV were acquired using a T2* GRE multi-echo sequence. To detect myocardial fibrosis, late gadolinium enhanced (LGE) images were acquired in the same views as used for cine images after the gadobutrol (1.0 mol/l) (0.2 mmol/kg) intravenous administration. During image analysis the 17-segment LV model of the standard AHA/ACC was taken into account. On the cine images, segmental wall motion was visually assessed by skilled observers (with at least 5 years of experience in CMR) and scored as 1=normal, 2=hypokinesia, 3=akinesia and 4=dyskinesia. The T2* value in all segments as well as the global value were calculated. Presence/absence of enhancing area was assessed for each segment.

Table 1.

	Patients with abnormal wall motion	Patients with normal wall motion	P-value
Age (years)	33.1 ± 8.3	30.4 ± 8.5	0.014
Sex (M/F)	37/29	500/526	0.248
Global Heart T2 (ms)*	22.5 ± 14.7	30.4 ± 8.5	0.001
N. of segments with T2 <20 ms*	8.6 ± 7.5	4.6 ± 6.1	<0.0001
Global heart T2 <20 ms, N (%)*	38 (57.6)	376 (36.6)	0.001
LV end-diastolic volume index (ml/m2)	99.0 ± 24.5	87.5 ± 18.9	<0.0001
LV end-systolic volume index (ml/m2)	49.3 ± 18.7	34.0 ± 11.6	<0.0001
LV mass index (g/m2)	66.3 ± 14.1	58.4 ± 13.3	<0.0001
LV ejection fraction (%)	51.1 ± 9.1	61.7 ± 6.6	<0.0001
LGE, N (%)	34 (51.5)	162 (15.8)	<0.0001

Results: Abnormal motion of LV was found in 66 (6%) patients: 60 were hypokinetic while 6 were dyskinetic. Our data demonstrated predominant involvement of wall motion abnormalities in the medium anterior, anterolateral and septal segments. Table 1 shows the comparison between patients with normal and abnormal motion. Patients with abnormal motion were significantly older, they had significantly lower global heart T2* value and a significantly higher number of segments with T2*<20 ms. Left volumes and mass indexed by body surface area were significantly higher in patients with abnormal motion while the EF was significantly lower. LGE areas were detected in 196 patients (18%) and they were predominantly located in the mid-ventricular septum. There was a significant correlation between the presence of enhancement and the abnormal motion.
Summary / Conclusion: Movement abnormalities in the left ventricle were not really frequent in TM patients but were associated with age, MIO, LV dilation and dysfunction, and myocardial fibrosis.

P405

CLINICAL CHARACTERISTICS AND EVOLUTION OF 78 DELAYED TRANSFUSION REACTION AMONG SICKLE CELL DISEASE PATIENTS

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Background: Patients with sickle cell disease (SCD) are exposed to delayed hemolytic transfusion reaction (DHTR), which is a life-threatening complication The diagnosis is based on the analysis of the post transfusional hemoglobin A level.
Aims: Our objective was to describe the clinical characteristics and outcome of DHTR episodes among adults with SCD.
Methods: We retrospectively analyzed 78 episodes of DHTR that have occurred in 54 patients with SCD (52 hemoglobin S/S, 1 S/C and 1 S/ ß) followed in our national referral center over a 12 years period.
Results: Transfusions were indicated to treat an acute complication such as an acute chest syndrome in 46.2% of the cases, or to avoid any complication (cerebral vasculopathy, during pregnancy..) in 53.8% of the cases (see table). Regarding the characteristics of the DHTR episodes, in 52 over 78 cases (66.6%), a secondary hospitalization after discharge was required, while the other patients stayed hospitalized beween the transfusion and the DHTR. The diagnosis was seldom made at the emergency department. The clinical presentation was a painful vaso-occlusive crisis in 75% of cases, an acute chest syndrome in 34% of cases. The median delay between transfusion and the first clinical sign of DHTR was 11 days [3-22]. The most frequent clinical manifestation was the presence of reddish urine (73/78). The mean lower hemoglobin level was 5.5 +/- 3.5 g/dl observed on average at 12 +/- 5 days after the transfusion. The mean highest level of lactico-dehydrogenase level was 1715 +/- 1283 UI/l at day 11+/-4. A management in intensive care unit was required in 34 (43%) cases and five patients died. In 30 cases (38%), another transfusion was decided, including 21 patients for whom the diagnosis of DHTR was not initially made. Five transfusions were decided because of multiple organ failure. The analysis of the hemoglobin A level revealed that 80% of the transfusions were ineffective at day 15. An allo-antibody was identified in 41% of cases. In term of management, recombining erythropoietin (Epo) either alone was used in 36

cases (46%) cases, intravenous immunoglobulin in 4 cases (combined with Epo in 2 cases), corticosteroids and Epo in 3 cases and rituximab + Epo in a single case.

Summary / Conclusion: DHTR is a severe and often misdiagnosed complication of transfusion in SCD patients with a high mortality rate. The indication for transfusion in patient with a history of DHTR must be highly restricted taking carefully into account the risk/benefit ratio. The early identification of DHTR is of major importance in order to rapidly start supportive care measures and to avoid any other harmful transfusion. Studies are ongoing to find the best therapeutic approach for DHTR.

Table 1.

Reason for transfusion	n
Acute complication	46
Acute chest syndrome	19
Vaso-occlusive crisis	15
Anemia	5
VOC during pregnancy	5
priapism	1
Stroke	1
Macular ischemia	1
Preventive transfusion	43
Before surgical treatment	18
Pregnancy	19
Anemia during chronic renal failure	4
Leg ulcers	2

Clinical sickle cell disease and other anemias

P406

RELATIVELY LOW MORTALITY OF SICKLE CELL PATIENTS WITH ELEVATED TRICUSPID REGURGITANT JET FLOW VELOCITY AFTER EXTENDED FOLLOW UP IN A DUTCH COHORT

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Background: Elevated tricuspid regurgitant jet flow velocity (TRV) is reported to be an independent risk factor for early death in patients with sickle cell disease (SCD) with mortality rates as high as 40% after a median follow up of 40 months. We previously reported a mortality rate of 8% in a Dutch cohort of 85 ambulatory sickle cell patients with an elevated TRV (≥2.5 m/s) using trans-thoracic echocardiography after a follow up of 53 months.

Aims: The aim of the current study was to determine the mortality rate after an extended follow-up period and to investigate the relation between baseline elevated TRV and mortality in this well described cohort.

Methods: After obtaining informed consent, consecutive patients with SCD (HbSS, HbSC, HbSβ^0 or HbSβ^+) were included in a prospective observational study and were followed by regular outpatient visits, including laboratory testing and repeated echocardiography. Baseline TRV, as well as laboratory values, were related to outcome. For statistical analysis, patients were divided in patients with relatively severe genotypes (HbSS/HbSβ^0-thalassemia) and patients with relatively milder genotypes (HbSC/HbSβ^+-thalassemia). The study protocol was approved by the local medical ethical committee.

Results: In 81 of 85 patients baseline echocardiography was performed. In 25 patients (31%) a TRV ≥ 2.5 m/s was measured (39% in HbSS/HbSβ^0-thal patients and 12% in HbSC/HbSβ^+-thal patients). A TRV >2.9 m/s was measured in 2 patients (both HbSS). Median follow-up for the whole group was 82 months (IQR: 75-85). Four patients were lost to follow-up, including 1 patient with a baseline TRV ≥ 2.5 m/s (2.65). During follow-up twelve patients (11 HbSS and 1 HbSβ^0-thal) died. The death rate for patients with a TRV ≥2,5 m/s was 20% versus 12.5% for patients with a TRV <2.5 m/s (Hazard Ratio of 1.6 (CI 0.5-5.2, P=0.4). In two of the patients who died without an elevated TRV at baseline, a TRV ≥2.5 m/s was measured during follow-up. Median age at death was 53 years (37-60) as compared to a median age of 34 (28-47) in survivors. NT-proBNP and BNP levels were significantly higher at baseline in patients who deceased (177 pg/mL; 77-871 and 179 pg/mL; 40-340) versus levels in survivors (52 pg/mL; 34-108, P<0.001 and 31 pg/mL; 17-71, P<0.001 respectively). Hazard ratio for patients with NT-proBNP ≥160 pg/mL at baseline was 10 (CI 2.9-34.4, P<0.001) versus patients with NT-proBNP levels <160 pg/mL at baseline. No difference in baseline HbF%, leukocyte count, or total bilirubin level was observed between survivors and non-survivors while haemoglobin concentration (P=0.005), LDH (P=0.017) and ferritin (P<0.001) differed significantly.

Summary / Conclusion: After a median follow-up of 82 months, we observed a mortality rate of 20% in patients with a TRV ≥2.5 m/s which, in contrast to previous studies, appeared not to be a risk factor for death in our study. NT-proBNP plasma level > 160 pg/mL appeared to be a strong risk factor for early death.

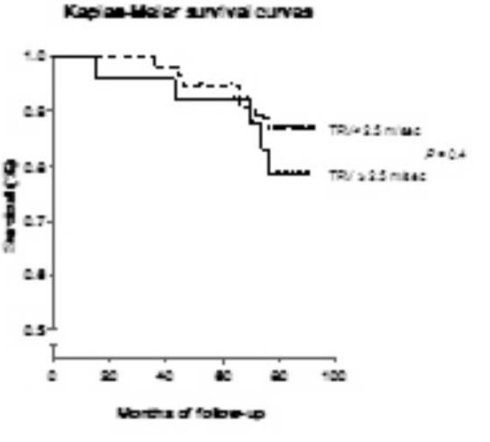

Figure 1.

P407

PRASUGREL IN CHILDREN WITH SICKLE CELL DISEASE: PHARMACOKINETIC AND PHARMACODYNAMIC CHARACTERISTICS FROM AN OPEN-LABEL, ADAPTIVE-DESIGN, DOSE-RANGING STUDY

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Background: There are few approved treatments for children with sickle cell disease (SCD) who experience painful vaso-occlusive crises (VOC). Evidence suggests a pathophysiologic role of platelets in VOC. Thrombocytosis is common and markers of platelet activation are elevated in SCD. Platelet activation is partially mediated by adenosine diphosphate (ADP) released as a result of inflammation induced by chronic hemolysis in SCD. Therefore, platelets are a possible therapeutic target to decrease the frequency and severity of VOC. We studied prasugrel, an irreversible $P2Y_{12}$ platelet ADP-receptor antagonist that reduces platelet reactivity and aggregation, in children with SCD.
Aims: The primary aim was to characterize the relationship between prasugrel dose, exposure to prasugrel active metabolite (Pras-AM), and platelet inhibition in children with SCD.
Methods: We conducted a two-part Phase2, open-label, multi-center, adaptive-design, dose-ranging, pharmacokinetic (PK) and pharmacodynamic (PD) study of prasugrel in children (2–17 years) with SCD (HbSS and $HbS\beta^0$-thalassemia genotypes). PD was assessed using vasodilator-associated stimulated phosphoprotein (VASP) and VerifyNow® (VN) P2Y12 assays. PK analysis of Pras-AM was performed by calculating area under the concentration-time curve. Part A: Patients received up to 3 single doses of prasugrel, separated by 14±4 days. Treatment was initiated with prasugrel doses expected to be sub-therapeutic, and doses were then modified based on PD responses to previous doses. Part B: Doses were administered once daily for 14±4 days. The initial dose was chosen to target ~30% platelet inhibition at steady state. The second dose was titrated up or down for each patient based on the response to the first dose.

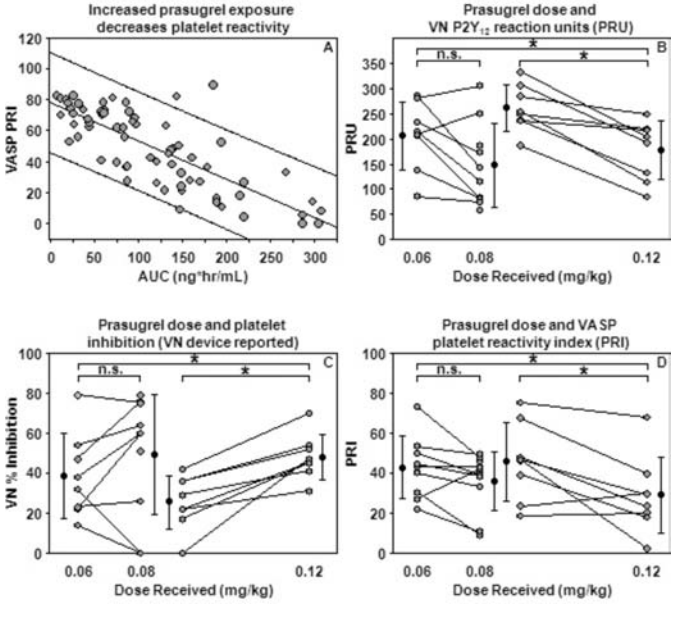

Figure 1. (A) regression analysis with 95% PI. (B,C,D) comparison with mixed model analysis for all treatments.

Results: Data were collected from a total of 24 patients in Part A and 18 patients in Part B. In Part A, a single-dose range of 0.30–0.50 mg/kg led to a VN $P2Y_{12}$ reaction units (PRU) of 197±96 (mean±SD), VASP platelet reactivity index (PRI) of 41±22, and platelet inhibition (VN device reported) of 41±27%. Pras-AM exposure increased with dose and correlated significantly with PRU (r=-0.72) and PRI (0.-78; Figure 1A). In Part B, first dose produced ~30–60% platelet inhibition for 6 patients (~33%), and over the 2 dosing periods with the titration strategy, no patient failed to meet the minimum PD response of 30% on the 0.12 mg/kg dose, and only 1 patient exceeded the maximum PD response of 60% on the lowest dose of 0.06 mg/kg. PRU and PRI were significantly decreased and platelet inhibition was significantly increased in patients given 0.12 mg/kg compared to 0.06 or 0.08 mg/kg (Figure 1B-D;* P<0.05). Overall, six serious adverse events related to SCD occurred in 4 patients. There were 3 mild hemorrhagic adverse events in Part B (epistaxis, eyelid bleeding, wound hemorrhage); 2 were possibly related to prasugrel. No patients discontinued study participation due to an adverse event.
Summary / Conclusion: Prasugrel appeared to be safe and well tolerated in this small patient sample. The trend toward higher exposure and platelet inhibition with increasing dose and variability in PD responses was generally consistent with previous studies in adult subjects. A majority of patients (11/18; 61%) were titrated to a prasugrel dose that resulted in 30–50% platelet inhibition. These results support the initiation of a Phase 3 trial designed to assess efficacy of prasugrel in reducing VOC in children with SCD.

P408

A MONOCENTRIC, PROSPECTIVE, OBSERVATIONAL STUDY ON VASO-OCCLUSIVE CRISIS (VOC) IN ADULT SICKLE-CELL DISEASE (SCD). CHARACTERISTICS AND PREDICTIVE SCORE OF SECONDARY ACUTE CHEST SYNDROME (PRESEV STUDY)

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Background: Vaso-Occlusive Crisis (VOC), the most common manifestation of SCD, is the first cause of death, particularly when complicated by an acute chest syndrome (ACS). However, no data are available on follow-up differences between patients hospitalized for uncomplicated VOC and those who will develop ACS. We report results on hospitalization for VOC from patients enrolled in the PRESEV study.
Aims: Our aims were to better characterize hospitalized VOC and find a predictive score of secondary ACS
Methods: This prospective, monocenter, observational trial included homozygous SCD patients, ≥18 years old with severe VOC requiring admission to our university hospital's Adult Sickle-Cell Referral Center. This study was conducted in accordance with the Declaration of Helsinki principles, Good Clinical Practice guidelines, and local laws and regulations. Severe VOC was defined as pain or tenderness, affecting at least 1 part of the body, e.g. limbs, ribs, sternum, head (skull), spine and/or pelvis, that required opioids and was not attributable to other causes. ACS was defined as the association of 2 criteria among chest pain, radiologic infiltrate and auscultatory abnormality. Patients could be enrolled in the trial more than once if their hospitalizations were separated by ≥1 months. Exclusion criteria were: ACS on day of inclusion, pregnancy, hospitalization for >24 h, chronic blood-exchange transfusion (BET), transfusion impossibility, severe complication requiring transfusion at admission, proven sepsis, surgery <15 days earlier. Patients were divided into 2 groups: VOC (without ACS) and those with secondary ACS; all were treated according to the French guidelines. Steady state was defined as a consultation ³1 months after an acute clinical event (VOC, infection, ACS, or any other clinical event requiring hospitalization and/or blood transfusion) and ³3 months after last blood transfusion. Results are expressed as mean±SD.
Results: 250 VOC were included. Among them, 18.8% developed a secondary ACS which appeared a mean of 3.2±2.75 days after admission. For the VOC and ACS groups, respectively, mean ages were comparable (30.8±7.1and 31.1±8 years), as were F/M sex ratios (1 and 1.18). The percentage of patients treated with hydroxyurea at inclusion in the two groups was similar (31.5% in VOC group, 38.3% patients in ACS group). Lengths of hospitalization were respectively 5.24±3.9 and 9.98±5.8 for the VOC and ACS groups. BET was required in 2.5% VOC and 44.6% ACS, according to French guidelines. Patients were readmitted within 2 weeks after hospitalization discharge in 12.3% VOC and 10.6% ACS (6.9±3.4 and 8.8±2.9 days respectively). No patient died. The multivariate analysis provided a predictive score for ACS at admission with a negative predictive value of 94.6%.This composite score includes one clinical and two easily available biological parameters.
Summary / Conclusion: This observational prospective trial on adult SCD patients gives new insights in ACS incidence during hospitalization for VOC and provides for the first time a predictive score for secondary ACS. This score should be to validate in an international study.

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FINAL RESULTS FROM THE MULTICENTER COMPACT STUDY OF COMPLICATIONS IN PATIENTS WITH SICKLE CELL DISEASE AND UTILIZATION OF IRON CHELATION THERAPY: A RETROSPECTIVE MEDICAL RECORDS REVIEW

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Background: The life expectancy of individuals with SCD is increasing. As these individuals age, they are at risk of developing significant morbidities and SCD-related complications. Throughout their course of care, individuals with SCD may receive acute, episodic and chronic blood transfusions, which may result in multi-organ iron burden. Substandard treatment of iron toxicity is directly related to increased morbidities and complications in all age groups. Early detection and appropriate intervention may prevent or delay the onset of damaging health outcomes associated with iron overload.
Aims: Describe the SCD complication rates, blood transfusion patterns, iron chelation therapy (ICT) use, and associated resource utilization in SCD patients ≥16.
Methods: Medical records of SCD patients ≥16 were retrospectively reviewed between 08/2011 and 07/2012 at three US tertiary care centers. Patients were observed from their first visit after age 16 until the earliest of death, loss to follow-up, or last patient record. Three patient cohorts were defined based on cumulative units of blood received and ICT history: <15 units of blood and no ICT (Cohort 1 [C1]), ≥15 units of blood and no ICT (Cohort 2 [C2]), and ≥15 units of blood and ICT (Cohort 3 [C3]). SCD complications recorded from patient charts per patient per year (PPPY) were reported and compared among cohorts using rate ratios (RRs). For the young adult subset, only complications and resource utilization observed between ages 16 and 30 were analyzed.
Results: Cohorts1,2, and 3 consisted of 69, 91, and 94 patients, respectively. Mean (range) age at index date was similar across cohorts (27 years [16-65]). Mean length of observation was shorter among patients in C1 (years, C1: 6.6; C2: 8.2; C3: 8.1). The rate (95% CI) of any SCD complications PPPY was highest in C2: 3.02 (2.89-3.14), followed by C3: 2.26 (2.16-2.37), then C1: 1.66 (1.54-1.77). For all patients, pain was the most frequent SCD complication (74%) and the most common reason for IP (76%) and ER (82%) SCD complication related visits. Among transfused patients (C2, C3), those not receiving ICT were more likely to experience any SCD complication (RR [95% CI] C2 vs. C3: 1.33 [1.25-1.42]), and pain (RR [95% CI] C2 vs. C3: 1.55 [1.44-1.68]) than those who did. Similar trends were observed in ER and IP visits associated with any SCD complications (RR [95% CI], C2 vs. C3, ER: 1.94 [1.70-2.21]; IP: 1.61 [1.45-1.78]) and pain (RR [95% CI], C2 vs. C3, ER: 2.05 [1.78-2.37]; IP: 1.89 [1.67-2.13]), but not in outpatient visits. These trends were more pronounced in young adults (any SCD complications: RR [95% CI], C2 vs. C3, ER: 2.45 [2.10-2.87]; IP: 1.93 [1.69-2.21]; pain: RR [95% CI], C2 vs. C3, ER: 2.48 [2.08-2.97]; IP: 2.14 [1.82-2.50]). Similarly, pain was the most frequent SCD complication (71%), and the most common reason for ER (80%) and IP (77%) SCD complication related visits; followed by infections [ER (8.3%), IP (7.0%)].
Summary / Conclusion: SCD complication rates, including pain, and associated IP and ER visits were higher among young adult transfused (C2, C3) SCD patients. Among transfused patients, those receiving ICT were less likely to experience complications than those without ICT. This trend, pronounced in young adults, underscores the necessity to avoid an interruption in the management of care as they transition from a pediatric to an adult doctor. Our results reinforce the importance of screening and treatment for iron overload in transfused individuals.

Table 1. Rate and rate ratio of SCD complications by type of setting.

	All Patients			Rate Ratio (RR) – Cohort 2 vs. Cohort 3				
				All Complications		Pain Only		
	Cohort 1 n = 69	Cohort 2 n = 91	Cohort 3 n = 94	All Patients	Patients 16–30	All Patients	Patients 16–30
Total SCD Complications								
Mean PPPY ± SD	11 ± 13	25 ± 53	18 ± 38	RR	1.33	1.63	1.55	1.89
Median PPPY [range]	8 [0 - 61]	7 [0 - 308]	4 [0 - 225]	[95% CI]	[1.25 - 1.42]	[1.50 - 1.78]	[1.44 - 1.68]	[1.65 - 2.08]
Rate PPPY [95% CI]	1.66 [1.54 - 1.77]	3.02 [2.89 - 3.14]	2.26 [2.16 - 2.37]					
Outpatient/Clinic								
Mean PPPY ± SD	4 ± 6	7 ± 14	6 ± 16	RR	1.01	0.85	1.00	0.89
Median PPPY [range]	2 [0 - 34]	1 [0 - 85]	1 [0 - 99]	[95% CI]	[0.90 - 1.13]	[0.70 - 1.02]	[0.86 - 1.16]	[0.67 - 1.17]
Rate PPPY [95% CI]	0.65 [0.57 - 0.72]	0.80 [0.74 - 0.87]	0.80 [0.73 - 0.86]					
Hospitalization								
Mean PPPY ± SD	3 ± 4	10 ± 22	6 ± 13	RR	1.61	1.93	1.89	2.14
Median PPPY [range]	1 [0 - 19]	2 [0 - 135]	1 [0 - 81]	[95% CI]	[1.45 - 1.78]	[1.69 - 2.21]	[1.67 - 2.13]	[1.82 - 2.50]
Rate PPPY [95% CI]	0.43 [0.36 - 0.49]	1.22 [1.14 - 1.30]	0.76 [0.70 - 0.82]					
Emergency Room								
Mean PPPY ± SD	3 ± 6	7 ± 23	4 ± 13	RR	1.94	2.45	2.05	2.48
Median PPPY [range]	1 [0 - 29]	0 [0 - 154]	0 [0 - 85]	[95% CI]	[1.70 - 2.21]	[2.10 - 2.87]	[1.78 - 2.37]	[2.08 - 2.97]
Rate PPPY [95% CI]	0.51 [0.45 - 0.58]	0.88 [0.82 - 0.95]	0.46 [0.41 - 0.52]					

Notes:
IP = Per Patient.
PPPY = Per Patient Per Year.
Cohort 1: Patients who have received < 15 units of blood in their lifetime and have not received ICT treatment in their lifetime.
Cohort 2: Patients who have received ≥ 15 units of blood in their lifetime and have not received ICT treatment in their lifetime.
Cohort 3: Patients who have received ≥ 15 units of blood in their lifetime and have received ICT treatment in their lifetime.

P410

NEWBORN SCREENING FOR SICKLE CELL DISEASE IN BRUSSELS, A PROGRAM WITH AN ONGOING CLINICAL OUTCOME IMPROVEMENT

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Background: Early identification of sickle cell disease (SCD) by newborn-screening (NS) is well established to be an efficient and practical tool in enhancing the health care of affected patients with SCD.
Aims: The aim of our study, conducted in Brussels Region, was to assess whether there is an ongoing improvement of clinical outcome of children with SCD detected by the NS program.
Methods: Universal NS was progressively implemented in Brussels starting within a few maternity wards in 1994 and extending to all maternity wards in 2000. Children identified with SCD progressively benefited from comprehensive expert medical care in three dedicated reference centers. Care included education, prevention, emergency and specific out-patient and in-patient treatments. To evaluate the improvement in comprehensive care, we reviewed data of children born from January 1st 2000 to December 31st 2003 (group A) and from January 1st 2005 to December 31st 2008 (group B). All data were recorded from January 1st 2000 to December 31st 2005 for group A and from January 1st 2005 to December 31st 2010 for group B. Both groups had the same follow-up period accounting for 118 patient-years in group A and 259 patient-years in group B. Median follow-up was 3.5 yrs (range 2.06-5.83 yrs) and 4.1 yrs (range 2.08-5.96 yrs) in group A and B respectively. The major events such as septicemia, anemia, dactylitis, vaso-occlusive event (VOC), acute chest syndrome (ACS), symptomatic neurological events and hospital days were reviewed and compared during the study follow-up between the two groups. The reasons for hospitalization that were selected were: septicemia, pneumonia, urinary tract infection, osteomyelitis, gastroenteritis, VOC crisis, dactylitis, ACS, acute splenic sequestration, aplastic episodes and neurologic events. Several biological parameters were also reviewed.
Results: Among the 98 patients identified with SCD at birth, 33 (16 girls and 17 boys) and 65 (37 girls and 28 boys) belonged to group A and B, respectively. In group A, 25 children were HbSS, 2 HbSβ$^+$ and 6 HbSC. In group B, 53 were HbSS, 5 HbSβ°, 5 HbSβ$^+$ and 2 had an other genotype. Most of the patients developed at least one major adverse event during the study period. The proportion of patients having presented severe anemia and acute chest syndrome was significantly lower in group B than in group A. The higher rate of septicemia in group A could be due to the delayed implementation of national vaccination for *Streptococcus pneumonia* or to the poor prophylactic penicillin compliance. No difference was observed between both groups for dactylitis, VOC and clinical neurological event. No patient died during the study period. Hematological parameters at one year of age were not different between both groups.
Summary / Conclusion: In conclusion, newborn screening is obviously recognized as a precious tool to identify patients with SCD. However, it must be part of a comprehensive care program. Our results demonstrated that its sustained effectiveness is really and clearly proven when it is coupled with a comprehensive and dedicated treatment program including close and regular parent education. This ongoing assessment should be performed to monitor and improve the screening program. Thereby the progressively implementation of comprehensive care has improved over time the quality of SCD management and then the outcome of patients in Brussels Region.

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ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE INTRONE4 VNTR POLYMORPHISM IN SICKLE CELL DISEASE AND TRANSFUSION-DEPENDENT B-THALASSEMIA MAJOR: RELATION TO CARDIO-VASCULAR COMPLICATIONS

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Background: Impaired nitric oxide (NO) bioavailability represents the central feature of endothelial dysfunction, and is a common denominator in the pathogenesis of vasculopathy in sickle cell disease (SCD) and thalassemia. Endothelial NO synthase (eNOS), an enzyme that generates NO, is encoded by a gene located on chromosome 7q35-36 and expressed constitutively by vascular endothelium. Some evidence indicates the contribution of 4a allele of the eNOS gene to cardiac disease. However, eNOS gene polymorphism has not been explored in SCD or thalassemia.
Aims: We aimed to study the 27 base pair tandem repeat polymorphism in intron4 of eNOS gene in young patients with SCD and β-thalassemia major patients and to assess its potential relation to cardio-vascular complications.

Methods: The study is a cross sectional study done in the Pediatric Hematology Unit , Children's Hospital,Ain Shams University,Cairo,Egypt.It included 30 patients with SCD and 50 transfusion-dependentβ-thalassemia major patients aged 12-18 years,compared with 60 age- and sex-matched healthy controls.Data were collected including: transfusion history, splenectomy, sickling crisis, thrombotic events, chelation/hydroxyurea therapy.Investigations included hematological profile ,serum ferritin, study the 27 base pair tandem repeat polymorphism in intron4 of eNOS gene by polymerase chain reaction , as well as Doppler echocardiography and assessment of carotid intima media thickness by Doppler ultrasound.
Results: SCD patients had significantly higher frequency of aa genotype and eNOS4a allele compared with healthy controls (P<0.001). We found that 13.5% of SCD patients had history of cerebral thrombosis, 35% had pulmonary hypertension defined as pulmonary artery pressure ≥25 mm Hg and 15% had cardiomyopathy defined as cardiac functional impairment with ejection fraction ≤50%. The frequency of the eNOS4a allele (aa and ab) was significantly higher in SCD patients with history of cerebral thrombosis, pulmonary hypertension or cardiomyopathy compared with bb genotype (P<0.001). SCD patients with frequent history of sickling crisis (≥5 attacks in the last year prior to the study) showed higher frequencies of the eNOS4a allele(P<0.05). In β-thalassemia, 29% of patients had pulmonary hypertension and 33% had cardiomyopathy. β-thalassemia patients had significantly higher frequency of aa genotype and eNOS4a allele compared with healthy controls (P<0.001) and the frequency of the eNOS4a allele (aa and ab) was significantly higher in those with pulmonary hypertension and cardiomyopathy compared with bb genotype (P<0.001). There was no significant difference between the SCD and thalassemia patients as regards aa genotype (P>0.05).
Summary / Conclusion: We suggest that eNOS intron4 gene polymorphism is potentially related to endothelial dysfunction and cardiovascular complications observed in SCD and β-thalassemia patients .Its value as a possible genetic marker for prediction of increased susceptibility to cardio-vascular diseases in those patients needs further confirmation by large longitudinal studies.

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ANTENATAL SCREENING PROGRAMME FOR HAEMOGLOBINOPATHY IN A LOW PREVALENCE AREA: IS IT EFFECTIVE?

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Background: In England Sickle cell & thalassaemia are among most commonly inherited serious genetic disorders. Recent data confirms that in England, Sickle cell disease affects more people than Cystic Fibrosis. To reduce the burden of the disease, the National Health Service plan in England published in 2000 recommended implementation of a linked antenatal and newborn haemoglobinopathy and sickle cell disease screening programme by 2004. The NHS Sickle Cell and Thalassaemia Screening Programme were set up as a consequence of this policy statement to target offering antenatal screening by 10 weeks' gestation. Antenatal screening is intended to identify pregnancies that are at risk of an affected fetus. If the mother is identified as a carrier, testing is offered to her partner, with a view to offering prenatal diagnosis (PND). The recommendations depend on whether the antenatal unit is in a high or low prevalence area. For low prevalence areas (fetal prevalence of sickle cell disease <1· 5 per 10 000 pregnancies), screening is based on determining the family origin of the woman and her partner.
Aims: The aim of our analysis was to confirm the effectiveness and to identify deficiencies of the current antenatal screening programme.
Methods: This was a screening test evaluation study of a group of patients undergoing a selective antenatal screening programme, which was conducted at East Kent Hospitals NHS Foundation Trust. The Trust serves a population of around 750,000 in Kent and has a well-established haematology department providing a selective antenatal screening programme. East Kent has a low prevalence of residents from ethnic minorities and within this population there were approximately 7225 births/year, and according to the 2011 census 11.6% of the population were from ethnic minorities. We retrospectively analysed data from 49,700 antenatal patient samples booked between June 2007 and February 2013. As per the BCSH guidelines all women had a family origin questionnaire for her and the baby's father along with a full blood count at booking. All samples were analysed with a full blood count and red cell indices as an initial screening test for thalassaemia. Haemoglobin variants were analysed in those in whom red cell indices were abnormal (MCH <27 pg) or based on their family origin (confined to those women whose own or the baby's father's family origin is not Northern European or is unknown). The initial test used in the lab was HPLC and if an abnormal variant was identified samples were sent to Kings College Hospital (KCH) for confirmation.
Results: Out of 49,700 antenatal patient samples, 10,974 samples were identified for screening. Of these, nine women refused screening and 10,965 samples were analysed. 124 women had a significant haemoglobinopathy. Thirty of them were diagnosed with Beta thalassaemia trait and thirty seven women were diagnosed with possible alpha 0 thalassaemia from high risk ethnic groups; thirty two women were diagnosed with sickle cell trait (AS), twelve women were diagnosed with suspected HbD Punjab trait, six women diagnosed with HbC trait and seven with HbE trait. Hospital notes were available for analysis in 119/124 women, and sufficient documents could not be found in 5/124. 111/119 partners underwent testing, 8/119 had no partner result as the partners either moved to a different area or a different country, no longer with the partner or refusal to disclose partner's details. Seven couples had results indicating the fetus was at risk of a major haemoglobinopathy. Three accepted pre natal diagnosis which confirmed that the fetus was not at a risk of a significant haemoglobinopathy. Two women did not have PND due to late booking, one woman declined PND and one woman had moved to another area. No follow up data were available for these four women.
Summary / Conclusion: This data confirms that we fail to targ*et all* the screen-positive women and fail to implement follow up. In addition we doubt the value of a screening programme in a low prevalence area as only 3 out of 10,965 of women screened had PND of which none had a significant haemoglobinopathy.

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FG4592, A NOVEL INHIBITOR OF THE PROLYL HYDROXYLASE OF HYPOXIA-INDUCIBLE FACTOR (HIF-PH) ELICITED LINEAR-EXPONENTIAL DOSE-RESPONSE PROFILE ON PLASMA ERYTHROPOIETIN (EPO) LEVELS

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Background: Hypoxia stimulates hemoglobin (HgB) production. FG4592 is a HIF-PH inhibitor that simulates hypoxia and is being developed to treat anemia associated with chronic kidney disease (CKD).
Aims: Two studies were performed in Chinese healthy male/female volunteers to investigate the pharmacokinetics (PK) and pharmacodynamics (PD) of FG4592 after single dose (study A) and repeated doses (study B).
Methods: Both were double-blind, randomized, placebo-controlled studies. After provision of informed consent, four cohorts of 10 subjects received single oral doses of FG4592 (40 mg, 100 mg, 160 mg, and 200 mg) or placebo in an 8:2 ratio in study A; three cohorts of 15 subjects received FG4592 (40 mg, 160 mg, or 200 mg) or placebo thrice-weekly (TIW) for two weeks in a ratio of 13:2 in study B. Plasma and urine samples were collected for bioassays of FG4592 and EPO after dosing in study A and following the first and the sixth doses in Study B. Safety and laboratory measures such as hemoglobin (HgB) were evaluated throughout each study.

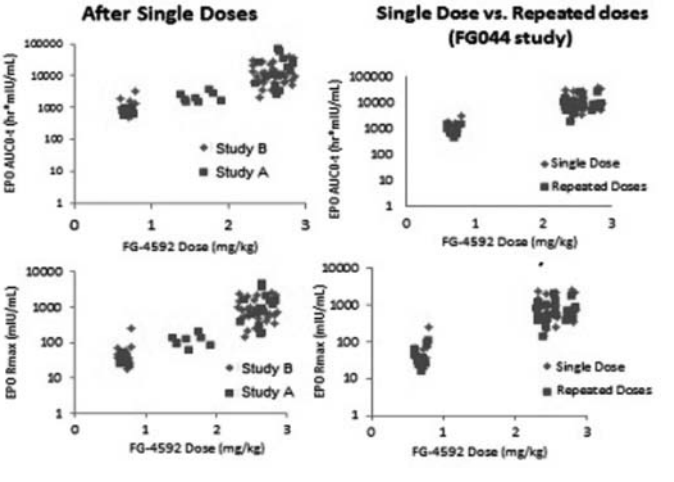

Figure 1. Semi-log-linear plotting between EPO exposure parameters and weight-adjusted FG4592 dosed by dose and dosing regimens, respectively.

Results: FG4592 reached a mean±standard deviation peak plasma concentration (C_{max}, μg/mL) of 3.48±0.81 (40 mg, N=21), 8.11±1.65 (100 mg, N=8), 13.65±2.93 (160 mg, N=21), and 14.81±2.65 (200 mg, N=21) within a median time of 2-3 hours after oral administration and had a mean elimination half-life of 7.4-8.5 hours among the groups. The plasma exposure of FG4592 increased

dose-proportionally from 40 mg to 200 mg (point estimate (90% confidence intervals)) for both AUC_{0-t} (0.936 (0.828,1.045)) and C_{max} (0.938(0.847,1.028)). TIW dosing in Study B did not lead to significant accumulation. The average urinary -recovery of FG-4592 was 5.14% (for 160 mg) to 6.22% (for 40 mg) of the administered dose in study A. Plasma EPO levels rose to maximal increments from baseline (R_{max}, mIU/mL: 40mg: 34.1±9.7; 100mg: 119.0±45.3; 160mg: 615.0±475.1; 200mg: 2110.0±1341.9; placebo: 18.1±6.4) at 16:00hr to 20:00hr of the day after either FG4592 or placebo and returned to baseline by hour 48. The exposures of EPO (AUC_{0-48h}, hr*mIU/mL) increased with doses of FG4592 (40 mg, N=21: 1019.7±619.8; 100 mg, N=8: 2103.7±726.1; 160 mg, N=21: 11390±8310; 200 mg, N=21: 20729±16918) and were all higher than that after placebo (N=14: 622.6±200.2). Plotting of EPO responses against weight-adjusted FG4592 doses suggested an exponential-linear relationship (Figure 1). The differences of EPO response between single dose and multiple doses seemed minimal in the semi-log-linear graphs although some subjects had lower EPO responses after repeated doses of FG4592. In Study B, overall HgB changes from baselines were significantly more positive with FG4592 160 mg (P=0.054) and 200 mg (P=0.034) compared to placebo between day 8 and day 21. A number of 6 (incidence, 46.2%), 4 (30.8%), 2 (15.4%) and 1 (16.6%) subjects had at least two consecutive higher-than-baseline post-dose HgB measurements in the group of 160mg, 200mg, 40mg FG4592 and placebo, respectively. FG4592 was safe and well tolerated in both studies.

Summary / Conclusion: Oral doses of FG4592 between 40mg and 200mg resulted in linear pharmacokinetics and an exponential-linear EPO-dose profile. FG4592 appeared to be safe and induced slight increase of HgB with short-term dosing in non-anemic healthy volunteers. These results support further exploration of the clinical effects of FG4592 in patients with CKD.

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THE NATURAL HISTORY OF COLD AGGLUTININ DISEASE

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Background: Cold agglutinin disease (CAD) is a rare and poorly understood disorder accounting for 15% of patients with autoimmune hemolytic anemia. Treatment has been controversial with few studies addressing safety and efficacy of various treatment regimens.

Aims: This study aimed to elucidate the clinical features, prognosis and management of cold agglutinin disease.

Methods: A retrospective analysis of Mayo Clinic medical records since 1970 was performed to identify all cases of CAD. Initial appraisal identified 89 patients after which an in depth review of clinical notes, laboratory evaluations, and treatment regimens was performed. Statistical analysis was performed via descriptive statistics and Kaplan-Meier survival.

Results: Eighty-nine patients with cold agglutinin disease were identified. The median age at symptom onset was 65 years (range, 41-83 years), whereas the median age at diagnosis was 72 years (range, 43-91 years). Median survival of all patients was 10.6 years, and 68 patients (76%) were alive 5 years after the diagnosis. The most common symptom was acrocyanosis (44%), and many had symptoms triggered by cold (39%) or other factors (22%). The most common disorder identified was monoclonal gammopathy of undetermined significance in 49%. Thirty-six patients (40%) received red blood cell transfusions during their disease course and 82% received drug therapy. Seventy-three patients (82%) required treatment for CAD and the median time from diagnosis to treatment was 11.4 months (range, 0-375 months). Treatment characteristics and responses are displayed in Table 1.

Table 1.

Characteristic	Single Agent Prednisone	Rituximab	Purine Analog	Alkylating Agent
Patients treated	24	44	8	37
Duration of therapy, median (range), mo	3.0 (0.1-13.9)	1.0 (0.1-107.7)	4.0 (1.0-24.5)	3.2 (0.0-46.4)
Tolerated therapy, (%)	83	92	90	83
Confirmed response to therapy, (%)	42	79	63	46
Duration of response, median (range), mo	51.6 (7.0-210.8)	24.0 (1.0-135.6)	18.5 (12.0-41.7)	11.3 (0.4-146.8)
Further treatment required, (%)	69	55	60	68

Summary / Conclusion: This is the largest study of patients with cold agglutinin disease to date. Symptoms were frequently ill-defined resulting in delay of diagnosis. Although drug therapy was frequently indicated, many patients were successfully observed without treatment. New treatment agents including Rituximab demonstrate promising response rates higher than seen with corticosteroids, especially in patients with underlying hematologic abnormalities. These results support consideration of CAD as part of the differential diagnosis in the setting of new onset anemia and re-enforces the importance of evaluation for underlying B Cell abnormality in this patient population.

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DEVELOPMENT OF A LABORATORY PATHWAY TO ASSIST THE DIAGNOSIS OF HEREDITARY HAEMOCHROMATOSIS IN PRIMARY CARE

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Background: Hereditary Haemochromatosis (HH) is a common, treatable condition. Large population studies have determined global distribution of the C282Y and H63D mutations with highest prevelance in North European populations: 0.5% C282Y homozygotes. Clinical penetrance, defined by biochemical evidence of iron overload, is around 50%. HH is however poorly recognised and there is a need to improve the detection of clinically relevant HH. The aim of this study was to develop a hospital laboratory pathway to improve the diagnosis in Primary Care. Serum ferritin (SF) is a reliable marker of body iron stores and is sensitive to iron overload in HH. Greater Glasgow and Clyde (GG&C) is the largest Health Board in Scotland, annually processing some 70,000 SF samples from Primary Care. These samples provided the study population.

Aims: The aim of this study was to utilise samples sent from Primary Care to develop a hospital laboratory pathway to improve the diagnosis of HH.

Methods: Samples were recruited from laboratories across GG&C from Jan 2011 to August 2012. Samples were selected from patients aged ≥30years with serum ferritin >200µmol/l (normal range 20-300µmol/l). Transferrin saturation was performed on all samples. HFE genotyping was carried out on samples with Tsat >30%.

Results: At final analysis, 3734 samples had been recruited. Males n=1657 (44%), median values; age 63yrs, SF 578 µmol/L, Tsat 36%. Females n=2077 (56%), median values; age 68yrs, SF 421 µmol/L, Tsat 31%. 1745 samples had Tsat >30% and were submitted for HFE genotyping.134 patients were homozygous C282Y (7.7% of those genotyped). Males; 878 samples were genotyped. 594 (68%) Tsat >30-50%; 284 (32%)Tsat >50%. 58 C282Y homozygotes were detected. 52 (90%) of these C282Y homozygotes had Tsat >50%. The detection rate was 18.3% (52/284) for patients with Tsat >50% and 1% (6/594) for Tsat >30-50%. 98.3% of C282Y homozygotes had SF >300 µmol/l. Females; 867 samples were genotyped. 657 (76%) Tsat >30-50%; 210 (24%) Tsat >50%. 74 C282Y homozygotes were detected. 57 (77%) of these C282Y homozygotes had Tsat >50%. The detection rate was 27.1% (57/210) for patients with Tsat >50% and 2.6% (17/657) for Tsat >30-50%. The detection rate was 5% (11/205) for Tsat >40-50% and 1% (6/452) for Tsat >30-40%. The detection rate is 16.3% (68/415) for Tsat >40%. 28% of C282Y homozygotes had SF 200-299 µmol/L and 78% had SF >300 µmol/L. Serum Ferritin; 1180 patients (31.6%) had SF 200-299 µmol/L. 148 males (12.5%) and 1032 females (87.5%). 22 C282Y homozygotes were detected in this group; 1 male and 21 females. 150 (39%) female patients with SF 200-299µmol/l had Tsat >40%. 21 C282Y homozygotes were detected in this group. Detection rate 14% (21/150).

Summary / Conclusion: 807 male patients had SF ≥300 µmol/L. HFE genotyping of samples with Tsat >50% revealed 18.3% C282Y homozygotes i.e 1 in 5.5 patients genotyped was affected; a 73 fold enrichment over population screening (0.5% C282Y homozygotes with 50% clinical penetrance). In female patients with SF ≥200 µmol/L and Tsat >40% the detection rate was 14% (56 fold enrichment).We would now propose: The standard normal range for SF in female patients should not exceed 200 µmol/L. The large number of SF requests sent from Primary Care can be utilised to improve the diagnosis of HH. The following algorithm should be applied to samples sent from Primary Care in areas with a North European population. Patients ≥30yrs; females SF >200 µmol/, males SF >300µmol/l should have iron studies performed. If Tsat >40% (females) or Tsat 50% (males) samples should be referred for HFE genotyping.

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DIRECT AGE EFFECT ON B12 DEFICIENCY IN HOSPITALIZED PATIENTS: A SINGLE INSTITUTION STUDY ON 14.904 SAMPLES.

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Background: B12 deficiency was considered as a rare pathology for a long

time, but more recently, the recognition of "middle" deficiencies with low-intermediate range of Cobalamin concentrations in the blood individualized a more frequent disorder. The high proportion of such B12 deficiency found in several studies performed in outpatients underlines a public health problem. However, identification of such deficiencies in large cohorts of hospitalized patients as well as correlations with other blood parameters just like hemoglobin level, MCV, homocystein or folate concentrations have not yet been reported.
Aims: The aims of this study were to screen a large cohort of inpatients for which B12 concentration measurement was performed in order to i-analyze population distribution according to age and the different biological values, ii-evaluate potential correlation between B12 and homocystein concentrations, iii-compare subjects according to B12 and folate profiles and iv-compare biological values distributions and metabolic profiles according to age.
Methods: The studied population included all consecutive patients admitted from January 1st2011 to December 31th2011 in 5 hospitals and who had centralized serum B12 concentration measurement during this period. Duplicate blood measurements for a given patient were excluded, thus the final analytic sample consisted of 14904 measurements of different subjects.
Results: Patients were aged 70.3+/-19.5 years. Low B12 concentration (<200 ng/L) was observed in 4.6% of cases, 24.2% had middle B12 (200 to 350 ng/L), 11.4% were true B12 deficient (B12<350ng/L associated to tHcy>17 microM/L), 20.4% had low folate concentration (folate<4 microg/L), 9.8% were true folate deficient (folate<4 microg/L associated to tHcy>17 microM/L) and 4.1% of patients were both B12 and folate deficient. Anemia and macrocytosis were not predictive factors of B12 deficiency. Significant increase in MCV and tHcy concentrations with age and decrease in B12, folate and hemoglobin levels with age were observed. At least, frequencies of true B12 deficiency and patients with low B12 concentration without true cellular deficiency increased significantly with age ranging respectively from 9.61% (patients aged 30 to 60 years) to 14.19% in patients over 90 years (P<0.05) and from 3.44% to 6.38% respectively (P<0.05).
Summary / Conclusion: This study demonstrates clearly an increase in true B12 deficiency according to age and demonstrates also that this increase is directly correlated to age decade. We also found a high rate of double deficiency (B12+Folates). It also suggest that B12 (or folates) deficiencies have a direct impact on MCV but not in hemoglobin level for a long period of time. It also confirm that about half the low B12 concentrations measured in routine practice are not "true" B12 deficiency since tHcy is normal but correspond probably to transcobalamin I deficiency. Moreover, we found that this sub-group of possible transcobalamin I deficient patients (B12<200 ng/L associated to normal tHcy level) was stable with age after 30 years. This suggests that tHcy measurement in clinical practice could be used only in the context of low B12 measurement to confirm the cellular deficiency and eliminate transcobalamin I deficiency that do not need to be treated with B12 supplementation.

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EPOETIN BIOSIMILARS IN THE MANAGEMENT OF CHEMOTHERAPY-INDUCED ANAEMIA (CIA) IN PATIENTS WITH LYMPHOMA AND MYELOMA: A SUBANLYSIS OF THE ORHEO STUDY

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Background: Chemotherapy may induce anaemia with potentially serious consequences, leading to discontinuation or interruption of the chemotherapy agents. The treatment of these CIA requires transfusion or epoetin administration. The ORHEO (place of biOsimilaRs in the therapeutic management of anaemia secondary to chemotherapy in HaEmatology and Oncology) study examined the post-marketing efficacy and safety of epoetin alpha biosimilars for the treatment of CIA.
Aims: To evaluate the efficacy and safety of epoetin alfa biosimilars (EAB) for the treatment of CIA in oncology and haematology in the clinical setting.
Methods: ORHEO was a French observational, prospective, multicentric study. CIA (Hb <110g/l) patients (pts), >18 years old, with solid tumors, lymphomas or myelomas and eligible for Epoetin Alpha Biosimilars (EAB) treatment were included to receive EAB according to drugs approval recommendations. Baseline patient characteristics and anaemia-related data including baseline Hb level, target Hb level, prescribed EAB brand and dose, any other concomitant treatments prescribed, were recorded. The primary study endpoint was the rate of responders (defined as increase in Hb levels to 100 g/L or at least 10 g/L since inclusion visit, or reaching target Hb level set by the physicians at start of study, without any blood transfusions in the 3 weeks prior to measurement) at +3 months (M+3). Other endpoints included rate of responders at +6 months (M+6) and safety (NCI-CTC V2.0) evaluation. A total of 2310 patients were included in the study. Here we present data for pts with lymphoma and myeloma.
Results: 472 pts (301 with lymphoma and 171 with myeloma) were included in this analysis. 100% of these pts received epoetin zeta (median dose 30 000 IU / week). 1.3% of pts with lymphoma and 1.2% of pts with myeloma received iron supplementation in addition. In the 301 pts with lymphoma, the mean age was 68.6 years; 62.7% of them had stage IV lymphoma. At baseline, 34.2% of pts had grade 2 anaemia and the mean Hb level was 95.5 g/l. For 71.3% of patients the target Hb level wasset between 120 and 129 g/l by the physicians. At M+3 and M+6, respectively 29.4% and 34.2% of patients reached the target Hb level, 80.8% and 86.0% were respondersand the average increase in Hb level was 17.2 and 21.2 g/L. At M+6, the transfusion rate was 10.3% and 15.4% of pts with lymphoma reported adverse events (AE); the most frequent was infection (8.8%), whereas the rate of thrombotic events was 1.1%. No EAB-related death was reported. In the 171 pts with myeloma, the mean age was 70.7 years; 88.6% of them had stage III myeloma. At baseline, the majority of patients (37.4%) had grade 2 anaemia and the mean Hb level was 95.7 g/l and. For 70.8% of patients the target Hb was set between 120 and 129 g/l. At M+3 and M+6, respectively 35.8% and 26.1% of patients achieved the target Hb level, 86.2% and 83% had a response to treatment and the average increase in Hb level was 20.3 and 15.7 g/l. At M+6, the transfusion rate was 7.5% and the rate of reported AE was 18.8%; themost frequent was also infection reported in 6.9% of patients, whereas the rate of thrombotic events was 5%. No EAB-related death was reported.
Summary / Conclusion: EAB therapy is effective and well-tolerated in the management of CIA in pts with lymphoma and myeloma.

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INTRAVENOUS IRON THERAPY FOR TREATMENT OF ANEMIA DURING PREGNANCY IS ASSOCIATED WITH IMPROVED MATERNAL QUALITY OF LIFE, LESS POSTNATAL DEPRESSION AND LONGER BREASTFEEDING

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Background: Currently, there are no data available concerning quality of life outcomes and other long-term effects of intravenous versus oral iron therapy of anaemia during pregnancy, particularly the physical impact of iron deficiency anaemia (IDA) on pregnant women as well as the impact of iron therapy on wellbeing and health-related quality of life (HRQoL) during and after pregnancy. IDA is a potential risk factor for many complications during and after pregnancy, and may be associated with inferior maternal and neonatal health.
Aims: To assess the long-term effect of iron therapy on HRQoL during and after pregnancy, in particular postnatal depression and duration of breastfeeding.
Methods: We conducted a follow-up study between January 2010 and January 2011 of an earlier randomized open-label clinical trial of intravenous and oral iron versus oral iron for pregnancy-related iron deficiency anemia. We useda modified version of the SF-36 questionnaire together with the original prospective HRQoL data collected during and after pregnancy. This study is approved by the Tasmanian Human Ethics Committee, Australia. The study was registered prospectively in the Australian New Zealand Clinical Trial Registry (http://www.ANZCTR.org.au) under ACTRN 12609000177257 and in the World Health Organization Clinical Trials Registry (http://*www.who.int/trialsearch/trial. aspx?trialid=ACTRN12609000596202)*. We assessed HRQoL data on 126 pregnant Caucasian women randomized to receive oral iron or a single intravenous iron polymaltose infusion during pregnancy followed by oral iron maintenance. The participants were followed-up 4 weeks after treatment, pre-delivery, and post-delivery for a median period of 32 months (range, 26-42) with a wellbeing and HRQoL questionnaire using a modified SF-36 QoL-survey and child growth charts as set by the Australasian Paediatric Endocrine Group (APEG).
Results: Patients who received intravenous iron demonstrated significantly higher hemoglobin and serum ferritin levels (P<0.001). There were strong associations between iron status and a number of the HRQoL parameters, with improved general health (P<0.001), improved vitality (physical energy) (P<0.001), less psychological downheartedness (P=0.005), less clinical depression (P=0.003), and overall improved mental health (P<0.001). The duration of breastfeeding was longer (P=0.046) in the intravenous iron group. The babies born in both groups recorded similarly on APEG growth chart assessments.
Summary / Conclusion: Our data suggest that HRQoL is improved during and after pregnancy in anemic pregnant women by repletion of their iron stores during pregnancy. About 80% of the intravenous iron group showed a maintained normal ferritin until delivery with long-term benefits such as prolongation of the breastfeeding period and less postnatal clinical depression. this study reports a novel finding in terms of a correlation between both postnatal depression and the breastfeeding period with iron status. There are no data available concerning the quality of life during and after pregnancy, which makes the scientific input of the current study important. Further studies to confirm these findings are warranted.

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ESTIMATION OF THE RELATIONSHIP BETWEEN SERUM FERRITIN AND LIVER IRON CONCENTRATION IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background: As magnetic resonance imaging (MRI) is not widely available to assess liver iron concentration (LIC), serum ferritin (SF) can be used as a surrogate marker of liver iron overload. In a previous pooled analysis of iron-overloaded patients with myelodysplastic syndromes (MDS) nearly half of patients had SF<2500ng/mL. A similar proportion of patients overall had severe liver iron accumulation with LIC≥15mg Fe/g dw (Gattermann *et al. Haematologica* 2012). These data suggest that, in this selected patient sample, liver iron burden appears to be more severe than indicated by SF in some patients with MDS.
Aims: To better understand patterns of iron accumulation in MDS patients and to evaluate the predictive value of various SF cutoffs to estimate severe liver iron burden.
Methods: Data were pooled from iron-overloaded MDS patients having Low/Int-1 risk or a life expectancy >1 yr and who completed 1-yr deferasirox treatment in four open-label single-arm studies. Patient distribution by baseline iron loading categories LIC < and ≥15 mg Fe/g dw was summarized descriptively. A receiver operating characteristic (ROC) analysis was conducted on all patients with available SF and LIC at baseline, for the LIC threshold of 15mg Fe/g dw. Positive predictive values (PPV; the percentage ratio of true positives; ie % patients with LIC≥15 in all patients whose SF>cutoff) and negative predictive values (NPV; the percentage ratio of true negatives; ie % patients with LIC<15 in all patients whose SF≤cutoff) were generated. SF cutoff values were tested at 500 ng/mL intervals. Accuracy was the proportion of accurate assessments, either true positive or true negative.
Results: 71 patients with both SF and LIC measurements were included in this assessment. Patient distribution by baseline iron loading categories is shown (Table 1). Overall, in patients with SF≥1000ng/mL, 58.0% had LIC≥15 mg Fe/g dw. The largest proportion of patients had SF 2500–<5000ng/mL (36.6%); among these patients 65.4% had LIC≥15mg Fe/g dw. The ROC analysis identified that a SF level of 2000ng/mL was the strongest predictor of an LIC of 15mg Fe/g dw, with an accuracy of 70.4% (PPV 68.6 & NPV 75.0). The probability of a patient having an LIC≥15 mg Fe/g dw when SF>2000ng/mL was 68.6% (PPV). SF<2000ng/mL was 75% predictive of an LIC<15mg Fe/g dw (NPV).
Summary / Conclusion: Patients with MDS in this pooled analysis showed severely elevated LIC despite only moderately elevated SF levels. Evaluating patient distribution by iron loading categories, over half of patients with SF≥1000 ng/mL showed evidence of severe liver iron accumulation in excess of 15 mg Fe/g dw. ROC analysis showed that SF levels of 2000 ng/mL can effectively predict an LIC of 15mg Fe/g dw, although a quarter of patients with SF<2000 ng/mL would have LIC≥15mg Fe/g dw. Underestimation of LIC by SF in this heterogeneous MDS patient population may be connected to variability in hepcidin production with the underlying disorder (Santini *et al. PLoS One* 2011). Ineffective erythropoiesis leads to inhibition of hepcidin production, thereby increasing iron absorption from the gut with periportal hepatocellular iron accumulation, but with depletion of macrophage iron. Consequently, SF levels, which mainly reflect macrophage iron, can be low relative to total LIC, with underestimation of hepatic iron overload. This analysis is limited by low patient numbers in other iron burden categories, which precluded assessment at different thresholds. SF remains a good marker of iron overload in MDS, but liver iron appears to accumulate more quickly than previously understood.

Table 1. Summary of MDS patient distribution by LIC an SC categories at baseline.

SF categories (ng/mL)	LIC categories (mg Fe/g dw), n (%)*		All patients, n (%)†
	<15	≥15	
<1000	2 (100.0)	0	2 (2.8)
≥1000 to <1500	9 (75.0)	3 (25.0)	12 (16.9)
≥1500 to <2000	4 (66.7)	2 (33.3)	6 (8.5)
≥2000 to <2500	6 (42.9)	8 (57.1)	14 (19.7)
≥2500 to <5000	9 (34.6)	17 (65.4)	26 (36.6)
≥5000	1 (9.1)	10 (90.9)	11 (15.5)
All patients, n (%)†	31 (43.7)	40 (56.3)	71 (100.0)

*% refers to the proportion of patients within each SF category
†% refers to the proportion of patients overall

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STUDY OF IRON STATUS AS A CONTRIBUTING FACTOR TO ANEMIA IN PEDIATRIC CANCER PATIENTS

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Background: The prevalence of anemia approaches 50% in patients with cancer and may increase to more than 90% in patients with certain types of cancer and in those undergoing chemotherapy or radiation therapy. *The* term functional iron deficiency (FID) has been applied to the situation characterized by iron-restricted erythropoiesis in the presence of iron stores. This is an additional cause of anemia in patients with various chronic diseases including cancer.
Aims: The study aimed to evaluate the etiology of anemia in cancer patients in relation to the iron status and the prevalence of iron restricted erythropoeisis.
Methods: All patients with childhood cancer on chemotherapy regularly following at the Pediatric hematology-oncology unit, Children's hospital ,Ain-Shams university ,in the period from June 2012 to January 2013, were screened by routine blood picture for the presence of anemia. Patients with hemoglobin level ≤10.5 gm/dL were subjected to history and revision of hospital records for collecting data including their age, sex, symptoms of anemia, dietary recall, bowel habits, any blood loss and/or blood transfusion, date at diagnosis of cancer, its type and chemotherapy protocol; then physical examination were done for signs of anemia and any evidence for infection. Laboratory investigations included complete blood picture, reticulocyte count, Iron profile: serum iron, serum ferritin, transferrin, total iron binding capacity, CRP , and reticulocyte hemoglobin content(**CHr**). Patients with malignant bone marrow infiltration, patients admitted for infection or having febrile neutropenia , and patients infected with hepatitis C virus were excluded from study. Cut-off level for CHr was 28pg and transferrin saturation (Tsat) 20% [1]. Patients were classified into 4 categories according the level of transferrin saturation (Tsat) and CHr content [1]. Category 1: with no iron deficiency (Tsat >20%)and no FID (CHr >28pg); category 2 : iron deficiency (Tsat<20%) and no FID(CHr>28pg); Category 3: replete iron (Tsat>20%) and FID (CHr ≤28pg) and category 4 both levels below cutoff: Iron deficiency anemia.
Results: Forty patients were included in the study, 22 males(55%) and 18 females (45%). Their mean age was 4.9± 3.9 years (range 1.3-17). Twenty two (55%) had acute leukemia and 18 (45%) had solid tumors and lymphomas. Their mean hemoglobin level was 9.8± 1.7 gm/dL(range 8.2-10.4). The mean serum ferritin was 904.1±723 ng/mL, Median 757 ng/ml (Range 55-2420 ng/mL). Nine (22.5%) patients had CHr ≤28 pg/mL.Patients had the following iron status categories: no ID and no FID were 29(72.5%), Mild ID and no FID was only 1(2.5%), Replete iron stores and FID was present in 8 patients (20%), and IDA was present in 2 patients (5%).Patients with FID were successfully treated with combined EPO and iron, while iron therapy was offered for the three patients with ID anemia.
Summary / Conclusion: Functional iron deficiency represents an appreciable etiology for anemia in childhood patients with cancer on chemotherapy. Serum ferritin level is not a reliable marker for assessment of iron status in children with cancer.The transferrin saturation and assessment of the reticulocyte hemoglobin content are accurate indicators of true and functional iron deficiency in cancer patient.

Reference

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A PHASE I, DOSE ESCALATION, PLACEBO-CONTROLLED STUDY IN HEALTHY INDIVIDUALS TO ASSESS THE SAFETY, TOLERABILITY, PHARMACOKINETICS AND PHARMACODYNAMICS OF FBS0701, AN IRON CHELATOR IN DEVELOPMENT

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Background: Chronic iron overload in patients with transfusion-dependent anemias can lead to organ failure if not effectively treated. Parenteral dosing, frequent daily dosing and side effects of current therapies reduce adherence and compromise clinical benefit. FBS0701 is an orally administered, tridentate, iron chelator in clinical development. Previous studies showed that once-daily FBS0701 (3–40 mg/kg) was well tolerated in healthy volunteers and iron-overloaded patients.
Aims: To characterize the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics of FBS0701 in healthy volunteers across the anticipated therapeutic dose range.
Methods: This was a phase I, single-dose, randomized, double-blind, placebo-controlled, dose escalation study of FBS0701 in healthy volunteers. Healthy

individuals 18–50 years of age with body mass index of 18–30 kg/m^2, serum ferritin of >20 ng/mL and ≤300 ng/mL, and erythrocyte indices within the normal range were eligible for inclusion. All participants provided written, informed consent. Oral FBS0701 was administered at 6 dose levels (30, 40, 50, 60 and 75 mg/kg once daily [q.d], and 20 mg/kg twice daily [b.d.]) with at least 4 participants per dose and an additional individual randomized to placebo. Dose escalation occurred in the absence of serious adverse events (SAEs). Blood samples were collected pre-dose on day 1 and at 15 intervals post-dose (15 min to 96 h). For twice-daily dosing, samples were collected pre-dose and at 6 time points up to 12 h following the first dose; blood sampling following the second dose was as per once-daily dosing. Urine volume and concentrations of FBS0701, creatinine and protein were measured at pre-dose and up to 24 h post-dose. The assay measured the total of free and Fe(III)-bound FBS0701.

Results: Thirty participants were enrolled with 5–8 per cohort; 5 participated in 2 cohorts. Mean age was 26 years (range, 18–47 years). FBS0701 plasma concentrations indicated dose-related but less than dose-proportional increases in exposure from 30–60 mg/kg; the 75 mg/kg exposures (mean C_{max}, 84 575 ng/mL) were lower than expected based on linearity (Table 1). Mean FBS0701 plasma concentrations for 40 mg/kg q.d. and 20 mg/kg b.d. were consistent with the different dosing regimens. Terminal half-life and renal clearance of FBS0701 20 mg/kg at 12 h intervals (i.e. twice daily) were consistent with those from the single-dose cohorts. No clinically significant urine creatinine or urine protein results were noted. AEs that were possibly/probably treatment related were reported in 4 of 30 participants (13%) and included dysgeusia, hyperchromic urine, loss of appetite, fatigue and headache, which were all mild. AEs were also reported in 1 of 5 individuals receiving placebo. There were no SAEs, deaths or withdrawals due to AEs, and no relationship between drug dose and incidence, severity or causality of AEs.

Summary / Conclusion: The bioavailability of oral FBS0701, based on the total of free and Fe(III)-bound plasma concentrations, appeared dose-related as reported for lower doses. PK parameters were consistent when FBS0701 was administered as either a once-daily or twice-daily dose. The half-life was similar to that observed in a previous study of healthy volunteers. FBS0701 was generally well tolerated and warrants further investigation in treating transfusional iron overload.

Table 1. PK results.

Parameter	Once-daily dosing	Twice-daily dosing
FBS0701 doses, mg/kg	30, 40, 50, 60, 75	20
Mean C_{max} per dose group, ng/mL	68 171, 87 900, 91 714, 82 325, 84 575	52 900
Mean C_{max} at same total daily dose, ng/mL	87 900 (40 mg/kg q.d.)	52 900 (20 mg/kg b.d.)
Range of mean T_{max}, h	1.0–1.5	0.75
Range of mean $t_{1/2}$, h	8.4–18.6	9.6
Range of mean urinary recovery of FBS0701 over initial 24 h, %	~38–55	NA

b.d., twice daily; C_{max}, maximum plasma concentration; NA, not available; PK, pharmacokinetics; q.d., once daily; T_{max}, time to maximum concentration; $t_{1/2}$, terminal half-life.

P422

THE SAFETY, TOLERABILITY, PHARMACOKINETICS AND PHARMACODYNAMICS OF FBS0701, AN IRON CHELATOR IN DEVELOPMENT, IN PHASE I STUDIES OF HEALTHY VOLUNTEERS AND PATIENTS WITH TRANSFUSIONAL IRON OVERLOAD

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Background: Despite advances in chelation therapy, the effective treatment of chronic iron overload is compromised in many patients. Parenteral dosing, frequent daily dosing and side effects of current therapies reduce adherence and limit clinical benefit. FBS0701 is an orally administered, tridentate, iron chelator in clinical development.

Aims: To characterize the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of FBS0701 in healthy volunteers and in patients with transfusional iron overload.

Methods: Two studies were conducted in distinct populations. Study 1 was a phase I, randomized, dose-escalation, double-blind, placebo-controlled study in healthy volunteers. Participants were randomized to a single-dose of oral FBS0701 at 4 dose levels (3, 6, 10 and 16 mg/kg) or placebo (cohorts 1–4). Healthy males aged 18–45 years with body mass index 18–27 kg/m^2, serum ferritin >20 ng/mL and ≤250 ng/mL, and erythrocyte indices within the normal range were eligible for this study. Study 2 was a phase I, dose-escalation study assessing FBS0701 in patients with chronic transfusional iron overload. Patients received a single oral dose of FBS0701 (6, 10, 16 or 32 mg/kg). Male patients with β-thalassemia aged 18–50 years with iron overload requiring treatment with an iron chelator (serum ferritin >300 ng/mL and ≤5000 ng/mL) were eligible for this study. In study1, blood samples were collected up to 96 h post-dose for PK and PD analyses; blood samples were collected up to 72 h post-dose in study 2. In both studies, urine samples were collected up to 24 h post-dose for PK and PD analyses. The total of free and Fe(III)-bound FBS0701 was measured in both studies. All participants provided written, informed consent.

Results: Seventeen individuals were enrolled into study 1; 12 received FBS0701 and 5 received placebo across the dose cohorts. Mean age was 23.5 years (range, 19–36 years). Mean AUC_{0-24} and C_{max} were linear across the dose range (Table 1). Nine treatment-emergent adverse events (AEs) were reported in 5 of 17 participants (29%); all 5 participants had received FBS0701. There were no AEs deemed to be probably/definitely related to FBS0701, and there were no deaths or withdrawals due to AEs. Four AEs were possibly related to the study drug and included moderate gastroenteritis, flatulence and headache. There was no evidence of a relationship between drug dose and the incidence, severity or causality of AEs. In study2, 4 patients were enrolled with 1 patient per dose. Mean age was 36.3 years (range, 33–41 years). AUC_{0-24} and C_{max} seemed dose-related but less than dose-proportional (Table). Three treatment-emergent AEs were reported in a single patient (bruising at venepuncture and cannula sites); none were related to the study drug. There were no deaths, SAEs or withdrawals due to AEs.

Summary / Conclusion: The bioavailability of FBS0701 seemed dose-related in both healthy individuals and patients with β-thalassemia. It is anticipated that there would be minimal accumulation of FBS0701 with repeated once-daily dosing. FBS0701 was generally well tolerated. Further investigation of FBS0701 in patients with transfusional iron overload is warranted.

Table 1. PK results across dose cohorts.

Parameter	Study 1 (n=12)	Study 2 (n=4)
Population	Healthy volunteers	Patients with β-thalassemia
FBS0701 doses (mg/kg)	3, 6, 10, 16	6, 10, 16, 32
Mean C_{max} per dose group (ng/mL)	5413, 12 233, 16 633, 34 833	14 900, 35 400, 38 500, 47 600
Mean AUC_{0-24} per dose group (h*ng/mL)	12 104, 24 720, 42 692, 74 620	45 664, 69 216, 121 781, 157 183
Mean T_{max}, h (range)	1.1 (0.9–1.3)	0.9 (0.8–1.0)
Mean $t_{1/2}$, h (range)	12.8 (3.8–35.2)	21.6 (16.0–30.0)
Plasma concentration 24 h post-dose, % of C_{max}	<0.5	<1
Mean urinary recovery of FBS0701 over initial 24 h, % (range)	59 (19–83)	39 (28–51)

AUC_{0-24}, area under the curve versus time; C_{max}, maximum plasma concentration; PK, pharmacokinetics; T_{max}, time to maximum concentration; $t_{1/2}$, terminal half-life.

P423

LONG TERM EFFICACY AND SAFETY OF DEFERASIROX IN TRANSFUSION DEPENDENT MYELODYSPLASTIC SYNDROME (MDS) PATIENTS WITH IRON OVERLOAD: RESULTS FROM THE EPIC EXTENSION STUDY

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Background: The prospective, 1-year EPIC study conducted in a large cohort of patients with transfusion-dependent anemias (including 341 MDS patients) demonstrated the efficacy and safety of iron chelation therapy with deferasirox (DFX) by reducing serum ferritin (SF) levels and proving a manageable safety profile. This EPIC extension study was conducted to further evaluate the long term efficacy and safety of DFX in these patients.

Aims: The objective of this sub group analysis was to evaluate the long-term efficacy and safety of DFX in MDS patients enrolled in the EPIC extension study.

Methods: Patients who had completed the core EPIC study continued to receive DFX for a maximum of an additional 18 months or until the drug was locally available, whichever came first. The inclusion and exclusion criteria were

earlier defined in Cappellini *et al.,* 2010. Initial extension dose was based on the last dose they received during the core period and dose adjustments were made based on safety and efficacy markers. The primary efficacy endpoint was change in SF from baseline (BL) to the end of extension (EOE), analyzed using the Student's *t-test.* Safety assessments consisted of monitoring and recording all adverse events (AEs), serious adverse events (SAEs) with their severity and relationship to drug, study discontinuation, and deaths.
Results: Of the 341 MDS patients enrolled in the core study, 175 patients completed the core and 166 patients discontinued, of which 47% were due to AEs (n=78) and were mainly gastrointestinal (GI) related (n=25). From the core study, 45 patients continued into the extension phase for an additional 18 months or until the drug was locally available. Cumulative data, core plus extension, is being reported here. In this cohort, 51% of patients were ≥65 years old (n=23). The average daily dose of DFX was 20.6 mg/kg/day and mean duration of exposure was 74 weeks (SD: 19). Compliance >80% was noted in most of the patients (n=29, 64%). Overall, significant reductions in median SF levels from BL to EOE were observed with DFX treatment in MDS patients (n=44, median change -393.5 ng/mL, P= 0.0161). Maximum reduction in SF levels (-736 ng/ml) was observed in patients receiving average daily dose of DFX 25 - <35 mg/kg/day. AEs related to study treatment were reported for 55.6% of patients from BL to EOE, similar to the rate observed in the core MDS patients (66.3%). The most frequently reported drug-related AEs were GI disorders (n=14, 31.1%), of which 64% were mild in severity and none lead to discontinuation of treatment. Increases of >5 x ULN for AST or ALT were not reported with exception of one patient with an elevated BL ALT who subsequently experienced two consecutive ALT increases of >5 x ULN post-BL values. At BL, the majority of patients had normal levels of serum creatinine (SCr) (n=35, 77.8%). In this cohort of 45 patients, 11 (24.4%) had two consecutive values >33% increase and >ULN of SCr. Increases in SCr or liver transaminases reported were mainly mild in severity, transient, non-progressive, and were effectively managed with dose adjustment. None of the 45 patients reported SAEs related to study drug and two patients had non-drug related AEs leading to discontinuation (acute myeloid leukemia, respiratory failure).
Summary / Conclusion: This extended follow up showed that DFX significantly reduced SF levels from BL in the MDS patients who completed core and extension phases of the EPIC study. DFX treatment had a manageable safety profile. This additional extension analysis confirms the overall safety and efficacy of DFX treatment in transfusion dependent MDS patients.

Infectious diseases, supportive care

P424

FOUR MEASURES TO REDUCE THE RATE OF HEALTH CARE-ASSOCIATED BLOODSTREAM INFECTIONS RELATED TO THE USE OF NEEDLESS MECHANICAL VALVE CONNECTORS IN LONG-TERM CENTRAL VENOUS CATHETERS
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Background: Mechanical valve needless connectors (MVC) are devices known to be associated with augmented risk for bloodstream infections (BSI). This risk is even superior when positive pressure MVC (PP-MVC) are compared with neutral pressure MVC (NP-MVC). One reason for this augmented risk seems to be related with the formation of a biofilm on the surface of the MVC, so measures taken with to reduce this biofilm could probably decrease BSI rate.
Aims: To compare the BSI rates registered in onco-haematological patients with long-term central venous catheter (CVC), before and after the introduction of measures aimed at reducing biofilm formation on the MVC.
Methods: We analysed the BSI rates for a period of 18 months after adoption of new rules for MCV manipulation: 1-changed the use of a PP-MVC to a NP-MCV; 2- replaced the MVC two times a week instead of just one time; 3- replace the MVC after each blood cultures collection to study a new episode of fever; 4- changed the cleaning solution from alcohol 70% to chlorhexidine 2%/alcohol). The rates obtained were compared with the rates registered in the 6 months immediately preceding this change of procedures.
Results: The number of CVC days analysed before and after the introduction of the MCV manipulation measures was 2111 and 6756, respectively. The evaluation of the number of blood cultures collected between the two groups revealed a reduction of 35% with the introduction of the measures (107 vs. 70 blood cultures/1000 CVC). The mean rate of BSI in the control group (without measures) was 35.5 / 1000 CVC days, whereas in the study group was 9.4 / 1000 central venous catheter days, corresponding to relative risk of 0.27 (CI 95%, 0.27-0.37; P<0.001). Using differential time to positivity to identify bacteraemias originating from CVC, the BSI rate decrease from 17.5 to 4.7 / 1000 CVC days after adopting the measures, relative risk of 0.27 (CI 95%, 0.16-0.43; P=0.006). We also found that the reduction in BSI rates was essentially at the expense of gram positive microorganisms (51 vs. 21%), indicating that the measures taken have affected mainly infections originating in catheters.
Summary / Conclusion: We have shown in this study that measures taken with the objective of reducing biofilm formation, including the substitution of PP-MCV with NP-MCV, can result in a significant decrease of BSI related with long-term CVC in haematological patients.

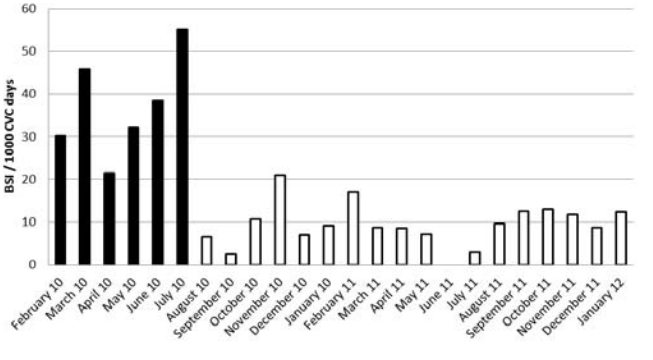

Figure 1.

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CHANGING PATTERN OF INFECTION DURING TREATMENT OF PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)
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Background: Acute myeloid leukemia (AML) patients during chemotherapy

induced neutropenia are at high risk of bacterial and fungal infections. The first induction course is associated with high susceptibility to infectious complications leading to increased morbidity and mortality.
Aims: In the present study, the incidence and pathogenesis of neutropenic fever were analyzed during the first induction therapy and compared with the following post-remission courses.
Methods: 223 consecutive newly diagnosed AML patients with a median age of 51 yrs (18-70) were treated with standard induction chemotherapy with Idarubicin, Citarabine and Etoposide (ICE). A complete remission (CR) was achieved in 169 pts (76%), whereas 40 pts (18%) were resistant and 14 (6%) died because of infections. Patients entering CR were treated with a second induction course with Idarubicin and Citarabine followed by a third cycle of intermediate Cytarabine (IDAC) with peripheral blood progenitor cells harvest. The post-remission therapy consisted in repeated (up to three) cycles with high-dose Cytarabine (HDAC) and PBSC rescue. Sixty-seven (30%) non low-risk patients with a suitable donor received an allogeneic stem cell transplant and were censored at the time of transplantation. A total of 774 neutropenic episodes was analyzed. All patients received anti bacterial and antifungal prophylaxis with levofloxacin and itraconazole or posaconazole. Four (1.8%) pts died in the post-remission phase due to infections.
Results: During the first induction course 19 patients (8%) had no infections whereas 87 (39%) developed a fever of unknown origin (FUO), 40 (18%) had clinically documented infections (CDI), 62 (28%) microbiologically documented infections (MDI) and 15 (7%) probable or proved invasive fungal infections (IFI). In the post-remission courses febrile episodes were significantly reduced compared to the first induction course. Analysis of post-remission neutropenic episodes showed no infection in 301 (54.6%), FUO in 87 (15.8%), CDI in 17 (3.1%), MDI in 139 (25.2%) and IFI in 7 (1.3%) (P=.0000). Forty-eight(21.5%) pts developed pneumonia during induction vs. 33 (6%) in the post-remission phase (P=.0000). Gram-positive bacteriemia (s. epidermidis 50%, enterococcus 16,6%, s. aureus 7%) was significantly predominant in induction, while Gram-negative bacteriemia (e. coli 76%, p. aeruginosa 5.5%, enterobacter 4%, k. pnemoniae 2.2%, s. maltophilia 2%) was mostly detected in post-remission. Gram positive vs Gram negative isolates were 65% vs 33% in induction and 35% vs 67% in post-remission (P=.00003). During induction in 15 (6.7%) pts a respiratory support with continuous positive air pressure (CPAP) ventilation was required. CPAP support was necessary in 8 (1.4%) out of 551 post-remission aplasias (P=.0000). Eight (3.6%) of 223 inductions were complicated by severe sepsis/septic shock vs 8 (1.45%) of 551 post-remission aplastic periods. Two pts required ICU admission in induction and 4 in post-remission.
Summary / Conclusion: The results of the present study show a significantly changing pattern of infection during treatment of AML patients. This observation should be considered in the choice of empiric antibiotic therapy during consecutive neutropenic episodes.

P426

PNEUMOCOCCAL VACCINE RESPONSES IN B CELL MALIGNANCIES AND DYSFUNCTIONS

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Background: Pneumococcal infections are a common cause of disease and death in patients with B cell malignancies. Vaccination with the 23-valent polysaccharide vaccine has been clinical praxis among these patients; however, more recent vaccination schedules suggest the use of conjugate pneumococcal vaccines.
Aims: To compare antibody responses and functional antibody activity as measured by opsonophagocytosis after vaccination with either polysaccharide or conjugate pneumococcal vaccine in elderly patients with multiple myeloma (MM), Waldenstrom's macroglobulinemia (WM) and monoclonal gammopathy of undetermined significance (MGUS).
Methods: Fifty-six patients > 60 years of age with a diagnosis of MM (*n* = 24), WM (*n*=15) and MGUS (*n*=17), and 20 age-matched controls were randomized to receive a single dose of either the 23-valent pneumococcal polysaccharide vaccine (PPV) or a 7-valent conjugated vaccine (PCV7). Sera were collected prior to and 4-8 weeks after immunization and analyzed with a serotype-specific ELISA for IgG antibodies to pneumococcal serotypes4, 6B, 9V, 14, 18C, 19F, and 23F, and with an opsonophagocytosis killing-type assay (OPA) for functional antibody activity towards serotypes 4 and 14. Written informed consent was obtained from all study participants.
Results: Pre- and post-vaccination sera revealed the lowest antibody and OPA titers among MM patients, followed by WM patients, MGUS patients and healthy controls. Statistically significant increased levels of IgG antibodies were seen for one pneumococcal serotype among MM patients, three serotypes among WM patients and four serotypes among MGUS patients and healthy controls post-vaccination. Geometric mean IgG and OPA titers did not differ significantly between the vaccine subgroups for any of the study groups although there was a tendency towards higher IgG antibody fold increases in PCV7 vaccinees among MM and WM patients. A few MM patients had very high IgG antibody levels to pneumococci in ELISA. However, corresponding OPA titers were low, indicating the presence of cross-reactive non-functional antibodies.
Summary / Conclusion: A suboptimal response to pneumococcal vaccination was confirmed in MM patients in particular. However, also WM and MGUS patients had lower IgG and OPA titers than healthy controls. No obvious difference between PPV and PCV7 given in single dosage was observed. OPA analysis seems to be a more reliable method than ELISA for evaluation of humoral pneumococcal vaccine responses in MM patients.

P427

A RETROSPECTIVE STUDY OF THE RELATIONSHIP BETWEEN VITAMIN D SUPPLEMENTATION AND THE RISK OF FEBRILE NEUTROPENIA(FN) IN ADULTS WITH HEMATOLOGICAL MALIGNANCIES IN EAST KENT HOSPITALS(EKUFHT)

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Background: Prior studies (1950-2000) regarding the use of vitamin D as part of treatment/prevention of infections opened the door to intensive scientific and clinical research, over the past decade, into the non-endocrine (autocrine/paracrine) role(s) of vitamin D, particularly its impact on the function and maintenance of both the innate and adaptive parts of the immune system. None of these studies looked specifically at the relationship between vitamin D supplementation and the risk of infections amongst adults with haematological malignancies (or with a diagnosis of cancer), an increasing cohort of patients who are particularly vulnerable to FN. Vitamin D deficiency is common in adults in the United Kingdom (UK). The UK National Diet and Nutrition Survey (NDNS), in the key findings from years one and two (2008/2009 – 2009/2010) of its rolling programme, states that there is evidence of low vitamin D status in UK adults, both male and female. The NDNS collected fasting blood samples. The UK Department of Health recommended daily allowance (RDA) for vitamin D is 10 micrograms (400 IU) for people over 65 years and those with limited exposure to sunlight. Between 01/01/2009 and 31/12/2010, there were 585 episodes of admissions with FN in patients who also have a diagnosis of cancer to EKUFHT (serving a population of 0.72 million) with an average hospital stay of 8.03 days. These episodes were caused by 297 patients. Of those 122 patients (41.2%) had hematological malignancies, which breaks into 65 patients (53.3%) with Lympho-Proliferative Neoplasms (LPNs) and 57 patients (46.7%) with Myelo-Proliferative Neoplasms (MPNs). *[FN is defined by the presence of fever (38 degrees Celsius or higher for at least 1 hour) and a peripheral blood neutrophil count of less than 0.5x10^9/L (high risk category) / 0.5-1.0x10^9/L (low risk category) as per EKUFHT guidelines].* The NHS UK, estimates the average cost of one day stay in secondary health care facilities at £670 for the financial year 2010/2011(>£1.5 million/year for EKUFHT).
Aims: To test the hypothesis; that regular vitamin D supplementation at the UK RDA reduces the risk of FN in adults with Haematological malignancies.
Methods: This is a follow-on sub-study from a large retrospective, single centre, hospital based, case-control study to assess the relationship between exposure to prescribed daily vitamin D and the risk of developing FN, in adults with cancer in EKUFHT(William Harvey, Queen Elizabeth Queen Mother, and Kent and Canterbury hospitals).*(The main study was submitted and accepted as the thesis part of a MSc. degree in clinical oncology issued to the author by the school of cancer sciences-CRUK, University of Birmingham, UK, in July 2012 - full Thesis available on request*). The risk of developing FN in adults with haematological malignancies was estimated by calculating the Odds Ratio (OR), with 95% Confidence Interval(CI), for prescribed daily vitamin D3(Yes/No).
Results: Vitamin D supplementation in adults with haematological malignancies was associated with a clinically significant reduction in the risk of developing FN with an unadjusted OR of 0.690 (95% CI, 0.159, 2.524).*[For those with a diagnosis of myeloma the unadjusted OR was 0.656 (95% CI 0.037, 2.877)]. [In the main study including all adults with a diagnosis of cancer the unadjusted OR was 0.44 (95% CI, 0.26, 0.74), with a p-value of 0.003, which equals a reduction in the risk of developing FN from 11.4% in those not taking vitamin D to 5.4% in those on regular vitamin D].*
Summary / Conclusion: These results suggest that vitamin D supplementation may significantly reduce the risk of FN in adults with haematological malignancies. Large double-blinded randomised controlled clinical trials for specific cancer sub-types, are necessary to further study the relationship between vitamin D supplementation and the risk of FN. The potential for preventative and therapeutic use of vitamin D to boost the human innate immune responses is exciting and warrants further studies.

P428

INFECTIONS CAUSED BY CARBAPENEMASE PRODUCING KLEBSIELLA PNEUMONIAE IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Background: Carbapenemase producing *Klebsiella pneumoniae* (KPC) is an increasingly prevalent pathogen in Greek hospitals. The mortality rates are very high in patients with hematologic malignancies who are infected by this microorganism. This is partly due to the fact that initial empiric antibiotic therapy is inappropriate. There is a need for new diagnostic and preventive strategies to control KPC infections in this vulnerable population.
Aims: The aim of this study was to assess the prevalence of KPC colonization among hematologic patients as well as the impact of weekly colonization screening for KPC in combination with adapted empiric treatment on a. the frequency of KPC infections among hematologic patients and b. the outcome of these infections.
Methods: Infections caused by multiresistant pathogens have been recorded in our hospital for approximately a three year period (March 2010-January 2013). Since March 2012 perianal swabs were obtained weekly from 204 consecutive patients with hematologic malignancies and cultured in meropenem supplemented Mac Conkey agar plates. The patients who were KPC+ were isolated or cohorted. Patients who were colonized or infected by KPC after being transferred to the Intensive Care Unit were excluded from the study.
Results: KPC colonization was present in 36 out of 204 screened patients at some time during their (often multiple) hospitalizations (17.6%). In a 3-year period 40 KPC related infections have been recorded. Thirty infections occurred in 21 patients before the commencement of weekly screening for KPC (during a 24-month period). Ten infections were reported in 9 patients after the commencement of weekly screening for KPC (during a 9-month period). Five deaths due to KPC were reported before the screening period while only one death occurred after screening was implemented (in a patient with relapsed disease). Most patients who recovered from KPC infection or were just colonized by KPC went on to receive additional chemotherapy without any life threatening KPC infection occurring. Furthermore, four patients who were previously found to be colonized by KPC received allogeneic stem cell transplantation without any life threatening KPC infection.
Summary / Conclusion: The prevalence of KPC colonization in our hospital is high among patients with hematologic diseases. Weekly screening and cohorting of KPC+ patients didn't reduce the rate of infections so far. Prompt initiation of adapted empiric therapy could reduce the mortality caused by KPC infection.

P429

MANAGEMENT OF HBV INFECTION IN ONCOHEMATOLOGIC IMMUNOSUPPRESSED PATIENTS: LAMIVUDINE PROPHYLAXIS AND RESCUE THERAPY OF OCCULT HBV INFECTION REACTIVATION.

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Background: Hepatitis B virus (HBV) overt and occult infection reactivation in oncohematologic patients (OHPs) is a frequent event and it can lead to severe hepatitis and to liver acute failure even. The reactivation can occur from 5 to 36 months (m) after the start of chemotherapy. Patients with serological markers of resolved HBV infection (HBsAg-, HBcAb+, HBsAb -/+) which receive highly immunosuppressive chemotherapy are at high risk of viral reactivation. Antiviral prophylaxis is recommended but the optimal length, monitoring and the mechanisms of reactivation in these patients are still uncertain.
Aims: 1) to evaluate the efficacy and safety of lamivudine (Lam) prophylaxis given for 18 m after discontinuation of chemotherapy; 2) the efficacy of antiviral standard treatment in OHPs chronic HBV carriers; 3) we also report 11 cases of HBV reactivation who had not received Lam prophylaxis and were treated with antiviral rescue therapy.
Methods: 1) Forty-eight OHPs (M/F:33/15; median age yrs:65; range 29-82) were studied: 28 non Hodgkin lymphoma (NHL), 1 Hodgkin lymphoma (HL), 9 chronic lymphocytic leukemia (CLL), 10 multiple myeloma (MM). At the Hematology Unit admission, before starting chemotherapy, all were screened for HBsAg, HBsAb, HBcAb, HCV-Ab, HAV-Ab and ALT values. HBsAg and ALT were monthly monitored and serum HBV-DNA was tested every 3 m after the start of chemotherapy. 2) Eleven pts. with ongoing HBV reactivation were hospitalized in the Liver Unit. Patients received standard highly immunosuppressive chemotherapic protocols for the hematologic malignancies.
Results: Following serological screening pts. were distinguished in two groups. *Group A* including 9/48 (18%) pts. (M/F:7/2; median age: 67 yrs (34-71) which resulted HBsAg/HBV-DNA pos (2 HBeAg+, 7 HBeAg-). One of these was HCV-Ab/HCV-RNA pos and all were HAV-Ab (IgG) pos. *Group B* including 39/48 (82%) HBsAg neg pts. (M/F: 26/13; median age yrs:65; range 29-82). Nine of 39 (23%) presented isolated HBcAb positivity and 29/39 (74%) HBsAb/HBcAb positivity. Five of 39 (13%) were HCV-Ab positive and 38/39 (99%) HAV-Ab (IgG) positive. *Group C*: 11 pts. (M/F: 7/4; median age 68 yrs), 6 with severe clinical reactivation (jaundice and high ALT levels) and 5 with mild/moderate disease. 4 pts. were HBsAg neg/HBV-DNA pos. *Standard therapy: Group A* pts. (4 inactive and 5 active carriers) received antiviral therapy (5 entecavir (Ent) 0.5 mg/d, 3 Lam 100 mg/d, 1 Lam/adefovir combination); all cleared HBV-DNA (antiviral median time months:11; range 4-24), normalized ALT and completed chemotherapy but are still HBsAg+. *Lam prophylaxis: Group B* pts. started Lam 100 mg/d for 18 m after the last chemotherapy cycle. Twenty of 39 (51%) pts. have completed 18 m of Lam prophylaxis and, among these, 14/20(70%) have passed 12 m after discontinuation of Lam prophylaxis. Median time after discontinuation of chemotherapy and Lam is 30 (1-58) and 19 (1-54) m respectively. None case of HBV reactivation has been observed. Five pts. of Group B died because hematologic malignancy. *Rescue therapy:* Group C pts. received Ent (6) and Lam (5). One died because liver failure, 3 because hematologic disease but were still HBV-DNA pos. 7/11 cleared HBV-DNA.
Summary / Conclusion: HBV reactivation is life threatening condition and must be prevented. These preliminary data show that: 1)prolonged 18 months of Lam prophylaxis is safe and effective in preventing HBV reactivation and in permitting the completion of chemotherapy; 2)Standard anti-HBV treatment is effective to treat chronic HBV carrier with hematologic disease.

P430

CLINICAL CORRELATIONS AND MOLECULAR ANALYSIS OF HUMAN CYTOMEGALOVIRUS (HCMV) INFECTION IN BRAZILIAN PATIENTS WITH SICKLE CELL DISEASE, BETA-THALASSEMIA MAJOR, AND BLOOD DONORS

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Background: There is a significant lack of information regarding the impact of the human cytomegalovirus (HCMV) infection on patients with sickle cell disease, and beta-thalassemia major, despite of the serious clinical effect of this virus. For example, the knowledge of HCMV impact on patients with sickle cell disease is limited to a unique case report describing a fatal HCMV pneumonia in a young man with sickle cell disease. Such information for HCMV in patients with hemoglobinopathies can be crucial in the time when new approaches for the treatment of these diseases by stem cells are gaining ground as the most promising therapeutic tools.
Aims: To examine the molecular characteristics and clinical impact of the HCMV infection on patients with sickle cell disease and beta-thalassemia major and healthy blood donors.
Methods: Blood samples were collected from 144 patients with sickle cell disease, 39 with β-thalassemia major, and 100 healthy blood donors at the Regional Blood Center of Ribeirão Preto, Faculty of Medicine of Ribeirão Preto, University of São Paulo (the Southeast Brazil). The specimens were submitted to viral load quantitation, sequencing analysis, genotyping and phylogenetic analysis of the UL55 gene. The HCMV molecular findings were related to the clinical records of the positive patients.
Results: HCMV DNA was detected in 13.8% of the sickle cell disease patients, 7.6% of the patients with beta-thalassemia major, and 3% of the donors at a mean viral load of $3.8x10^3$ copies/mL. Nevertheless, infections with higher viral load and accompanied by a different hematological findings, and even retinopathy were also observed. HCMV genotype gB2 was detected predominantly (90.9%), followed by genotype gB1 (9%).
Summary / Conclusion: This study examines for the first time the clinical impact and the molecular characteristics of HCMV in patients with hemoglobinopathies. The high rates of HCMV detection among patients with sickle cell disease compared to the patients with beta-thalassemia major could be due to their relative immune suppression. The extensive spread of genotype gB2 among patients with hemoglobinopathies is probably due to geographic characteristics and could not be related to higher virulence, specific patient group or clinical symptoms. Nevertheless, special attention should be paid for the monitoring of the HCMV infection in patients with sickle cell disease, and β-thalassemia major, once this virus can have serious impact on them due to their altered immune surveillance and the specificity of the underlying diseases.

P431

ABNORMAL SPLEEN FUNCTION ASSESSED BY THE STUDY OF "PITS" AND SUBMEMBRANOUS VACUOLES IN ERYTHROCYTES IN PATIENTS WITH SPLENOMEGALY OF DIFFERENT ETIOLOGY

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Background: Patients with enlarged spleen may present a hyposplenism
Aims: To evaluate the spleen function in patients with benign and malignant blood diseases presenting splenomegaly.
Methods: 100 patients were studied (54 males 46 females, median age 39 years (range:16-84 yeas) (group I: 14 autoimmune processes, group II: 6 hereditary red cell disorders, group III: 23 lymphoproliferative disorders, group IV: 10 chronic myeloproliferative neoplasms, group V: 10 liver cirrhosis, group VI: 37 splenic traumas (22 conservative treatment and 15 splenectomy). The control group was formed by 28 "normal subjects": 12 males 16 females, median age 32 years (range: 22-50). The following clinical and analytical data were recorded: age, sex, spleen size, diagnosis, complete blood count and erythrocytic formula. The methods used were: a) study of red cell "pits" (Nomarsky optic) and b) study of the red cell submembranous vacuoles (transmission electron microscopy).
Results: There was a correlation between the measurements of "pits" and the submembranous vacuoles in the whole series (R>0.68, P<0.001). The correlation was better in the cases with pathology (R>0.82, P<0.001) than in the normal subjects. In the group of healthy people (negative control)the median percentage of red cells with "pits" was 2.1% (0.5-8.8) and with submembranous vacuoles 1.4% (0.4-3.8). The median number of "pits" per red cell was 0.02 (0.01-0.12) and of submembranous vacuoles 0.02 (0.00-0.20). In the traumatic spleen group (group IV), in patients in which splenectomy was performed without autologous transplantation (9 cases) (positive control), the median percentage of red cells with "pits" was 54.7 (extremes 29-64) and the median of "pits" per red cell 1.4 (0.6-1.8). Both median values were statistically different from those of the negative control group (P<0.001). The results of the pathological groups are depicted on the attached Table 1.
Summary / Conclusion: a) Patients with splenomegaly due to chronic lymphoproliferative and myeloproliferative disorders present a hyposplenism that may contribute to severe infections; b) An altered splenic function is also observed in patients with autoimmune diseases and with hereditary red cell disorders although with less intensity; c) Splenic congestion characteristic of patients with liver cirrhosis presents a normal spleen function; d) Spleen function is more related to the splenic red pulp infiltration than to spleen size.
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Table 1.

PATHOLOGICAL GROUPS

	MEDIAN PERCENTAGE OF RED CELLS WITH "PITS"			MEDIAN OF "PITS" PER RED CELL		
	Result	Comparison with positive control	Comparison with negative control	Result	Comparison with positive control	Comparison with negative control
Grups						
I	5.70 (0.20-24.60)	(p<0.001)	(p=0.011)	0.065 (0.00-0.38)	(p<0.001)	(p=0.033)
II	7.80 (3.10-13.10)	(p<0.001)	(p=0.001)	0.10 (0.03-0.20)	(p<0.001)	(p=0.002)
III	5.30 (1.10-87.40)	(p<0.001)	(p=0.001)	0.10 (0.01-2.7)	(p<0.001)	(p<0.001)
IV	4.05 (0.80-22.30)	(p<0.001)	(p=0.012)	0.065 (0.01-0.49)	(p<0.001)	(p=0.011)
V	2.25 (0.60-4.90)	(p<0.001)	(pns)	0.03 (0.01-0.08)	(p<0.001)	(pns))

P432

NEUTROPENIC ENTEROCOLITIS AND VANCOMYCIN RESISTANT ENTEROCOCCUS AS PREDISPOSING FACTOR

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Background: Neutropenic enterocolitis (NEC) is a life-threatening disease with high rates of morbidity and mortality, seen primarily in patients with hematologic malignancies. The frequency of NEC has increased with the use of chemotherapeutic agents causing severe gastrointestinal mucositis. Neutropenic patients with fever and abdominal symptoms (pain, distention, diarrhea, GI bleeding), should be evaluated for bowel wall thickening of ≥4 mm, the hallmark of NEC. Management includes bowel rest, correction of cytopathies and coagulopathies, and broad spectrum antibiotics and antifungal agents. Surgical intervention may be necessary to manage complications such as hemorrhage and perforation and should be delayed, if possible, until recovery from neutropenia. Vancomycin resistant enterococci (VRE) are an increasingly common and difficult to treat cause of hospital-acquired infection. Long hospitalization periods, use of broad-spectrum antibiotics and immunosuppression are major risks for VRE colonization.
Aims: The aim of our study is to evaluate our patients' characteristics and factors which may contribute to VRE colonization as well as the role of colonization for the development of NEC
Methods: 140 patients who have been hospitalized in the Hematology inpatient clinic between April 2012 and September 2012 were enrolled in the study. Ages of the patients ranged between 19 and 84 years (mean 54.81) and gender of the patients were 63 female (45%) and 77 male (%55). Diagnosis of the patients were grouped as acute leukemias, lymphomas (both Hodgkin's and non-Hodgkin's), chronic leukemias an myeloproliferative disorders and benign hematological disorders. Leucocyte count, initial questioning for gastrointestinal problems and the presence of hypogammaglobulinemia were recorded. After discharge, VRE status and neutropenic enterocolitis and diarrhea were also recorded.
Results: VRE colonization was observed primarily in patients with acute leukemias. Initial leucocyte count was not related with VRE status, thus VRE colonization was significantly related with the presence of hypogammaglobulinemia. History of previous gastrointestinal problems were significantly related with VRE colonization. And last o all, Both neutropenic enterocolitis and neutropenic diarrhea were significantly related with VRE status.
Summary / Conclusion: VRE colonization is a fast spreading, not easily managed problem especially in Hematology clinics. Patints with hypogammaglobulinemia is observed to be susceptible for colonization. Serious and life-threatening complications of hematological malignancies and their treatment such as neutropenic diarrhea and enterocolitis are observed to be more frequent in patients who have VRE colonization.

Table 1.

Parameters		VRE positive (# of patients)	VRE negative (# of patients)	Total (# of patients)	P values
Total number of patients		28	112	140	
Hypogammaglobulinemia at the time of diagnosis		27	20	47	0.000
Complaints about gastrointestinal system at the time of diagnosis		13	9	22	0.000
Diagnosis	Acute leukemias	20	26	46 (32.9%)	
	Lyphomas	6	36	42 (30.0%)	
	Chronic leukemias and myeloproliferative disorders	2	40	42 (30.0%)	
	Benign hematological disorders	0	10	10 (7.1%)	
Neutropenic diarrhea		23	7	30	0.000
Neutropenic enterocolitis		14	2	16	0.000

P433

ANAEROBIC BLOODSTREAM INFECTIONS AMONG HAEMATOLOGICAL CANCER PATIENTS: EPIDEMIOLOGY AND OUTCOME. RESULTS OF AN 8-YEAR SURVEILLANCE PROGRAM AT A SINGLE INSTITUTION

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Background: Anaerobic bloodstream infections (BSI) are a rare event among haematological cancer patients (pts). Due to its low incidence, few large studies have been published concerning cancer pts as a whole, but only few small series referring to haematological cancer pts. Frequent association with polymicrobic BSI, oral/gastrointestinal mucositis and life-threatening complications were reported.
Aims: In order to better define the clinical characteristics of anaerobic BSI (AnBSI) among haematological pts, we analyzed all BSI occurring to consecutively admitted haematological pts to our Institute during a period of 8 years.
Methods: Since June 2004 a program of active epidemiological surveillance is ongoing at our Institute. Levofloxacin prophylaxis was given to pts with a ≥7day-expected neutropenia. All pts showing fever or signs/symptoms of infection underwent at least two sets of blood culture; empiric antibiotic treatment consisted of beta-lactam+amynoglycoside association. Data concerning anaerobic BSI occurring were analysed.

Results: From June 2004 to December 2012, 691 cases of BSI were recorded. Gram-negative (G-) pathogens accounted for 395 cases (57.2%), Gram-positive (G+) for 235 (34%), fungi (all *Candida* spp) for 11 (1.6%). In 50 cases (7.2%) BSI etiology was polymicrobic (PMB). In 17/691 (2.5%) cases an anaerobic pathogen was isolated, in 4 cases they were observed in PMB BSI. Incidence of AnBSI was constant over time. G+ anaerobic bacteria were observed in 9 cases (6 *Clostridium* spp, 1 *Gemella* spp, 1 *Eubacterium* spp, 1 *Lactobacillus* spp), whereas G- in 8 cases (5 *Bacteroides* spp, 2 *Campylobacter* spp, 1 *Prevotella* spp). Enterococci were the most frequent bacteria associated with anaerobic pathogens in PMB AnBSI. AnBSI were associated with uncontrolled underlying haematological disease (13/328, 4.0% vs 4/363, 1.1%; OR 3.72 [IC 1.2-11.51]) and PMB BSI (4/50, 8% vs 13/641, 2%; OR 4.19 [IC 1.31-13.38]), whereas neither type of disease (acute leukaemia or not) nor neutropenia or prophylaxis with levofloxacin were considered risk factor for AnBSI. Antibiotic susceptibility profile was available in 11/17 cases. None of the *Clostridium* and *Bacteroides* spp bacteria showed metronidazole resistance (4 and 3 cases, respectively), whereas fluoroquinolone resistance was observed in the *Campylobacter* susceptibility available test. Overall, 30-days mortality was 54/691 (7.8%). AnBSI mortality was significantly higher (4/17, 23.5%) in comparison with G- (32/395, 8.1%) (P=0.05) and G+ (5/235, 2.1%) (P=0.0015); it was similar to mortality due to other PMB BSI (9/46, 19.6%) and fungal BSI (4/11, 36.3%) (P=ns). Life-threatening complications was observed in two cases, 11.1% (admission to ICU for respiratory distress and compartment syndrome in 1 case each).
Summary / Conclusion: Incidence of AnBSI was similar in our study to those reported by literature in unselected series. Anaerobic pathogens are frequently associated to other bacteria, particularly enterococci, also among haematological cancer patients, and typically occur with an uncontrolled underlying disease. Our data also confirm that incidence of complications is high as well as mortality rate, which is similar to those of candidemia and other PMB BSI. Further studies are warranted in order to better clarify predisposing factors for AnBSI among haematological cancer pts.

P434

COMPARISON OF THE BD GENEOHMVANR ASSAY TO CULTURE FOR IDENTIFICATION OF VANCOMYCIN-RESISTANT ENTEROCOCCI IN RECTAL AND STOOL SPECIMENS IN PEDIATRIC MALIGNANCY PATIENTS
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Background: Active screening for vancomycin-resistant enterococci (VRE) in rectal and stool specimens has been recommended to limit the spread of antimicrobial resistance within certain high-risk populations including oncology-hematology wards.
Aims: The aim of this study is to compare the diagnostic performance of the rapid real-time PCR test; BD GeneOhmVanR assay (BD Diagnostics,SanDiego, CA) BD with the conventional screening cultures for VRE
Methods: Direct swab specimens were tested by the molecular assay and compared with direct culture. 306 rectal swabs and stool specimens were evaluated with BDGeneOhmVanR assay (BD GeneOhm,SanDiego, CA) that detects the presence of vanA and/or vanB genes. The rectal swaps were inoculated directly to ChromID VRE agar (Biomerieux-France) and enterococci were identifiedusing standard biochemical tests and VITEK 2 automated microbiology system (Biomerieux-France). The susceptibility of the enterococci to vancomycin and teicoplanin was determined using an Etest (Biomerieux-France).
Results: VRE were initially isolated from 37 cultures, and the RT-PCR assay was positive in detected 37 of the 306 samples. The sensitivity and spesificity of RT-PCR was 91.8%(34/37) and 93.6%(252/269) respectively. The positive and negative predictive values for GeneOhmVanRassay was 66.6% and 98,8%.
Summary / Conclusion: VRE colonization is an important health-care problem however infections were mostly common in high-risk patients. Early diagnosis of VRE colonization will help cohorting the patients, transmission of VRE patients and improving infection control measures. In our study; GeneOhmVanR assay is an useful tool for this usage; however its specifity was found to be more impressive compared to its sensitivity.

P435

REASONS FOR TREATMENT SEEKING DELAY IN NEUTROPENIC SEPSIS
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Background: Treatment seeking delay (TSD) may be divided into emergency medical services delay, hospital delay and patient presentation delay. In the United Kingdom hospital delay has been a recent focus of guidelines and standard development for the common and potentially fatal chemotherapy complication of neutropenic sepsis (NS). However, studies of TSD in other settings suggest most TSD is due to patient, not medical service delay. Since NS commonly arises in the out-patient setting, patient factors in TSD represent a substantial but under-researched clinical issue
Aims: To identify the patient processes involved in seeking medical treatment for potential NS in chemotherapy patients and identify a model for TSD which could inform future efforts to improve outcome.
Methods: Between May 2011 and April 2012, all patients receiving chemotherapy at our haematology service received the same written and verbal instructions, consistent with national guidance with 24 hour contact details and advice to seek immediate medical attention for any self-recorded temperature exceeding 38C or after 2 hours of being unwell without fever. Patients admitted with suspected NS who had not followed these instructions were invited to participate in the study. This involved an audio-taped semi structured interview with a psychologist. A social constructionist version of grounded theory was used to explore the processes underlying patient experiences, emotions and identity and to contextualise theory development for this clinical scenario.
Results: 50% (16) of patients admitted with suspected NS had not followed the instructions given to them. 12 agreed to participate in the study. Six theoretical constructs were generated from interview: Recall of Treatment Advice, Symptom Monitoring Behaviour, Symptom Interpretation, Impact of Emotions, Influence of Social Networks and, finally, Preparation and Journey Time. TSD appeared to be shaped by a complex interplay between these constructs comprising 3 stages (Figure 1): 1) Adherence to treatment advice (monitoring signs and symptoms), 2) Contacting hospital, 3) Presenting at hospital. Only in stage 1 is patient recall of advice and instructions a dominant factor, combined with the degree of acceptance that the concept of neutropenic sepsis is relevant to them. In the more complex stage2, monitoring behaviour, interpretation of symptoms – in turn driven by understanding and prior experience of illness – emotion (e.g. fear, denial) and social network influence (e.g. opinion and advice of family or friends) are the key decision making modifiers. Stage3, Presenting at Hospital, centres on practical considerations around the journey such as transport, packing and preparing the rest of the family.
Summary / Conclusion: This study permits a provisional 3 Stage Model of TSD for NS in chemotherapy patients to be developed (Figure 1). There are implications at two levels: Health policy / clinical practice - there is often poor recall of FN information. Future guidelines on NS could recognise that information provision is insufficient and this provision should be structured to maximise recall, for example at different stages rather than during one outpatient visit. Lay population - contextual social factors and social networks heavily influence help seeking behaviour. Patients are more likely to seek treatment for NS if they view it as a real danger and accept previous health beliefs may not apply. Chemotherapy patients also need to believe that seeking treatment would not inconvenience family/friends and healthcare staff.

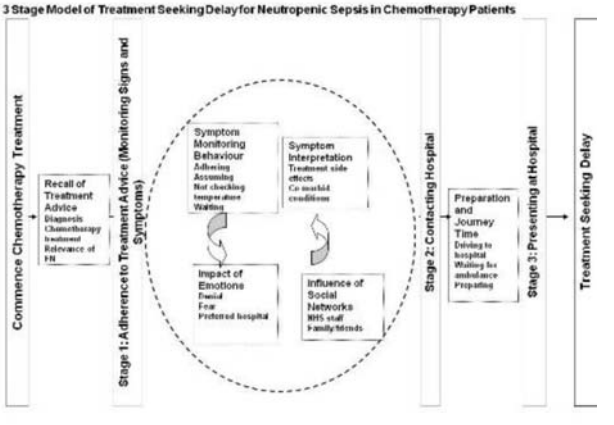

Figure 1.

P436

CEFEPIME-INDUCED ENCEPHALOPATHY IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: CLINICAL FEATURES AND RISK FACTORS
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Background: Cefepime is a widely used antibiotic for patients with hematological malignancies (HM). It can cause encephalopathy, which has been increas-

ingly described in the literature, occurring mainly in patients with impaired renal function. However, the risk factors and characteristics have not been fully elucidated in patients with HM.
Aims: The primary objective of this study was to measure the incidence of cefepime-induced encephalopathy and determine potential risk factors and clinical characteristics.
Methods: We conducted a retrospective cohort study in 222 patients using cefepime with HM at our hospital in the period from August 2011 to February 2013. Other causes of encephalopathy were excluded for all patients with clinically suspected encephalopathy. Head CT, MRI, cerebrospinal fluid examination, and electroencephalography (EEG) were performed to assist in the diagnosis. We also investigated the clinical features of patients with cefepime-induced encephalopathy. We used univariate analysis and assessed the risk factors for the development of cefepime-induced encephalopathy in HM patients. Statistical analysis included two-sampled *t* test and Fisher's exact tests. All calculations were performed using the program R 2.12.1.
Results: Among 222 HM patients using cefepime, 9 were diagnosed with cefepime-induced encephalopathy, thus indicating a cumulative incidence of approximately 4%. These cases corresponded to 4 men and 5 women with an average age of 70 years. With respect to underlying disease, 2 patients had myeloma, 4 had lymphoma, one had leukemia, and 2 had other hematological malignancies. Of 9 patients with encephalopathy, 7 patients had impaired renal function (4 on maintenance hemodialysis), and 1 had impaired liver function when cefepime was prescribed. The average creatinine level at the beginning of treatment was 5.02 mg/dL (range: 0.60-19.85) and the initial dose of cefepime was 3.66 g/day (range: 1.0-6.0). The average time between commencement of treatment and symptoms was 3.4 days (range: 1-5).The most common clinical manifestations were decreased level of consciousness (100%) and myoclonus (30.0%). The EEG was pathological in 6 cases where it was carried out, demonstrating periodic short-interval diffuse discharges with a predominance of triphasic waves in 4 cases, and slowed global activity with repetitive paroxysm in 2 cases. The head CT scan and MRI were normal in all cases. After diagnosis of encephalopathy, treatment with cefepime was discontinued immediately. Symptoms improved fully in all patients, and the average period between symptoms and recovery of encephalopathy was 5.4 days (range: 2-23). Univariate analysis showed that impaired renal function (creatinine, eGFR) ,acute worsening of renal function was significantly associated with development of cefepime-induced encephalopathy (P=0.0001, P=0.025, respectively). Age, sex, weight, liver function abnormality, hypertension, diabetic mellitus, total dose of cefepime per day and unadjusted cefepime dose were not identified as potential risk factors. Receiver operating characteristic (ROC) curves demonstrated that the threshold level of eGFR for cefepime-induced encephalopathy was 31.82 (area under the curve=0.849).
Summary / Conclusion: This study indicated that the development of cefepime-induced encephalopathy was associated with severely impaired renal function (eGFR<30 mL /min/1.73 m^2) in patients with HM. We should be aware of cefepime-induced encephalopathy, especially in HM patients with impaired renal function, and early withdrawal is needed when there is clinical suspicion of encephalopathy.

P437

DIVERSE CLINICAL PICTURE AND ORIGIN OF HEMOPHAGOCYTIC SYNDROME IN ADULT PATIENTS. SINGLE CENTER EXPERIENCE OF 18 PATIENTS

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Background: Hemophagocytic syndrome also called hemophagocytic lymphohistiocytosis (HLH) is a rare but devastating clinical syndrome characterized by fever, splenomegaly, cytopenias, hyperferritinemia, hypofibrinogenemia and hemophagocytosis leading to fatal outcome if untreated. It is frequently misdiagnosed as sepsis because resemblance of the symptoms and frequent secondary bacterial infection. For long time it was considered to be disease of children, where it occurs in mainly primary form produced by mutations affecting function of cytotoxic lymphocytes. In reported cases of adolescent and adult patients it was related either to infection (mainly aberrant EBV) or malignancy. However, the knowledge on its occurrence, diversity and course in adult patients is very limited.
Aims: The aim of the study was to describe relatively large group of patients with this condition referred to our Department.
Methods: For this patients records were evaluated and results reported.
Results: Since 2006 17 patients were referred to our Department, who fulfilled majority of criteria for this syndrome and additionally 1 patient who died in 2000 was retrospectively included. For majority of patients it was not possible to test them at diagnosis for soluble IL-2R and NK cells, so diagnosis was made in 12 patients after fulfilling 5 out of 6 remaining diagnostic criteria and in 6 patients after fulfilling 4 out of 6 diagnostic criteria of HLH. There were 9 cases of EBV-associated HLH, 8 cases of malignancy-associated HLH and 1 case where both PTCL and confirmed recurrent EBV infection contributed to the syndrome. Regarding malignancy-associated HLH there were 2 more cases of PTCL, 2 T-LGL, 1 ALCL ALK (-), 1 DLBCL, 1 AML, 1 CML-BC, and 1 with cancer of unknown origin. Eight patients have been referred in so advanced status that there was not possible to initiate treatment and they died early after admission. Out of 7 EBV-associated HLH patients who have received combination of etoposide, cyclosporine A and steroids (HLH 2004 protocol), 5 are alive and well up to 5 years after treatment. Two patients died of infection day 14 and day 18 of therapy with significantly alleviated symptoms. Patient suffering from both PTCL and EBV-associated HLH died of unrelated cause while in remission of both conditions after CHOP and DHAP treatment. Out of 8 malignancy associated HLH patients, 2 are surviving: one with DLBCL treated with R-CHOP and one with T-LGL treated with methotrexate + G-CSF. The most prominent features in all patients were hyperferritinemia (18/18), fever (17/18), and splenomegaly (18/18) with less regularly observed red cell, platelet and neutrophil deficiencies (18/18 but variable), hemophagocytosis (10/18) and hypofibrynogenemia (7/18) or hypertriglicerydemia (11/18). Many patients suffered from prominent mixed coagulapathy (16/18) with D-dimer level up to 24,6, decreased albumin (11/18) and increased liver enzymes: LDH (16/18), ASPAT (13/18), ALAT (13/18) and hyperbilirubinemia (11/18). Frequent were pleural and peritoneal effusions and skin changes such as panniculitis. Only one patient demonstrated severe neurologic deficits that resolved completely after HLH 2004 treatment.
Summary / Conclusion: Adult patients demonstrating fever, splenomegaly and deficiencies in complete blood count should be tested for ferritin level and if increased further followed for confirmation or exclusion of HLH. Since disease is fatal when untreated, therapy with either HLH 2004 protocol or other disease specific protocol should be initiated as promptly as possible.

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USEFULNESS OF GALACTOMANNAN ASSAY IN BRONCHOALVEOLAR LAVAGE SAMPLES IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES AND RISK OF INVASIVE PULMONARY ASPERGILLOSIS.

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Background: Invasive pulmonary aspergillosis (IPA) is a major cause of morbidity and mortality in patients with haematological malignancies. Definitive diagnosis of IPA is based on demonstration of the fungus in lung tissue by culture and / or histopathology. Clinical status of these patients makes so difficult to get a diagnosis with certainty and is where the role of biomarkers, such as galactomannan (GM) can be useful. GM is a wall component of fungal cell polysaccharide which is released during invasion by *Aspergillus* spp. and can be detected in body fluids. Its determination in serum of patients with haematological malignancies at risk of invasive fungal infection is useful in the early diagnosis of aspergillosis, but its presence in bronchoalveolar lavage (BAL) remains unclear in diagnosing IPA.
Aims: To determine the usefulness of galactomannan assay in bronchoalveolar lavage specimens and its value in clinical practice.
Methods: We conducted a retrospective and observational study, during 32 months (February 2010-November 2012). We got 33 BAL samples from 32 patients diagnosed with hematological malignancies in context of fever and suspected lung infection. Cases were categorized into low, intermediate and high risk of IPA; also diagnostic of IPA were made in base of EORTC/MSG criteria. A chest CT scan was performed in all patients prior to bronchoscopy and BAL. A serum galactomannan antigen (AGA) assay, prior and / or subsequent to the completion of BAL was measured. All patients were receiving or had recently received beta-lactam antibiotics or carbapenems. Samples obtained from BAL were conducted to determine galactomannan (GM), Gram stain, culture of aerobic bacteria and fungal, antigen *P. jirovecii*, PCR [respiratory syncytial virus (RSV), parainfluenza, adenovirus, rhinovirus, coronavirus, bocavirus, metapneumovirus, Influenza and Enterovirus], cytomegalovirus (CMV) Shell-Vial, CMV culture, auramine staining and culture of mycobacteria. The results of GM in BAL, were expressed as ratio, considering positive a value greater than 0.5 (GM +) extrapolating from serum values and its significance.
Results: Of the 33 cases studied, 6 were classified as IPA (5 probable and 1 proven). The proven IPA was based of identification by culture and histopathology of *A. fumigatus* in BAL and lung tissue. 22 cases (66%) were at high risk of IPA, 8 intermediate risk (23%) and 4 (11%) low risk. GM + was observed in 76% of cases (25/33) (range 0.62-6.60). In all cases of IPA, GM had elevated values >3 (range 3.02 - 6.25). In 19/33 cases of GM + BAL were not evidence of *Aspergillus* spp. in the diagnostic screening. In 12/33 cases was isolated an infectious agent, *Candida* spp. (1 case), *P. jirovecii* (2 cases), *E. faecium* (2 cases), CMV (2 cases), RSV (1 case), rhinovirus (2 cases), coagulase-negative *Staphylococcus* (1 case) and *Penicillium* spp. (1 case). CT scan showed variable findings in all patients, being pulmonary infiltrates and cavitation the most frequent radiological pattern in patients with IPA (4/6). Positive and negative predictive values of determination of GM in BAL was 24% (95% CI 11.5-43.4) and 100% (95% CI 67.6-100) respectively. Sensitivity and specificity of the test was 100% (95% CI 61-100) and 29.6% (95% CI 15.9-48.5).
Summary / Conclusion: In our experience determining galactomannan in BAL specimens using a ratio ≥ to 0.5 is useful to identify a group of patients at risk

for aspergillosis with very low probability of getting the infection. This limited series does not allow establishing other cutoff value to improve the specificity of the test, although it is noted that all IPA cases in this study, proven or probable, showed elevated levels of GM in BAL (ratio> 3). It is necessary to carry out studies with larger series to clarify these issues.

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RISK FACTORS RELATED TO MORTALITY IN HEMATOLOGICAL PATIENTS ADMITTED TO INTENSIVE CARE UNIT (ICU)

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Background: Patients admitted into the intensive care unit (ICU) because of complications in the treatment of a hematological malignancy have a poor prognosis classically. However, recent studies indicate that advances in managing critically ill cancer patients and in selecting them for ICU admission have improved their outcome.
Aims: To assess the main clinical features of this group of patients at discharge from the ICU in a tertiary university hospital and to identify possible risk factors associated to mortality such as invasive mechanical ventilation (IMV) requirement.
Methods: We retrospectively collected data of all patients with a hematological disease admitted to the ICU between January-2009 and April-2012. Recorded variables included: sex, age, hematological diagnosis, disease status, type of treatment received, evidence of sepsis criteria, major reason for ICU admission, need for IMV, use of vasopressors agents, ICU lenght of stay, laboratory values at admission, and APACHE II and SAPS II scores calculated within the first 24 h. Predictors for in-hospital mortality were evaluated using uni- and multivariate analysis.
Results: A total of 21 patients (median age 55, inter-quartile range IQR 15-80, and male 66.6%) were analyzed. Of these, 95.2% had a hematological malignancy, 47.6% with a recent diagnose, and only in two cases ICU admission coincided with disease progression. The main causes of admission were pneumonia associated with acute respiratory distress syndrome (ARDS) and sepsis of any origin (28.6% each). The average stay in the ICU was 12.4 days (IQR 1-60). Globally, 71% had received chemotherapy during the 2 weeks prior to admission. Severe neutropenia was present in 38% of patients, and 50% of them died during ICU stay. IMV requirement was recorded in 66.6% of patients, of which 50% died; however, only 14.3% of patients who did not require IMV were exitus. No statistically significant relationship was found between IMV and mortality (P=0.133). Fourteen patients (66.6%) required vasopressor drugs, of which 57% died; by contrast, all patients who did not need amines (33.3%) survived (P=0.018). The mean scoring systems APACHE II and SAPS II were 18.7 and 46.1 respectively, and a relationship between them and mortality was not observed (P=0.804). The overall mortality rate during ICU stay was 38%. Of the 13 patients alive at discharge from ICU (62% of total), two (15.4%) died of ARDS within a week, both in situation of hematologic disease progression. The overall in-hospital mortality was 47.6%, and overall survival at 6 months after ICU discharge was 47.6%.
Summary / Conclusion: The overall mortality rate in our population is slightly below average described in the literature. Regarding prognostic factors associated with mortality, we observed only statistically significant association between this and the need of vasopressor amines administration. Finally, although the sample size is small, mortality was not significantly associated with IMV, the degree of neutropenia or the haematological disease type.

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AUDIT OF POSACONAZOLE USAGE IN THE HAEMATO-ONCOLOGY UNIT AT ST BARTHOLOMEW'S HOSPITAL

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Background: Posaconazole, a triazole antifungal, is indicated for use in the prophylaxis and treatment of invasive fungal disease (IFD) in adults. In 2007, our antifungal management protocol was changed to include posaconazole for IFD prophylaxis in patients with graft versus host disease (GVHD) receiving high dose steroids (≥1mg/kg/day of prednisolone or equivalent) and to stop posaconazole at a steroid dose of <0.25 mg/kg/day.
Aims: To establish compliance with the protocalised use of posaconazole.
Methods: Data was collected retrospectively with posaconazole use identified from an existing database, F.A.T. (fungal audit tool) and pharmacy records. 67 patients received posaconazole between January 2006 and December 2011. Review of their records revealed 100 episodes of posaconazole prescriptions. Toxicity was assessed from the clinician reported information, as well as laboratory parameters.
Results: Data was captured for 99 prescriptions, of which 63% were for prophylaxis in the setting of GVHD, 19% for suspected IFD, 8% for prophylaxis during chemotherapy, 6% for previous IFD (secondary prophylaxis) and in the remaining 3% episodes indications were not documented. In terms of the dosing schedule, 68% (n=66) were prescribed 600mg per day for prophylaxis as per protocol in GVHD; 28% (n=28) were prescribed 800mg per day, which included all "treatment" use, but also prophylactic use in 9% of episodes. The average duration of a posaconazole prescription was 61.5 days, which was longer in the outpatient setting (69.9 days) than for inpatients (13.5 days). Steroids were prescribed in 55 episodes, all for GVHD management. Posaconazole was discontinued appropriately in 32 episodes as per protocol. No stop date was available in 3 episodes. In the remaining 20 prescriptions, 7 prescriptions were for the treatment for IFD and hence not stopped at the same time as steroids; however, the protocol was not followed in 13 cases and prophylactic posaconazole was continued, representing a cost of £34.533. Evidence of symptomatic toxicity was seen in 8% of episodes, which included tiredness, visual disturbances and vomiting, none of which required discontinuation. In 13 episodes, posaconazole was started despite hyperbilirubinaemia and/or transaminitis (>5x upper limit of normal): at the cessation of posaconazole, liver function had improved/returned to normal in 10; was stable in 1; and worse in 2 cases. Posaconazole induced hepatoxicity was documented in only one episode and led to cessation of treatment. Posaconazole was changed to a new antifungal in 13 episodes without evidence of toxicity, liver impairment or intolerance: Ambisome in 6 cases; caspofungin in 3; voriconazole in 4. Although this is indirect evidence, it suggests a maximal breakthrough rate of suspected IFD of 13%.
Summary / Conclusion: The greatest use of posaconazole was for IFD prophylaxis in the setting of GVHD as per protocol. Posaconazole use appeared to be safe even with significant liver impairment. One third of posaconazole use was non-protocolised, mainly prophylaxis for chemotherapy. Overall, this audit highlights appropriate prescribing by clinicians. However, the failure to stop posaconazole prophylaxis in the GVHD setting on reduction/stopping of steroids has a significant budgetary impact. The results of this audit have led to the design of a specific request form for all outpatient prescriptions for posaconazole (and voriconazole), with monthly monitoring to ensure appropriate management in terms of indications and duration of therapy.

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CHILDREN WITH MACROPHAGE ACTIVATION SYNDROME: A SINGLE CENTER EXPERIENCE

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Background: Macrophage activation syndrome (MAS) is a severe, potentially life-threatining complication of childhood systemic inflammatory disorders. Recognition of MAS in patients with JIA and SLE is often challenging because MAS may mimic all signs and symptomps even laboratory features of the underlying disease.
Aims: We aimed to document all relevant clinical and laboratory features, treatment and outcome of MAS in our center.
Methods: This report presents a retrospective review of 22 patients diagnosed as MAS in Hacettepe University Ihsan Dogramaci Childrens Hospital. Our group (17 JRA and 5 SLE) included 12 girls and 10 boys and the median age of MAS onset was 11,5 years(range 16 months-16 years). The mean duration of underlying disorder was2,5. months(range 0-156,5 months) for the whole group however median duration was 0 months(range 0-156,5 months) for JIA and 12 months(10-24 months) for SLE. Patients with JIA were diagnosed to have MAS when they developed prolonged fever with organomegaly and worsening blood values especially platelet count which is progressively decreasing, decreased sedimentation rate, deranged liver functions, elevated ferritin level, elevated Vit B12 and LDH level, hyponatremia, hypoalbunemia, coagulopathy, encephalopathy and other diagnostic criterias for HLH -2004 with or without eveidence of hemophagocytosis in the bone marrow.
Results: Twelve patients with JIA were presented with MAS. At the time of the diagnosis fever at ≥38 C was observed 93% of the patients. Leucopenia, anemia and thrombocytopenia was found in 12%, 81% and 75% ,respectively. Median Hb, WBC and platelet count were 9,4 g/dL (range 6,4-15), 5100/mm^3 (range 900-70200) and 88500/mm^3 (range 21000-536000),respectively. When compare SLE with JIA at the time of MAS episode , it seems that patients with SLE had lower blood counts (median Hb, WBC and platelet count 7,6 g/dl(range 7-13,6), 1450/mm^3(1100-5000) and 65000/mm^3 (21000-99000/mm^3)) than JIA(median Hb, WBC and platelet count 10 g/dL (range 6,4-15), 10800/mm^3(900-70200) 995000/mm^3 (27000-536000/mm^3) respectively. Median ferritin level was 16728 µg/L (range 3747-150099). Vit B12 is available for 14 patients and 6(43%)of them were found to be elevated. The overall mortality rate was 40% in our group. Mortality rate for SLE was 60% (3 died, 2 alive) and it was 35% for JIA(6 died, 11 alive).
Summary / Conclusion: Literature review did show that we have relatively a large number of cases with MAS in single center experience beside there is an

accumulating case series in the recent years. According to our experience we can say that some of those patient has a very silent MAS clinic perhaps to due to underlying genetic feautures. On the other hand some of those patients rapidly worsened beside a prompt recognition and immediate therapeutic intervention. There were 3 patient presented in this study had consanguinity and none of them revealed specific mutations caused by primary HLH. In conclusion, MAS patients may presented with almost within a normal limit of blood values, however clinician kept in mind that platelet and sedimentation decrease will give a very important diagnostic clue. Bone marrow aspiration does not necessarily show hemophagocytosis at the first admission, serial examination with checking ferritin level regularly is helpful to put the diagnosis. Patient clinical status is also very important during these follow up period, in case of unexpected worsening of the patient even in the absence of any MAS criteria, prompt evaluation probably will be life saving.

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DIAGNOSTIC EFFICACY OF GALACTOMANNAN ASSAY AND COMPUTERIZED CHEST TOMOGRAPHY IN INVASIVE ASPERGILLOSIS OF FEBRILE NEUTROPENIC CHILDREN

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Background: Invasive pulmonary aspergillosis (IPA) is a leading cause of morbidity and mortality in children with haematological malignancy and it can result in various clinical presentations due to the characteristics of fungus. Early diagnosis is quite important for better prognosis, however it is difficult to achieve this through the conventional methods Detection of IPA represents a formidable diagnostic challenge and, in the absence of a 'gold standard', a combination of clinical data and microbiology with histopathology is helpful. Culture-based approaches rely on the availability of biopsy samples, but these are not always accessible in sick patients, Blood and radiologic tests are frequently used for diagnosis of invasive pulmonary aspergillosis, but it remains unknown which is more useful for its early diagnosis.
Aims: In our study, we aimed to evaluate the diagnostic methods, that are based on measurement of galactomannan levels and imaging with computerized chest tomography of neutropenic patients with persistent fever (> 5 days duration) and to evaluate the incidence of aspergillus infection depending on these methods.
Methods: In this study, children with haematological malignancies and fever were enrolled prospectively. Fifty-three febrile neutropenic episodes of 32 patients, who have been followed up because of hematologic malignancies in Pediatric Hematology and Oncology Department of Kanuni Sultan Süleyman Education and Research Hospital were eveulated. Microbiological sampling was performed to determine the etiology. Clinical samples were assessed through culture and direct microscopy. Aspergillus antigen was detected with serum galactomannan (GM) levels. Blood sample for GM was drawn on the day of admission; levels were measured with Platellia Aspergillus enzyme immunoassay. Computerized chest tomography is used for determining invasive pulmonary aspergillosis. Invasive fungal infection was diagnosed according to the latest EORTC/MSG criteria. Proven, probable and possible episodes were considered as the disease groups.
Results: The mean age was 7.52±3.82 years. Thiry-one patients were treated for acute lymphoblastic leukemia,seventeen patients for acute myeloid leukemia and five patients for solid tumors. Twenty-one of 53 (39.6%) episodes were diagnosed with IFI, while six of them were probable, fifteen of them (28.3%) were possible IFI . No IFI was detected in 32 of the patients. It is observed, that prolonged neutropenia is a risk factor for invasive aspergillosis (P<0.05). The coincidence of pulmonary lesions seen at X-rays and computerized tomography was only 42%. The findings on chest CT were halo sign (7.5%), cavitation (3.8%) and nodules (22.6%) and other nonspesific findings (49.1%). For the diagnosis of invasive aspergillosis halo sign is more sensitive than other findings (P<0.05). The progression of CT findings is a sign of poor prognosis (P<0.05).Thirty-nine of 228 (17.1%) samples were positive for galactomannan. The false positive results were present in 29.4%. A close relationship was found between CT findings and GM positivity (P<0.05). The mortality rate due to fungal infection was 14.3%.
Summary / Conclusion: Serologic follow up with galactomannan levels and chest CT are important for the successful diagnosis and management of invasive aspergillosis in febrile neutropenic children associated with hematological malignancies.

Transfusion medicine

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VESICLES OBSERVED IN LEUCOREDUCED AND NON LEUCOREDUCED CONCENTRATED RED BLOOD CELLS BY MEANS OF ADVANCED MICROSCOPES: POSSIBLE RELATION TO THE STORAGE LESION

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Background: Red Blood Cells (RBCs) constitute the basis of probably the most important blood product that is widely used in transfusions in clinical practice. Processed RBCs (pRBCs), leucoreduced (LR) or not (nLR), are isolated from the plasma, redispersed in a storage solution in concentrated form and finally stored at 2-6 ºC under sterile conditions. Normally, the storage duration does not exceed 42 days due to the pRBCs biochemical degradation that is accompanied by morphological modification of their membrane, a sequel of processes that is termed 'storage lesion'. Specifically, the morphological modification of the pRBCs membrane is established by a gradual reduction of both surface and volume that is commonly ascribed to the exocytosis of cytoplasm and membrane parts through the releasing of *spherical* micro/nano-vesicles (SmnVs).
Aims: To investigate the morphological characteristics of the released vesicles and deduce detailed information on their dimensions at the nanometer level we employed advanced microscopes.
Methods: Samples were drawn and investigated progressively (day 0, 1, 10, 20, 30, 40, 42 and 50) from two units (CPDAM: citrate, phosphate, dextrose, adenine and mannitol), one LR-pRBCs and one nLR-pRBCs both stored at 2-6 ºC. The imaging techniques employed in this study were the conventional Optical Microscope (OM) and the advanced Atomic-Force Microscope (AFM) and Scanning-Electron Microscope (SEM) that both have extreme spatial resolution. These microscopes except for the overall morphological characteristics of the complete cell at the micrometer level (1 μm=10^{-6} m) can reveal specific details of the cell membrane at the nanometer level (1 nm=10^{-9} m). We stress that both LR-pRBCs and nLR-pRBCs were investigated in their *intact* form with only minimum processing, carefully avoiding mechanical stress (intense centrifugation) and or biochemical shock (washing with any kind of solution). Specifically, for the AFM measurements the smears were completely *intact*, while for the SEM investigations a thin metallic overlayer was unavoidably deposited to the otherwise *intact* smear.
Results: SmnVs, but most importantly *cylindrical* mnVs (CmnVs), were observed in both LR-pRBCs and nLR-pRBCs by means of AFM (upper Figure 1) and SEM (lower Figure 1). The CmnVs most commonly had spherical ends. The cylindrical part had length within 0.2-10.0 μm and diameter within 70-400 nm. Regarding the spherical ends of the CmnVs they had diameter in the range 100-700 nm. The CmnVs were detected even during the early stages of storage (day 1), but exhibited a pronounced increase (two orders of magnitude) with storage duration (threshold day 10).
Summary / Conclusion: The observation of CmnVs in pRBCs is reported here for the first time. This finding raises questions on the origin of the relative SmnVs that are only observed by means of scanning/transmission electron microscopy in samples produced after intense processing such as centrifugation, fixation etc. The incompatibility of the morphological and geometrical characteristics of the extended CmnVs with the RBCs and the lack of microscopy snapshots that prove the releasing of the former from the later are against the scenario of CmnVs direct releasing from RBCs. Extra studies are surely needed to clarify the origin of the CmnVs reported here and the possible relation with the SmnVs that are commonly reported in the literature.

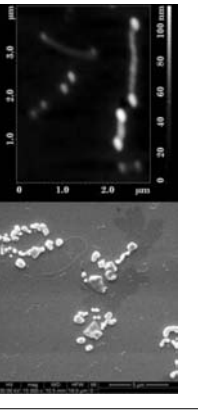

Figure 1.

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SUCCESSFUL STEM CELL RE-MOBILIZATION AND HARVEST IN PATIENTS WITH MULTIPLE MYELOMA RELAPSED AFTER A PREVIOUS MYELOABLATIVE AUTOLOGOUS TRANSPLANT.

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Background: Background: Autologous stem cell transplantation (ASCT) still remains a necessary component of a therapeutic program in patients affected by multiple myeloma and younger than 65 years. However, in relapsed patients, despite the improvements reported with novel drug therapies, outcome remains not satisfactory. Recently it has been shown that a second ASCT represents a viable option in individuals with chemosensitive relapse after a first ASCT; however in a proportion of patients an adequate storage of PBSC had not been planned at diagnosis in order to ensure a second salvage transplantation. Melphalan (MEL) is traditionally considered the best anti-myeloma drug and furthermore it is extensively used at myeloablative doses (200 mg/sm) in the conditioning regimen; however pretreatment with high dose of melphalan is a known risk factor for poor mobilization of PBSC, because it can damage bone marrow microenvironment and stem cell hematopoiesis, finally impairing future attempts of mobilization rate.
Aims: Aims: Aim of this study was to analyze retrospectively whether there was the possibility to perform a stem cell mobilization in a relatively rare category of multiple myeloma patients, who have already undergone a first myeloablative ASCT and do have an indication for a second one.
Methods: Methods: A research was carried on in the database of five italian hematologic centres between 1998 and 2012 and 9 patients (median age 59 years) were found with those charactheristics, namely with a chemosensitive disease, prolonged remission after a prior autograft, exclusion from an allogeneic transplant for older age at relapse (4 patients), patient's refusal (2 patients) or inability to find a matched donor (3 patients). The recommended schedule for mobilization included: 1) intermediate doses of chemotherapy at day 0 (4 patients) followed by daily injection of G-CSF (10 mcg/kg) starting from day 5 or 2) daily injection of G-CSF (10 mcg/kg) for four consecutive days. Peripheral CD34+ blood monitoring was started from the fifth day of G-CSF and in case of low blood count of circulating CD34+ (< 20/mcl), a single injection of Plerixafor was planned in order to prevent a mobilization failure
Results: Results: All patients have a chemosensitive relapse after a previous ASCT performed with MEL 200 mg/sm as conditiong regimen. Median time of remission after first ASCT was 5 years (range 2-12). Median circulating CD34+ at the day of starting leukapheresis was 30/mcl (range 25-500 CD34+/mcl). The collection goal of at least 2×10^6 CD 34/kg was achieved in all patients (median yeld of 3×10^6 CD34+/kg, range 2-19). Allowing for the small number of patients, there were no differences in terms of number of circulating and harvest of CD34+ cells, except for the number of aphereses requested for patients mobilized with G-CSF only (median 2 aphereses), compared with the addition of Plerixafor (1 apheresis for all patients).
Summary / Conclusion: Conclusions: To date, there is a very limited number of reports about successful stem cell mobilization and harvest in patients with multiple myeloma who have undergone a previous ASCT. In our retrospective analysis, we demonstrate that: i. Melphalan administered as conditioning regimen at myeloablative doses is no longer to be considered a limiting factor for future stem cell re-mobilization in patients without CD34+ back-up and in whom there is the need for a second ASCT. ii. Addition of Plerixafor could provide an increase of circulating CD34+ in order to assure stem cell yield while maximizing the cost and time effectiveness.

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ALLOANTIBODY FORMATION IN PATIENTS WITH SICKLE CELL DISEASE

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Background: Sickle cell disease (SCD) is a hereditary hemoglobinopathy, characterized by chronic anemia, recurrent pain and irreversible organ damage. Transfusion of red blood cells (RBC) is a common intervention to treat and prevent these complications. However, frequent transfusions may lead to erythrocyte alloimmunization, thereby complicating donor matching procedures and posing patients at risk for hemolytic transfusion reactions.
Aims: The primary aim of this study is to evaluate the cumulative incidence of first alloantibody formation in a Dutch cohort of transfused SCD patients, and to compare this with a general Dutch RBC-transfused population. In addition, potential clinical determinants of alloimmunization and the effectiveness of extended RBC matching protocols in reducing the incidence of alloantibody formation in SCD will be assessed.
Methods: We conducted a retrospective cohort study and collected data on SCD patients (genotypes HbSS, HbSC, $HbS\beta^0$ and $HbS\beta^+$ thalassemia), diagnosed in the Academic Medical Center, Erasmus Medical Center and Haga Hospital in the Netherlands, that received non-extended matched (ABO, RhD) RBC transfusions between 1984-2004 and extended matched (at least ABO, Rhesus phenotype, Kell) RBC transfusions between 2004-2011.
In addition, we compared this population with a general population of 3 042 patients that received non-extended matched (ABO, RhD) RBC transfusions between 2005-2009 in the Leiden University Medical Center (Zalpuri *et al*. 2012). Cohorts were not matched for ethnicity. Alloimmunization incidences and potential associations with clinical determinants were respectively estimated by Kaplan-Meier and Cox-regression analyses.
Results: A total of 294 SCD patients received 7 961 RBC units. Alloantibody formation occurred in 52 (17.7 %) patients. The cumulative incidence of alloimmunization was 9% after 5 RBC units, 17% after 10, 24% after 20 and 34% after 40 RBC units. Multivariate analysis, correcting for the number of transfusions received, demonstrated a significantly increased risk of alloantibody formation in our SCD cohort when compared to a general population of transfused patients (HR 7.5 (95% CI: 5.06-11.13), where the cumulative incidence of alloimmunization was 1.1% after$_5$, 2.4% after 10, 3.4% after 20 and 6.5% after 40 RBC units. No association was found between alloantibody formation and clinical determinants such as gender, SCD-phenotype or ethnicity. However, a significant reduction in alloimmunization was observed in SCD patients that received their first transfusion from the year 2004 onwards, after preventive matching for Rhesus phenotype and Kell was introduced for SCD patients (HR 0.51 (95% CI: 0.258-0.989). (Figure 1)
Summary / Conclusion: The overall rate of first RBC alloantibody formation in our SCD cohort was 17.7% and the risk of alloimmunization increased substantially with an increasing number of RBC transfusions. A unique comparison with a general cohort of Dutch transfused patients demonstrates a significantly higher risk of alloantibody formation in SCD, acknowledging earlier findings. Partially, this can probably be explained by the differences in RBC antigens between patients of African descent and the predominantly Caucasian donors. Besides the number of RBC units, no other clinical risk factors for alloimmunization in SCD could be identified. The effectiveness of extended RBC matching protocols in the prevention of alloimmunization for chronically transfused patients in the participating centers was confirmed.

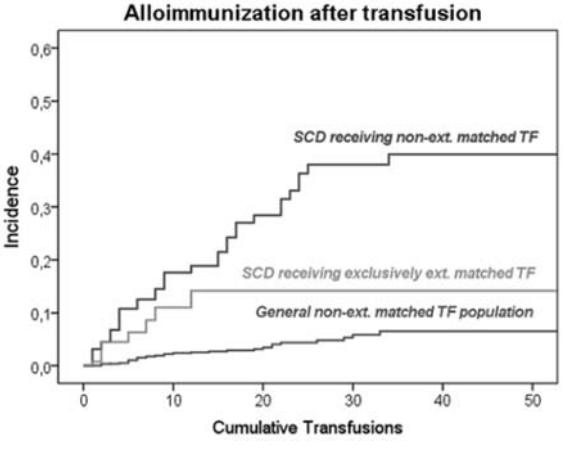

Figure 1.

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THE ROLE OF BLOOD TRANSFUSION IN BETA-THALASSEMIA: AN EX VIVO STUDY OF HEMOSTATIC PARAMETERS

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Background: Blood transfusion is one of the main therapeutic strategy for ß-thalassemia. Splenectomy is often required. In the last decades clinical trials report on a high rate of thromboembolic events, particularly in ß-thalassemia intermedia and in splenectomized patients. Regular transfusions seem to significantly reduce the relative risk of thrombosis.
Aims: To describe the post-transfusional modifications of hemostatic parameters in ß-thalassemia.

Methods: Consecutive patients followed-up at the Center of Immunohematology and Transfusion Medicine of Bergamo were screened. Inclusion criteria: ß-thalassemia requiring regular blood transfusions and acquisition of written informed consent. Exclusion criteria: ongoing therapy with anticoagulant or antiplatelet drugs. Seven thalassemia carriers (trait) and nine healthy donors were enrolled as a control group. Blood samples were obtained before the monthly scheduled blood transfusion and 60 minutes post-transfusion. The following tests were performed: hemocromocytometry by fully automated hematology analyzer XE-2100 (Sysmex); platelet function by Multiplate® platelet function analyzer with Adenosine DiPhosphate, arachidonic acid (ASPI), COLLagen or Thrombin Receptor Activating Peptide 6; flow cytometry to detect platelet activation (as a percentage of cells expressing surface P-Selectin, Tissue Factor, and/or Fibrinogen) by FACS Canto™ II (BD Biosciences) before and after ADP-stimulation. In addition, thrombin generation (TG) was performed by calibrated automated thrombography (CAT assay, Thrombinoscope™) in platelet-rich plasma spiked with 1 pM Tissue Factor or ADP (1.6 and 8.3 µM). TG results were described in terms of lag-time, peak height, time-to-peak and endogenous thrombin potential.
Results: Nine ß-thalassemia patients were enrolled from May to August 2012 (mean age 28.9, age range 5-48, ß-thalassemia intermedia 33%, splenectomy 44%, prior history of thrombosis 22%). Before blood transfusion, splenectomized patients had a higher increase of platelet surface TF expression upon ADP-stimulation (by flow cytometry) compared to healthy controls (P<0.05). In the same patients the platelet function was significantly higher than healthy donors (ADP P<0.01, COLL P<0.001, TRAP P<0.05, ASPI P<0.001), thalassemic trait subjects (ADP P<0.05, COLL P<0.01, ASPI P<0.05) and non-splenectomized patients (ADP P<0.05, COLL P<0.001). In splenectomized patients we observed a decrease (not statistically significant) in platelet aggregation capacity to all agonists after blood transfusion. In the same group of patients, the lag-time and time-to-peak of TG performed in the presence of TF or ADP were significantly shorter than in controls both before (lag time TF P<0.01, ADP P<0.05; time to peak TF P<0.001, ADP P<0.01) and after blood transfusion (lag time TF P<0.05, ADP P<0.05; time to peak TF P<0.05, ADP P<0.05). Differently, the hemostatic profile of non-splenectomized patients was similar to controls.
Summary / Conclusion: In routinely transfused ß-thalassemia patients, splenectomy is associated with a prothrombotic shift of hemostatic parameters (i.e. thrombocytosis, enhanced platelet reactivity and function, and earlier thrombin generation), which are not influenced on the short term by blood transfusions.

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AN EVALUATION OF PREDICTIVE SCORES FOR MASSIVE TRANSFUSION IN A TRAUMA COHORT CLINICALLY JUDGED TO BE AT HIGH RISK OF MAJOR HAEMORRHAGE

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Background: Major Transfusion Protocols (MTPs) provide a timely and co-ordinated response to traumatic Major Haemorrhage (MH). MTPs aim to prevent coagulopathy and must be activated during the early stages of resuscitation. The decision to activate an MTP is complex. Although predictive models for Massive Transfusion (MT) exist they are not widely employed. A senior clinician's judgment remains the most common trigger for MTP activation and de-escalation. Under-recognition of MH has grave consequences, however use of MTP in non-massive haemorrhage is associated with adverse outcomes. One pragmatic solution to balance these opposing risks is to escalate transfusion support early, but promptly de-escalate when the MTP is not required.
Aims: We sought to evaluate whether predictive scores for MT can determine the likelihood of progressing to an advanced phase of a MTP (> 6 red cell concentrates) in patients judged to be at risk of MH and already initiated on a MTP.
Methods: This work was undertaken at a single academic UK major trauma centre. The department's MTP is ratio-based and activated at the discretion of a senior trauma clinician. MTP pack 1 consists of 4 units of red cell concentrate (RCC) and 4 units of fresh frozen plasma (thawed from frozen). Prior to MTP pack 1 arrival, 2 units of group O RhD-ve RCC are available in the emergency department for immediate transfusion. MTP pack 2 and subsequent packs contain an additional adult dose of platelets. Data about blood component usage were prospectively recorded during every MTP activation. To derive the Trauma Associated Severe Haemorrhage (TASH) score and Assessment of Blood Consumption (ABC) score for each patient, data were retrospectively collected from the patient's electronic record, written notes, laboratory system and the regional trauma audit research network. Sensitivity, specificity and receiver operating characteristic (ROC) analysis were performed by defining a positive outcome as use of > 6 RCC units. This figure was chosen as it marked progression to an advanced stage of the MTP i.e. to MTP pack 2.
Results: A total of 118 trauma MTP activations were reviewed. The mean age of trauma cases was 40.8 years, 73% were male. The mean injury severity score was 27. Blunt injury accounted for 81% of cases, the remainder (19%) were due to penetrating trauma. The ABC and TASH scores were strongly associated with the total number of RCC transfused. Linear regression analysis confirmed that both the TASH score (R^2=0.63, P=< 0.0001) and ABC score (R^2=0.28, P=0.004) remained predictive of total RCC usage. A TASH score of ≥7 provided a sensitivity of 90% for progression to MTP pack two with a specificity of 67%. The TASH score had an area under ROC (AUROC) of 0.88 in our cohort (Figure 1). An ABC score of ≥2 had a sensitivity of 25% with a specificity was 96% for the same outcome. The AUROC was 0.8 for ABC.
Summary / Conclusion: We have demonstrated that both the TASH and ABC scores retain an association with the total volume of RCC transfused in a cohort of trauma patients at high risk of MH. The TASH AUROC analysis demonstrated that this model may be useful to predict the need to progress to MTP pack two (>6 RCC units) and beyond. Our data suggest that, in addition to MTP activation, the TASH score can objectively guide clinical decisions about continuation of a major transfusion protocol.

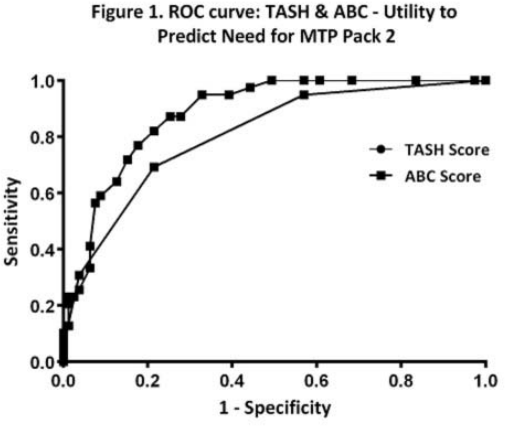

Figure 1.

P448

TRANSCRIPTIONAL PROFILING OF HUMAN ERYTHROID PROGENITORS FROM G-CSF MOBILIZED AND NONMOBILIZED PERIPHERAL BLOOD

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Background: Progenitor cells from a variety of sources including bone marrow, cord blood, and peripheral blood (PB) have been used for transplantation. Granulocyte colony-stimulating factor (G-CSF) mobilized PB (mPB) progenitor cells have been successfully used for both autologous and allogenetic transplants.
Aims: The functional and genomic characterization of primitive and more mature populations of mPB can be very useful in examining the role of these progenitors in engraftment following transplantation.
Methods: We correlated gene expression patterns of highly enriched steady-state PB- and mPB-CD34$^+$ cells by cDNA array technology, to identify molecular causes underlying the functional differences during *in vitro* erythroid differentiation. To better understand the transcriptional program that accompanies erythroid differentiation, we performed oligonucleotide microarray analysis of erythroid CD71$^+$ progenitors harvested after 6 days of culture.
Results: The total gene expression is almost doubled in mPB-derived (4417) compared to PB-derived erythroid progenitors (2246). The genes only present in PB-derived erythroid cells are NUP85, NOP10, SLC30A5, and CHD1L genes, while only present in mPB-derived erythroid cells are ESRRB, DTX4, and KIT genes. Comparative analysis of transcript levels shows differential expression of 88 genes between mPB- and PB-derived erythroid progenitors. We identify 13 genes (CRTC3, VIM, SERPINH1) that are downregulated in both pools of cells; per 7 genes (TM9SF2, TCP1, TGIF2) are downregulated in PB- and mPB-derived cells respectively, while 61 genes (ERAF, MTSS1, VAMP7) are upregulated in both pools of cells. Using Ingenuity pathways analysis we describe the network of genes linked to cancer, hematological diseases, cell growth and proliferation. The G-CSF receptors CSF2RA and CSF2RB are linked to STAT3 transcription factor influenced by upregulated erythroid-specific ALAS2, CLC and GATA2 genes preferentially more expressed in mPB-derived erythroid progenitors. GATA2 and ALAS2 transcription factors are linked to NUDC and GTF3A genes upregulated in PB-derived erythroid progenitors.
Summary / Conclusion: As expected, the G-CSF stimulated network has predominantly increased total gene expression in mPB-derived erythroid progenitors. This report provides an extensive transcriptional profile of erythroid progenitors and leads to a better understanding of diversity among the hematopoietic progenitor sources.

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IMPACT OF HLA ANTIBODY LEVELS ON PLATELET TRANSFUSION REFRACTORINESS

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Background: Poor post-transfusion platelet increment, PPI, (post-transfusion platelet count minus pre-transfusion platelet count) may be fatal in high dose treatments of malignant disorders. In most cases the poor increment is secondary to non-immune causes such as infections, splenomegaly or medications. Immunologically mediated refractoriness caused by alloantibodies against HLA-A and –B locus antigens is usually induced by previous pregnancies or non-leukocyte-reduced transfusions. In these cases the availability of HLA-matched platelets is crucial.
Aims: The aim of this study was to analyse the impact of the HLA class I antibodies on PPI.
Methods: We have analysed PPI of 270 platelet transfusions from HLA-identical or selectively mismatched donors of 40 adult patients with hematological disorder (22 AML, 6 MDS or MDS-AML, 3 ALL, 3 MM, 1 NHL, 5 SAA). 31 (78 %) of the patients were women and 9 (22%) were men. Since 2003, all blood components in Finland have been leukoreduced with filtration. One platelet transfusion comprises of 2 apheresis products, both equivalent to four units of pooled buffy coat platelet concentrates. The median number of platelet transfusions per patient was5, range 1-23. All transfusions were prophylactic. The presence of HLA class I antibodies was determined by Luminex-based IgG single-antigen-bead assay. Donors were selected with HLAMatchmaker computer program and the selection was modified with the HLA antibody profile of the patient. ABO incompatibility, an infection at the time of the transfusion, and body weight were also included in the analysis.
Results: The median pre-transfusion platelet count was $15x10^9$/l, range 1–86. PPI at one hour was available in 92 transfusions, and at 24 hours in 253 transfusions. The median PPI at one hour was $40x10^9$/L (range -16–91) and the median PPI at 24 hours was $28x10^9$/L (-61–123). The 24 h PPI was >$20x10^9$/L in 168 (65%), >$30x10^9$/L in 116 (49%) and >$40x10^9$/L in 78 (31%) out of 253 transfusions. In 103 transfusions (38 %) donors were HLA-compatible. They were either identical (54 cases) or they had no antigens against which the patent showed antibodies (49 cases). In 167 (62%) transfusions donor specific antibodies were present with the median level of 2026 mean fluorescence intensity, MIF (range 299-29203). Impact of the donor specific HLA antibody levels on PPI as an independent risk factor was highly significant (P=0.001). Antibody levels less than 1000 MIF had no negative effect on PPI. Other independent risk factors were the presence of an infection at the time of the transfusion (P=0.01) and the age of the product in days (P=0.002).
Summary / Conclusion: Simple measures, such as PPI, for platelet refractoriness are appropriate in everyday clinical practice. The availability of HLA identical platelets may be limited for patients with class I HLA alloantibodies. The selection of incompatible platelet donors using HLAMatchmaker computer program supported with the HLA antibody profile of the patient may improve PPI. In these cases, good co-operation between clinicians and blood service consultants is mandatory.

P450

MATERNAL AND FETAL OUTCOMES IN PREGNANT PATIENTS WITH SICKLE CELL DISEASE WHO APPLIED PROPHYLACTIC RED BLOOD CELL EXCHANGE PROCEDURE

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Background: Sickle cell disease (SCD) is one of the most common inherited diseases worldwide and is associated with anaemia and intermittent severe pain. Pregnant women who are affected have increased maternal and fetal mortality and morbidity. Maternal risks include painful prepartum and postpartum crises, urinary tract infections, pulmonary complications, anemia, gestational hypertension, pre-eclampsia or eclampsia, and death. Fetal complications include preterm birth and associated risks, intrauterine growth restriction, fetal distress during labor, and a high rate of perinatal mortality.
Aims: This retrospective study examines the pregnancy outcomes of SCD patients who underwent red blood cell exchange (RBCX) procedures.
Methods: A total of 45 pregnants underwent apheresis prophylaxis for mentioned complications. Exchange procedures were performed in 20 patients for prophlaxis of mentioned complications. Four patients underwent RBCX for treatment of vasoocclusive crisis. Prophylactic RBCX procedures were performed in the second or third trimester of pregnancy and before the caesarean.Two different continuous flow apheresis systems (Cobe Spectra V. 7.0, and Spectra Optia V. 7, Terumo BCT) were used to exchange 60–70% of the patients' red cells with crossmatched donor red blood cells of the same blood type with an aim of reducing the hemoglobin S level to less than 30%. Leukofiltered red cell suspensions preserved for 1–7 days by citrate-phosphate-dextrose and with a hematocrit level of about 75% were used. The target hematocrit levels were determined according to the steady-state hematocrit levels of the patients who were regularly examined as outpatients.
Results: No women and no babies died. HBS values were decreased to <30% and the hematocrit levels were increased, allowing for perfusion of the placenta as determined by Doppler USG. There was no crisis during pregnancy, among the SCD patients who underwent prophylactic RBCX procedures. After the delivery, the 5th minute apgar scores were 9.1±1.2.
Summary / Conclusion: This study shows that prophylactic RBCX during pergnancy seems to be a feasible and safe procedure for prevention of hemoglobin S associated complications in patients with SCD. Given the decrease in transfusion risks, RBCX is worthy of further study in future trials.

P451

PROSPECTIVE SCREENING FOR ANTI-PLATELET ANTIBODIES AND IMMUNE PLATELET REFRACTORINESS IN PATIENTS DIAGNOSED WITH ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) patients are treated with intensive chemotherapeutic protocols, suffer from *protracted* pancytopenias and need prolonged transfusion support. Long-term transfusion dependency exposes these patients to allo-immunization to both leukocyte (HLA) and platelet (HPA) antigens, expressed by immune platelet transfusion refractoriness, a major risk factor for bleeding-associated morbidity and mortality. The published incidence of platelet refractoriness in patients with hematologic malignancies, ranges between 7% and 34%. The frequency of platelet transfusion failure, at least once, in multiply transfused patients is reported to reach 70%. There is no data regarding the incidence of platelet refractoriness, whether immune or non immune, in the Israeli population.
Aims: To estimate the prevalence of platelet transfusion refractoriness (both immune and non-immune) among AML patients treated according to defined treatment protocols, to evaluate the incidence and "timing" of alloimmunization during treatment protocol and to evaluate weekly antibody screening as a tool to predict the risk for immune transfusion refractoriness.
Methods: Newly diagnosed AML patients were screened for the presence of anti-platelet antibodies and anti-HLA antibodies using flow cytometry. Antibodies were identified by the MAIPA assay. All patients were followed using weekly antibody screening. Clinical parameters of patients were consecutively registered. Patients received platelet transfusions according to the local transfusion policy: random or single donor platelets with continuous PLT increment monitoring. Patients' blood counts and platelet transfusion requirement were followed weekly, platelet refractoriness was determined when no increment was documented on two consecutive platelet transfusions.
Results: 64 newly diagnosed AML patients (35 males and 29 females) with age ranging between 25-60 years of various ethnic origins were included. Platelet refractoriness was found in 30 (47%) patients (10 males and 20 females). 26 patients (41%) developed new anti HLA and/or anti HPA antibodies, 21/26 (81%) were females. Immune platelet refractoriness was defined in 22/30 (73%) refractory patients (18 females and 4 males). 22/26 (85%) patients with antibodies developed refractoriness, 10/22 had anti HPA with (8) or without (2) anti HLA antibodies. 12/22 had only anti HLA antibodies. Average time to antibody production was 20 days from the beginning of treatment protocol.
Summary / Conclusion: although all AML patients receive irradiated and leukoreduced blood products at our centre, the prevalence of platelet refractoriness, specifically immune refractoriness, was higher than expected according to published data. Anti HPA antibodies were strongly correlated with the appearance of immune platelet refractoriness. Altogether our results demonstrate that immune platelet refractoriness is in a larger extent than previously described. These interesting results need to be confirmed in a larger cohort.

P452

ANCESTIM VERSUS PLERIXAFOR FOR THE COLLECTION OF PERIPHERAL BLOOD STEM CELLS

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Background: Autologous peripheral blood stem cells transplantation (APBSCT) has always had an important indication for patients with lymphoma or myeloma. The essential condition for this procedure is the existence of a sufficient graft, at least $2x10^6$ CD34 per kg of patient weight, according to the recommendations of the EBMT. For patients defined as 'poor mobilizers', two drugs with different mechanisms of action have been used to optimize the collection of PBSC.
Aims: We conducted a retrospective, monocentric, cohort study, to characterize and compare the effectiveness of ancestim and plerixafor, associated to G-CSF during mobilization of PBSC.
Methods: Between 2002 and 2011 we identified 48 patients who failed mobilization after classic stimulation with G-CSF. Among them, 32 patients received stimulation, in steady-state situation, by ancestim+GCSF and 16 patients with plerixafor+GCSF. We compared the successful rate of harvest, the total amount of CD34 + cells collected and the number of aphaeresis necessary to achieve the target.
Results: There were no significant differences in the initial characteristics of the patients between the two groups. The successful rate was 43% (14/32patients) for patients stimulated by ancestim+GCSF and 68% (11/16 patients) for patients with plerixafor+GCSF (P-0.0006). There were no statistically significant differences between the two groups of patients for the average number of CD34 + cells collected: $3.45x10^6$/kg for ancestim and $3.05x10^6$/kg for plerixafor (p-0.37) or the mean number of aphaeresis necessary to achieve the target 3.89 for ancestim and 3.28 for plerixafor (p-0.59). Similar results were observed in subgroups of patients according to the type of disease: multiple myeloma and lymphoma.
Summary / Conclusion: The two molecules have different mechanisms of action: ancestim is an agonist of c-kit (CD117), which allow a stimulation of cell proliferation and plerixafor is an reversible and selective chemokine receptor (CXCR4) antagonist which blocks binding of its ligand (SDF-1), enabling a break in the connection between the stem cells and their microenvironment. In situation of clinical failure to first mobilization, for PBSC harvest in view of ABSCT, in patients treated for lymphoma or myeloma, plerixafor seems superior to ancestim regarding the successful rate of harvest. By cons, there is no significant difference between the number of aphaeresis necessary to achieve the target or the number of CD34+ cells harvested.

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BONE MARROW ASPIRATE MORPHOLOGIC, IMMUNOPHENOTYPIC AND CYTOGENETIC EVALUATION IN RELATED DONORS PRIOR TO HEMATOPOIETIC STEM CELL DONATION

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Background: The current screening for eligibility of related marrow donor volunteers comprises a complete clinical check-up, personal and familiar history, a blood cell count, biochemical analysis and serological assays.
Aims: In our center, a potentially eligible donor is requested to perform an hematopoiesis evaluation by bone marrow aspirate. The donor who provides consent undergoes a bone marrow aspiration for morphological, cytogenetic and flow cytometric evaluation.
Methods: From April 2012 to February 2013, we analyzed bone marrow samples from 34 related donor candidates. Median age, at time of evaluation, was 44 years (range 18-72) and the male:female ratio was 50:50. Morphologic evaluation included staining with May Grunwald-Giemsa, PAS and PERLS. Cytogenetic analysis was performed using conventional G-banding karyotyping. Flow cytometric analysis was performed using a whole blood lysis technique and a panel of directly conjugated antibodies. Five-color flow cytometry was performed using an FC500 cytometer (Beckman/Coulter). Data, collected in list mode, were analyzed with FCS Express (DeNovo software). An immunological gating strategy utilizing CD45 versus side scatter was used to select the populations of granulocytes ($CD45^{bright}SSC^{high}$) and lymphocytes ($CD45^{bright}SSC^{low}$). CD2 and CD19 were used to identify the T and B lymphocyte subpopulations. Myeloblasts were primarily identified and gated as $CD45^{dim}SSC^{low}$ and further qualified and enumerated with CD34, CD117 and the myeloid marker CD33. Within the CD34+ cell compartment, the myeloblast related cluster (CD33+CD10-) and the B-progenitor related cluster (CD33-CD10+) were identified. In order to apply the low-grade-MDS score (Haematologica 2012; 97(8)), we considered the lymphocyte to myeloblast CD45 MFI ratio and the granulocyte to lymphocyte side scatter (SSC) peak channel ratio. The staining patterns of CD11b versus CD16, CD13 versus CD16 and CD11b versus CD64 were used to reveal abnormal antigen expression in the granulocytic population.
Results: All evaluated donors had normal a karyotype. Six subjects showed morphologic dysplasia of one, two or three lineages and 4 revealed an abnormal antigen expression of the granulocytic population. Table 1 describes the characteristics of the six donors with detected abnormalities. The median age of donors showing bone marrow dysplasia was 51 years and no alterations of blood cell count parameters were found. The history and the clinical check-up excluded co-morbidities potentially responsible of the morphological and cytometric changes observed.
Summary / Conclusion: These donors will be followed up with future checks. We believe that bone marrow analysis should be considered for a complete evaluation of eligibility of related donors prior to hematopoietic stem cell donation.

Table 1.

donor	Age (year)	Morphology (No. of observed dysplastic lineages)	CD117+(% of CD45+)	CD34+(% of CD45+)	abnormal antigen expression in granulocytic population	FCM score for low-risk MDS
TB	65	3	1.4	1.57	no	0
AG	29	2	1.5	2.1	yes	0
BO	44	1	1.5	1.37	no	0
AM	64	2	0.4	1.67	yes	0
DC	56	3	1.5	1.55	yes	0
DM	46	2	1.8	1.65	yes	0

P454

THE USE OF PREOPERATIVE ERYTHROPOIESIS-STIMULATING AGENTS (ESAS) IN PATIENTS WHO UNDERWENT KNEE OR HIP ARTHROPLASTY

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Background: Erythropoiesis-stimulating agents (ESAs) havebeen used preoperatively in orthopedic patients to reduce the need for allogeneic blood transfusion. However, their use for this indication is still not widely practiced.
Aims: The purpose of this review is to evaluate the efficacy of preoperative administration of ESAs on hemoglobin level at discharge and frequency of allogeneic blood transfusion in patients undergoing hip or knee surgery.
Methods: This is a systematic review of comparative randomized clinical trials that compared preoperative ESAs to other interventions or placebo in reducing the need for allogeneic transfusions and increase in hemoglobin levels at discharge.
Results: Pooled results of 26 trials with3,560 participants showed that the use of preoperative ESAs significantly reduced the need for allogeneic blood in patients undergoing hip or knee surgery [Relative Risk (RR):0.48, 95% CI: 0.38 to 0.60, P<0.00001)]. Hemoglobin mean difference between ESA and control groups was 7.16 (g/L) [95% confidence interval (CI)of 4.73 to 9.59, P=0.00001]. There was no difference in the risk of developing venous-thromboembolism between ESA group and the control groups [Risk Difference (RD): 0, 95 % CI: -1%>2%, P= 0.95; I^2= 0%].
Summary / Conclusion: ESAs offer an alternative blood conservation method to avoid allogeneic blood transfusion in patients undergoing hip or knee surgery.

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THE EFFICACY OF IMAGE MONITORING OF OPERATING ROOMS IN TRANSFUSION MEDICINE

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Background: Communication between operating rooms and the transfusion unit is important to provide safe and appropriate transfusions, especially in cases with emergency and mass bleeding. However, it was difficult for the transfusion unit to understand real-time information in operating rooms only by an interphone or an intranet transfusion management system.
Aims: The efficacy of image monitoring of operations has been reported in various situations. Recently, we placed monitors on the central board of the

transfusion unit, and linked it to each operating room. We could understand the perioperative needs by integration of image, voice communication and blood transfusion management system. Here, we analyzed the efficacy of this system, and discuss its importance in transfusion medicine.
Methods: Two 40-inch screen displays (Panasonic, Tokyo, Japan) linked to 11 operating rooms, as well as to the preparation and recovery rooms, were placed on the central board of the transfusion unit in January 2010. Each screen of the monitors can be divided into 16 sections, and each section can be enlarged to a full-size screen. We analyzed the amounts of blood ordered, blood used and blood returned to the transfusion unit and compared them during the 3-year periods before and after adoption of the image monitoring system.We also analyzed the amount and frequency of expired blood after surgeons had ordered blood in the preoperative period. Statistical analyses were performed using chi-square test or Student's t-test (SAS, Tokyo, Japan). All statistical analyses were two-tailed with a significance level of 0.05.
Results: Among all operations, the number of cases with expired red blood concentrates (RCC) significantly decreased from 17 cases to 2 cases (P<0.001); the amount of expired RCC decreased from 57 units to 3 units (P<0.001); and the frequency of expiration decreased from 0.73% to 0.05% (P<0.001). In cardiovascular operations in which 10 units or more of RCC were used, the amount of RCC, that was requested initially in the preoperative period, was 7.5±2.2 (mean±SD) units and 6.8±2.4 units before and after adoption of the image monitoring system, respectively, showing a significant reduction (P=0.02). The number of cases in which more than 30 units of RCC were requested initially, decreased from 24 cases (9.1%) to 1 case (1.7%) (P=0.03). The amount of blood that was requested in the perioperative period and the amount was returned to the transfusion unit significantly decreased (P=0.03 and P<0.001, respectively). The amount of expired blood by mishandling also decreased. The serious deficiency of blood in the transfusion unit was alleviated. All of the doctors realized the importance of image monitoring in this area.
Summary / Conclusion: The efficacy of an image monitoring system, which was linked to the operating rooms, was analyzed. It in combination with the existing communication procedure was effective in improving transfusion practice. It increased the safety of transfusion, and decreased the amount of expired blood. It is useful to establish a good partnership between clinicians and the transfusion unit.

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FAILURE ON MAINTAINING HAEMOGLOBIN LEVEL AFTER TRANSFUSION IN TRANSFUSION DEPENDENT THALASSEMIA PATIENTS RELATED TO ERYTHROCYTE ALLOANTIBODY AND AUTOANTIBODY PRODUCTION: PROPORTION AND REL

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Background: In transfusion dependent thalassemia patients who has got repeated transfusion for a period of time, the haemoglobin level after transfusion could not be maintained appropriately to be expected. The production of erythroyte alloantibody and autoantibody in transfusion dependent thalassemia patients has been reported before. These antibodies were probable related to the failure on maintaning haemoglobin level after transfusion.
Aims: To find related factors of failure on maitaining haemoglobin level after transfusion in adult transfusion dependent thalassemia patients related to erythroyte alloantibody and autoantibody production.
Methods: Cross sectional study of adult transfusion dependent thalassemia patient without others autoimune disease at Haematology and Medical Oncology outpatient clinic in Cipto Mangunkusumo hospital from July to September 2012 was done. The specimen was subjected to erythroyte alloantibody and autoantibody evaluation by column gel agglutination technique. Eleven cell reagent panel were used in screening and identification of alloantibody and autoantibody respectively. Positive alloantibody is defined as positivity of indirect antiglobulin test and positive autoantibody is defined as positivity of direct antiglobulin test. Statistic analysis between erythrocyte alloantibody and autoantibody positivity and sex, type of rhesus, feritin level, type of iron chelation, and alloantibody were done.
Results: From 88 subjects, there were 37,5% thalassemia patients that did not maintain haemoglobin level after transfusion. From 33 of those subjects, there were 78,6% subjects with alloantibody and 72,7% subjects with autoantibody. From 24 patients with autoantibody, there were 25% subjects with severe hemolytic anemia that clinically significant. Positif alloantibodi related to autoantibody production (P≤0,000). Positive alloantibody [odds ratio (OR) = 26,32; P≤0,000], positive autoantibody (OR = 11,99; P≤0.011), and feritin level ≥3000 ng/mL (OR = 6,36;P≤0,042) related to failure on maintaining haemoglobin level.
Summary / Conclusion: The proportion of failure on maintaining haemoglobin level in adult thalassemia patients were 37,5%. The proportion alloantibody and autoantibody production in adult thalassemia patients that failure on maintaining haemoglobin level were 78,6% and 72,7% respectively. Related factors of those were positive alloantibody and autoantibody, and feritin level ≥3000 ng/mL. Positive alloantibody related to autoantibody production.

P457

EFFECT OF USING LEUKOSTOP FILTER DURING BLOOD TRANSFUSION ON PULMONARY FUNCTIONS IN PATIENTS WITH THALASSEMIA MAJOR

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Background: Although pulmonary dysfunction is not the most significant clinical manifestation of patients with β-thalassemia major, a certain reduction in pulmonary volumes has frequently been reported.Its etiology is multifaceted including iron deposition, free radicals production and a leucocyte inflammatory reaction could be a contributing factor. In developing countries, the blood is not routinely filtered, and the use of bedside filters is limited to patients with leucocyte mediated complications.
Aims: The aim of this study was to determine the prevalence and patterns of lung dysfunction among patients with BTM after the application of the Leukostop filter during transfusion for a period of 6 months.
Methods: The study included 30 patients with transfusion dependent BTM sequentially recruited from the Hematology Clinic, Children's Hospital, Ain Shams University. They were 12 (40%) males and 18 (60%) females, their ages ranged between (7-17) years with a mean 12.4±3.2 years. They were sub divided into 2 groups according to their use of Leuckocyte filter. Group 1: included 15 patients with BTM were allocated to use the Leuckocyte filter before each blood transfusion for 6 months and group 2 (Control Group) included 15 patients with BTM using non Leuckocyte filtered blood. Patients with history of airway disease and smokerswere excluded. They were subjected to history taking and revision of hospital files recording age, sex, age at diagnosis, transfusion dependency period and frequency, previous transfusion reactions and history of Splenectomy. Chest X-Ray was done for each patient before the use of the Leuckocyte filter and after its use for 6 months .Pulmonary Function tests using spirometry was done at baseline and after 6 month-follow up. Forced expiratory maneuver was used for calculation of forced vital capacity (FVC), forced expiratory volume in one second (FEV1) , FEV1/FVC ratio(%), functional residual capacity (FRC), forced vital capacity (FVC), residual volume (RV) and Total lung capacity (TLC); then impulse oscillometry technique for assessment of small airway behavior was used.
Results: Group 1 (using leukostop) showed no significant difference at baseline evaluation in distribution pulmonary abnormalities with control group; the distribution of pulmonary disease significantly improved in group 1 (normal 40%, restrictive 33.3% and obstructive 26.67% at initial evaluation to 53.33, 33.3 and 13.33% respectively in the post-filter evaluation, P<0.05), however no change in pulmonary disease distribution in control group. A statistical significance improvement in FVC (L)1.72±0.48,1.85±0.51,P=0.043, FVC (%) 88.13±15.08,91.89±12.97, P=0.025, FEV1(L) 1.33±0.35,1.51±0.32, P=0.004, FEV1(%)79.31±14.24,88.56±12.86, P=0.001 and FEV1/FVC (%) 79.33±14.56,84.27±7.59, 0.041 in the 6 month evaluation compared to the initial evaluation in patients with BTM using bedside filter,while in the control group a decline in FEV1 (%)(87.09±12.11,80.91±8.87, P=0.001), FVC (L)(1.85±,1.55±0.33, P= 0.036) , FVC (%) (90.88±13.71,84.81±11.42, P<0.001), and no significant change in FEV1/FVC ratio. There was no correlation between Serum Ferritin and PFT results in the pre or post filter evaluation (P > 0.05).
Summary / Conclusion: Pulmonary function abnormalities mainly restrictive pattern although subclinical is not an unfrequent finding in patients with BTM. Leukofiltration although increases the cost of provided service in countries with limited health resources; yet it improves all PFTs and should be implemented to prevent long-term consequences.

P458

PLATELET TRANSFUSIONS IN HAEMATOLOGY PATIENTS

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Background: Platelet transfusion modality still constitutes an unsolved problem. The majority of hospitals routinely give prophylactic platelet transfusions to patients with long-term bone-marrow failure. Recent findings demonstrate that the platelet count threshold for prophylactic transfusion can be as low as 10,000/μL and a therapeutic rather than a prophylactic strategy of transfusion for bleeding manifestations only may be equally safe for most patients. Another recent study suggests that very low doses of platelet transfusions are as effective at preventing bleeding as much higher doses.
Aims: reduction number of platelet transfusions per subject and cost.
Methods: Our retrospective study was conducted on two groups of 15 patients each who treated for ALL or AML. We compared the quantity of platelet transfused, cost and hemorrhagic risks between both groups. Patients were given transfusion when the platelet count was ≤20×10^9/L. The first group received 0,5.10(11) platelets per 7 kg of weight, and in the second group patient received 0,5.10(11) platelets per 10 to 14 kg of weight.
Results: Among the 30 adults who were enrolled in total, only those in the second group received a decrease number of transfusions and fewer units of platelet transfusions per subject. No significant difference in the outcome and no hemorrhagic complication were detected between the two groups

Summary / Conclusion: Changes in transfusion practices such as maintain of prophylactic strategy but with the reduction of dose per transfusion is considerable potential for decreased use of platelet transfusions with a consequent improvement of cost reduction

P459

THE EFFECTIVENESS OF PLATELETS TRANSFUSIONS IN PATIENTS WITH DE NOVO ACUTE MYELOID LEUKEMIA (AML) DURING INDUCTION CHEMOTHERAPY (IC).

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Background: Platelets transfusions (PT) are the main condition to save patients' lives during and after IC period. The effectiveness of PT depends on different parameters including the age of patients, variant of AML, serious infectious complications and HLA-antibodies.
Aims: To determine the factors that influenced on the effectiveness of PT in patients with *de novo* AML during IC.
Methods: Retrospective analyses of 41 patients' IC were done. All patients were treated with 7+3 regimen. FAB classification was used to determine the morphological variant of AML. The prognostic variant of karyotype was managed by ELN criteria. Platelet transfusion was effective if the 24 hours corrected count increment (24h CCI) was more than $4.5x10^9$/L.
Results: Median age of patients was 42 y. (19-84). Morphological variants of AML were next: 5 M1, 17 M2, 3 M3, 6 M4, 7 M5, 2 M6 and 1 M7. Good karyotype was determined in 11 patients, intermediate in 25 patients and poor in 5 patients. Complete response was diagnosed in 25 patients (61.0%). The IC was ineffective in 13 patients (31.7%) and 3 patients (7.3%) died from infectious complications during the period of pancytopenia. Platelets transfusions were started when the count of platelets in peripheral blood was $19.0\pm11,8x10^9$/L (0-51) and were ended when the count of platelets $57.7\pm37.6x10^9$/L. The criteria to begin PT were thrombocytopenia $\leq20.0x10^9$/L (68.2%), hemorrhagic syndrome (36.5%) and DIC (48.7%). Two or three reasons to start PT have been taken place in 26.8% patients. The total number of PT transfused to all patients including in the trail was 459 units and most of them (88.7%) were prepared by discrete apheresis. The number of platelets units transfused to one patient was 10.2±5.8. There was no any association between PT effectiveness and age of patients. We did not find the role of prognostic variant of karyotype and the response to IC on the effectiveness of PT. At the same time the dependence of effective PT rate from morphological variant of AML was determined. Patients with M1 and M2 AML have got much more effective PT than patients with M4-M5 AML; P=0.022. There was association of overall survival (OS) and the level of platelets in peripheral blood before PT. The median of OS of patients with platelet count $\leq10x10^9$/L was 10.9 mo versus 7.0 mo in patients with platelet level $>10x10^9$/L.
Summary / Conclusion: The effectiveness of PT depends on morphological variant of AML. The reason is unknown. We suppose the role of high level of anti-HLA antibody. The early start of PT is a condition to improve OS of patients with *de novo* AML during IC.

P460

MEASUREMENT OF IRON CONCENTRATION IN HAIR AS AN INDICATOR OF BODY IRON LOAD

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Background: As in iron deficiency, iron excess in the body also precipitates metabolic disorders. Iron accumulates in tissues and organs and causes tissue damage during the course of treatment in patients with myelodysplastic syndrome and ß-thalassemia, which require regular blood transfusions. Iron accumulation, which is seen commonly in the liver, myocardium, pancreas, pituitary gland and joints, is associated with functional loss in these organs and leads to life-threatening complications. Currently, several tests including invasive laboratory evaluations are used to determine the amount of iron in the body. Blood count, serum iron concentration, total iron binding capacity (TIBC), percentage of transferrin saturation (TS) and serum ferritin levels form the basis of these evaluations, which are widely available at many centers and relatively affordable. Serum free transferrin receptor level, which shows iron deficiency at cellular level and is not affected by acute phase response, is used for investigational purposes, although it has limited use in clinical practice. Furthermore, magnetic resonance imaging and needle biopsies are alternative tests which are used to demonstrate iron accumulation in the organs. An invasive method, consisting of staining bone marrow aspirate with Prussian blue, is the best clinical test to show iron status.
Aims: In the literature, there is limited information suggesting that iron concentration in hair may be an indicator of body iron load. In a few studies, the authors failed to demonstrate a significant relationship, thus emphasizing the need for further studies. We sought an answer to the question as to whether iron concentration in hair could be used as an indicator for body iron load. We aimed to determine whether there is a relationship between the iron concentration in hair and indicators of body iron in patients with iron deficiency anemia in which body iron load varies; in those with transfusion-dependent anemia who require regular blood replacement; and in controls.
Methods: Patients being followed up at the Hematology Department of Erciyes University's, Medical Faculty and at Kayseri Teaching Hospital between September 2010 and October 2011 were included in this study. The study population was assessed in three groups. The groups consisted of patients with high and low body iron and healthy controls with levels iron with in the normal range. Twenty patients with ß-thalassemia and myelodyplastic syndrome, who received at least 30 units of erythrocyte suspension, were included in the transfusion-dependent anemia group with high body iron. Twenty three patients with a iron deficiency anemia were or included in the group with low body iron. Seventeen healthy cases with a percentage of transferrin saturation and ferritin levels with in the reference range and who did not meet anemia criteria were employed as the control group. The hair samples were then dried in an oven at 80ºC. Each hair sample, weighing 250 mg, was transferred to a digestion vessel of 10 mL capacity, and concentrated nitric acid (5 mL) was pipetted into the vessel. Hair samples were diluted with concentrated nitric oxide in a microwave oven (Berghof Speedwave Germany).
Results: Median age was 33 years (14-48) in the iron deficiency anemia group, 22 years (15-75) in the transfusion-dependent anemia group and 28 years (13-42) in the control group. Differences between groups were found to be statistically significant for all parameters other than age. The median iron concentration in hair was measured as 5.080 µg/g in the iron deficiency anemia group, whereas it was 28.886 µg/g in the transfusion-dependent anemia group and 12.986 µg/g in the control group. The differences in terms of iron concentration in hair were found to be statistically significant (P<0.001). The highest iron concentration in hair (89.41 µg/g) was found in patients with transfusion-dependent anemia, who had the highest ferritin values, while the lowest concentration (0.77 µg/g) was detected in the iron deficiency anemia group (P<0.001).
Summary / Conclusion: These findings indicate the usability of iron concentration in hair as an indicator of body iron concentration. Further studies should evaluate how iron in hair is affected in settings such as acute or chronic infection or inflammation, in which the ferritin value acts as an acute phase reactant and the relationship between iron concentration in hair and hepatic iron load should be measured by MR imaging and liver biopsy.

P461

REVIEW AND DESIGN OF A NEW BLOOD BOOKING PROTOCOL FOR EACH SURGERY AND A RESTROSPECTIVE STUDY OF THE DIRECT EXPENSE DECREASE WITH THE NEW PROTOCOL

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Background: Blood components are a limited resource with many adverse reactions and they suppose a significant economic impact in health management. Is therefore necessary to implement protocols for their optimal use.
Aims: Valoration of a new standarized transfusional protocol implementation study according to direct expense decrease.
Methods: During 2011 the potentially bleeding surgeries (n=1107) are analized in our hospital and classified according to original surgical units, blood booking application rates and blood transfusion rates during 48 hours after surgery. Urgent surgeries are not included. Transfusion probability is analized depending on employed protocol and a new protocol is defined based on transfusional probability of applied surgery procedure: If transfusion probability is < 3% a signed Informed consent is required, if transfusion probability is >3% but <10% a group analysis and indirect coombs is required, and if transfusion probability is >10% a standarized blood booking is required. Finally, patients with transfusion need, without previous blood booking but all pretransfusional laboratory tests done (ABO and RH D group, indirect coombs, blood crossmatches tests) with patients who would need a transfusion if the new protocol was applied are compared. Direct expense savings according to Osakidetza´s public rates and its retrospective application to analysed requestes are calculated.
Results: From the global analysed patients transfusion rate of requested blood (74.6%) was 16.9%. Only one surgery (0.09% 1/1107) needed blood transfusion without previous blood booking. If the new consensual protocol would have been applied 16 blood transfusions would have been done: 2.2% (8/359) from the Informed consent group and 6.4% (8/125) from the group analysis and indirect coombs group, but there would have been saved 11.447€ (6.528€ in blood crossmatches, 419 € in ABO and RH D group analyses, and 4.500 € in indirect coombs tests).
Summary / Conclusion: The developement of new surgical techniques fitted protocols carry on an increasement in human resources use efficiency, a better management of the booked blood avoiding blood blockade and direct and indirect expense decrease.

P462

A CANADIAN TALE OF TWO BLOOD GROUPS

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Background: A 75 year old gentleman fron Canada presented of a cruise ship to the acute medical take with lethargy. Blood counts revealed a pancytopenia, with a normochromic, normocytic haemaglobin of 5.7 g/dL.
Aims: Investigation of an extremely rare cause for mixed field blood grouping
Methods: Given symptom of cardiac compromise, i.e. shortness of breath and chest tightness, he was cross-matched for four unit of blood. His cross match revealed two distinct population of cells, some group O, and group A; some Rh(D) positive and some Rh(D) negative. The patient denied receiving any previous blood transfusions.
Results: Bone marrow biopsy revealed myelodysplasia with trilineage cytopenia and a complex karyotype. Reference Laboratory. Red cell samples were sent to Bristol Red Cell Reference Laboratory. Samples sent for cross matching revealed mixed field grouping reactions. Samples were then analysed at the regional transfusion laboratory. Two red cell populations were confirmed - O and A, Rh(D) positive and Rh(D) negative. DiaMed gel testing indicated double populations with anti-A, anti-A,B and both anti-D's. Gel Rhesus genotyping also showed a double population with anti-C. The patient's DAT was negative. These findings were confirmed by manual tests. Additional manual phenotypes were performed: Patient phenotype: Amf; Cmf, Dmf, E-, c+, e+, K-, k+, Fy(a+b+), Jk(a+b+), M+,Nmf, Smf, s+, P1+. Transfusion recommendation was group O Rh(D) negative red cell, and group A Rh(D) negative plasma containing products. PCR assay analysis excluded a weak subgroup A, predicted that the patient's genotype is O1 O1 (probable phenotype O).
Summary / Conclusion: This interesting case illustrates the challenge faced by both medical and laboratory staff in providing appropriate, safe blood products in a very unusual situation. The most common cause of a mixed field reaction in a patient not recently transfused is a weak subgroup A. This was excluded by molecular analysis. As there are two dual populations detected on phenotyping (ABO and rhesus), the mixed field phenotype result is actually chimaera i.e. two populations and transfer of DNA because of a resorbed twin in utero.

Platelets 1

P463

REDUCED HOSPITALIZATIONS AND BLEEDS IN ADULTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) RECEIVING ROMIPLOSTIM IN CLINICAL PRACTICE – INTERIM RESULTS FROM A LARGE, EUROPEAN, OBSERVATIONAL STUDY

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Background: ITP is characterized by platelet counts <100x10^9/L, with romiplostim recommended for second-line treatment of adult ITP. Patients (pts) with more severe disease may experience bleeding symptoms which require hospitalization. Such hospitalizations incur high direct and indirect economic costs.
Aims: Describe ITP-related hospitalizations in adult ITP pts receiving romiplostim in clinical practice.
Methods: Study eligibility criteria and outcomes have been described elsewhere (Wadenvik *et al*, 2012). Data on ITP-related hospitalizations were collected for up to 2 years before and after romiplostim initiation; bleeding data were collected for up to 6 months before and 2 years after initiation. Informed consent was obtained from all pts. Hospitalization and bleeding rates were calculated with adjustment for the varying observation periods.
Results: At an interim analysis conducted in September 2012, 310 pts had enrolled; 296 met the study inclusion criteria and were included in the Full Analysis Set. Of these, 44% (n=131) remained on study, 46% (136) had completed the observation period and 10% (29) had withdrawn, with death the most common reason (19 [6%]). At romiplostim initiation, median (Q1, Q3) age, weight and platelet count were 62.0 (46.0, 73.0) years, 75.00 (64.00, 85.00) kg and 19.0 (9.0, 35.0)x10^9/L. One-third (n=102) of pts were splenectomized, one-third (97) diagnosed <1 year previously (median [Q1, Q3] time since diagnosis 3.56 [0.42, 10.96] years) and 54% (161) female; 54% (159) had received ≥3 prior ITP therapies. Median (Q1, Q3) duration of romiplostim exposure was 65.0 (29.9, 104.9) weeks (maximum 109 weeks); 28% (84) of pts initiated romiplostim at doses above 1 µg/kg/week, 64% (190) received ≥1 romiplostim injection at home and 29% (86) self-administered. Median (Q1, Q3) average weekly dose was 2.8 (1.6, 4.4) µg/kg/week. Median (Q1, Q3) platelet counts rose to 86 (41, 150)x10^9/L after 4 weeks of romiplostim treatment and remained >50 x10^9/L thereafter. After romiplostim initiation, the rate of ITP-related hospitalizations, primarily for ITP treatment administration and bleeds, was 2-fold lower; the rate of all bleeds 3-fold lower; and grade ≥3 bleeds rare (Table 1).

Table 1. ITP-related hospitalizations and bleeding events.

	Full Analysis Set (N=296)	
	Before rom initiation	After rom initiation
Number of ITP-related in-pt hospitalizations* (rate/100 pt-yrs)	340 (83.3)	172 (37.1)
ITP-treatment administration	166 (40.7)	61 (13.2)
Bleeding event	102 (25.0)	66 (14.3)
Number of bleeds† (rate/100 pt-yrs)	318 (262.8)	336 (72.6)
Number of grade ≥3‡ bleeds (rate/100 pt-yrs)	16 (13.2)	8 (1.7)

pt, patient; rom, romiplostim; yrs, years; *Defined as requiring an overnight stay; collected for up to 2 yrs before and after rom initiation, with a total of 408 and 463 pt-yrs of observation, respectively. †Collected for up to 6 months before and 2 yrs after rom initiation, with a total of 121 and 463 pt-yrs of observation, respectively. ‡Defined using the WHO bleeding scale.

Ten pts reported a total of 13 serious adverse drug reactions (ADRs): 2 events each of pulmonary embolism and myelofibrosis (likely due to the underlying disease [bone marrow metastases in 1 subject each whose initial disease diagnosis was inconsistent with ITP]); 1 event each of acute myeloid leukemia (confirmed by bone marrow trephine biopsy), deep vein thrombosis, drug ineffective (clinical symptoms, thrombocytopenia), myocardial infarction, no therapeutic response (clinical symptoms, bleeding), phlebitis, platelet count decreased (platelets <20x10^9/L), retinal vein thrombosis, and (reversible)

thrombocytosis (platelets 477x10^9/L). No other thrombotic or bone marrow reticulin ADRs were reported. No fatal ADRs were reported.
Summary / Conclusion: With similar doses as reported previously (Kuter *et al*, 2008), and ADRs consistent with the known safety profile, pts with ITP of varying duration and severity receiving romiplostim in routine clinical practice achieved sustained increases in platelet counts and a reduction in ITP-related hospitalizations. The reduction in hospitalizations was likely due to fewer bleeds and less rescue medication/IVIg use, and could offer cost savings in clinical practice. Possible incomplete reporting before romiplostim initiation and imbalanced observation periods should be noted when interpreting these data.

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COMPLEMENT ACTIVATION IN THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background: Thrombotic Thrombocytopenic Purpura (TTP) is a microangiopathy associated with organ dysfunction and a high level of morbidity and mortality. Although dysregulation of complement is well established in the pathophysiology of atypical Haemolytic Uraemic Syndrome (aHUS), the potential role of complement in TTP has not yet been determined.
Aims: We carried out a prospective study to investigate the role of complement anaphylatoxins C3a and C5a in patients with TTP during acute episodes, and in remission, and compared these with normal controls. For both acute and remission groups we also investigated the cytokine response (Th1/Th2/Th17-involving IL-2, IL-4, IL-6, IL-10, TNF, IFN-Y and IL-17a) and association with disease state.
Methods: EDTA plasma was obtained from 21 patients (12F, 9M) with acute TTP, ADAMTS13 activity <5% and positive anti-ADAMTS13 IgG antibodies (median IgG 59%, range 8-180%). Also, EDTA Plasma from 50 patients (34F, 16M) previously treated for acute TTP, now in clinical remission (normal ADAMTS13 activity and a platelet count >150x10^9/L). Paired EDTA plasma was obtained from 12 patients (6F, 6M) for whom both acute and remission samples were available. Measurement of complement anaphylatoxins C3a (NR 32.5-56.1ng/ml) and C5a (NR 1.7-13.6ng/ml) was performed using BD™ Human Anaphylatoxin Kit, (BD Biosciences, San Jose, CA, USA) using a Cytometric Bead Array (CBA) method. Serum was obtained from acute and remission patients, enabling the measurement of cytokines, using BD™ Human Th1/Th2/Th17 Kit, (BD Biosciences, San Jose, CA, USA) also incorporating the CBA method.
Results: In acute TTP, median C3a and C5a were significantly elevated compared to patients in remission, C3a 66ng/ml vs 38.5 ng/mL (P<0.001) and C5a 17 ng/mL vs 10ng/ml (P<0.001), respectively. For the 12 patients with paired acute and remission samples, median C3a levels were significantly higher during the acute episode than in remission, C3a 45.5 ng/mL vs 35.8 ng/ml (P=0.041); there was a non-significant difference in C5a levels 15.2 ng/mL vs 10.6ng/ml (P=0.084). Compared to controls, remission median C5a levels were significantly higher 10ng/ml vs 5.81ng/ml (P=0.001); however remission C3a levels were lower than controls 38.5 ng/ml vs 43.7ng/ml (P=0.046). Within the acute TTP group, there was no significant difference in C3a or C5a levels for patients requiring ITU admission vs those not requiring ITU care or patients with or without neurological features at presentation. Both median IL-6 and IL-10 levels were significantly higher in the acute vs remission groups, IL-6: 8pg/ml vs 2 pg/ml (P=0.002), IL-10: 6pg/ml vs 2pg/ml (P<0.001). C3a levels were strongly correlated with both anti-ADAMTS13 IgG (r_s=0.731, P=0.002) and IL-10 (r_s=0.627, P=0.012) levels.
Summary / Conclusion: These results suggest complement anaphylatoxin levels are higher in acute TTP cases than in remission, and highlight the possibility that the complement response seen acutely may relate to both the degree of anti-ADAMTS13 IgG antibody level and cytokine (IL-10) levels. Factors triggering complement activation in TTP need to be elucidated. Complement inhibition at presentation in the acute phase may have therapeutic potential.

P465

A LONGITUDINAL PROSPECTIVE STUDY EVALUATING THE EFFECTS OF ELTROMBOPAG TREATMENT ON BONE MARROW IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA: INTERIM ANALYSIS AT 2 YEARS

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Background: Eltrombopag (epag), a thrombopoietin receptor agonist (TPO-RA), increases platelet counts in chronic immune thrombocytopenia (cITP) patients. TPO-RAs are associated with varying degrees of bone marrow (BM) reticulin increases.[1,2] Due to lack of pretreatment evaluations, the incidence and clinical significance of these findings are not established. Inconsistencies in specimen preparation, staining, and analysis across institutions further confound conclusions.
Aims: To assess the degree of BM fibrosis (reticulin and/or collagen) in cITP patients treated for up to 2 years with epag (NCT01098487).
Methods: BM biopsies were collected at baseline (prior to epag treatment) and 1y and 2y on treatment. Specimens were centrally processed and stained for reticulin (silver) and collagen (trichrome), and were reviewed by a central independent hematopathologist for cellularity; megakaryocyte, erythroid, and myeloid quantity and appearance; trabecular bone quality; reticulin grade (European Consensus scale-MF[3]) and presence of collagen.
Results: 167 patients were enrolled. Currently, 45 patients have completed all 3 protocol-specified BM biopsies (baseline, 1y [10-14 mo], and 2y [22-26 mo]). Median age is 39 (18-78) y; 34 (75.6%) are females; 51% Caucasian/European, 13% East Asian, and 36% South Central Asian. Median time since ITP diagnosis is 3.5 (0.6-45.7) y. All patients received prior ITP therapy, and 2 (4%) received prior TPO-RA treatment (both with epag), and the last dose was ≥6 months before enrollment. At baseline, 40 (89%) patients had reticulin grade 0 (MF-0), and 5 (11%) MF-1. At 1y, 26 (58%) patients had MF-0, 17 (38%) MF-1, and 2 (4%) MF-2. At 2y, 37 (82%) had MF-0, 6 (13%) MF-1, and 2 (4%) MF-2 (Figure 1). Compared with baseline, 33 (73%) patients had no change at 2y in MF grade, 7 (16%) a 1-grade increase, 4 (9%) a 1-grade decrease, and 1 (2%) patient had a 2-grade increase. Three patients had post-baseline MF-2; 2 (4%) patients at 1y, and 2 (4%) at 2y. One patient with MF-2 at 1y decreased to MF-0 while continuing on epag. One patient with MF-2 had collagen at 1y and 2y. None of the 3 patients with post-baseline MF-2 had adverse events or hematologic abnormalities considered related to impaired BM function, and none withdrew due to BM findings. Both patients with prior TPO-RA (epag) treatment had baseline reticulin of MF-0, and remained MF-0 at 1y and 2y.
Cellularity was normal in 86%, 71%, and 80% of patients at baseline, 1y, and 2y, respectively. No changes occurred in marrow cellular composition. In 2 of 3 patients with post-baseline MF-2, cellularity was increased from baseline. Trabecular bone thinning was found at baseline in 8 (18%) patients (most with prior steroid use), 24 (53%) patients at 1y, and 28 (62%) at 2y.
Summary / Conclusion: 11% of patients had MF-1 at baseline. After 2y of treatment, no increase or a mild increase in reticulin was observed in 73% and 16% of patients, respectively. No patient with an increase to MF-2 at 2y had clinical signs or symptoms indicative of BM dysfunction. Results were similar to those reported for EXTEND, an epag extension study (median treatment duration >2y[1]).
Conclusion: These data suggest that treatment with epag is generally not associated with clinically relevant increases in BM reticulin or collagen. The potential association of TPO-RAs and increased BM reticulin needs further study.

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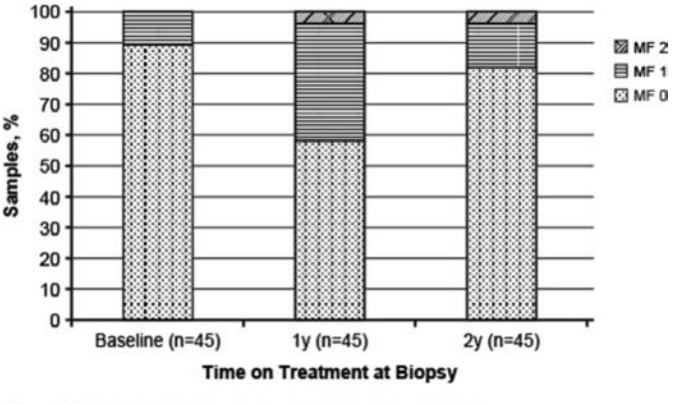

MF, myelofibrosis grade at each on-treatment time interval (samples); Y, year.
*European Consensus Scale.

Figure 1. Reticulin grade* at each time point.

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RECOMBINANT HUMAN THROMBOPOIETIN (RHTPO) IN COMBINATION WITH LOW-DOSE RITUXIMAB (LD RTX) IN SEVERE OR REFRACTORY PRIMARY IMMUNE THROMBOCYTOPENIA: A MULTICENTER PROSPECTIVE CLINICAL TRIAL

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Background: Adult primary immune thrombocytopenia (ITP) is an autoimmune disease characterized by autoantibody-induced platelet destruction, which affects around 1 in 10,000 people. 25%>30% of the patients achieve no response to the conventional therapy. Both rituximab and thrombopoietin are recommended as the second-line treatments. Standard-dose rituximab (SD RTX, 375 mg/m^2 ×4 weeks) is the mainstream for the treatment of ITP. Previous studies reported a response rate around 60%. Zaja *et al* evaluated LD RTX (100 mg dose×4 weeks) in 28 patients with ITP, which showed that the response rate (platelet count > 50×10^9/L) was 75%, which was similar to that with SD RTX, though the median time to response (or complete response) were longer than SD RTX. Conventional agents focused on the decrease of platelet destruction, while it was also discovered in recent years that many ITP patients had abnormalities in megakaryocyte growth and apoptosis, as well as lower level of thrombopoietin (TPO). The recombinant human full-length glycosylated TPO (rhTPO) is a TPO receptor agonist. The combination of rhTPO and rituximab could complement each other's advantages and exert powerful effect on platelet production, which may be a choice for the glucocorticosteroids-resistant/ relapsed patients.
Aims: We reported 86 adult ITP patients treated with rhTPO in combination with LD RTX from 12 centers. This trial focused on exploring novel therapeutic strategies for ITP. We assessed response rates and safety of rhTPO combined with low-dose rituximab in primary ITP who had failed to response to glucocorticosteroids or relapsed.
Methods: rhTPO was given 300U/kg in the first 14 days, with 100mg qw rituximab weekly for 4 weeks. The dose frequency of rhTPO was adjusted according to the platelet counts. Primary outcomes include response (R), complete response (CR), no response (NR) and relapse. R was defined as platelet count ≥30×10^9/L and at least two-fold increase from the baseline count and absence of bleeding; CR in case of platelet count ≥100×10^9/L and absence of bleeding; NR in case of platelet count <30×10^9/L or less than two-fold increase from baseline. Relapse was defined as a drop in platelet count to ≤30×10^9/L following an initial, partial or complete response. Secondary outcomes are listed below. Time to response and sustained time were considered as the duration from baseline to response, duration from response to relapse or the end of the study, respectively. Safety was reflected as adverse events graded according to the Common Toxicity Criteria. All of the patients were informed about the clinical trial and signed the consent forms. This clinical trial was registered at http://clinicaltrials.gov as NCT01506414.
Results: Response and complete responses were achieved in 26/86 (30.2%) and 35/86 (40.7%) patients respectively. In a follow-up of 3 months, 5 cases lost to follow-up, and 14/56 (25%) patients relapsed. Median time to response (or complete response) was 7 days (range 4-28 days). And the median sustained time was 79 days (range 3-86 days) in a total observation time of 90 days. There were several adverse effects reported, such as fatigue, pulmonary inflammatory, but almost all were below Grade 2. Two of the patients died in the process. One (>70 year old) died of cerebral embolism, however it has nothing to do with therapy of ITP, since his platelet count kept steady. The other was a patient relapse in the third month, and died of cerebral hemorrhage.
Summary / Conclusion: Our findings suggest that combination of RTX and LD RTX in ITP improves response rate and yields shorter time to response compared with monotherapy of RTX in reports. Thus, combination therapy may represent an effective treatment option for glucocorticosteroids-resistant or relapsed patients.

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EFFICACY AND SAFETY OF ROMIPLOSTIM IN PATIENTS ≥65 YEARS WITH IMMUNE THROMBOCYTOPENIA (ITP)

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Background: ITP is an autoimmune disease characterized by increased platelet destruction and suboptimal platelet production. ITP prevalence increases with age, and geriatric patients (pts) may not tolerate therapy for ITP as well as younger pts. Romiplostim is a thrombopoietin (TPO) receptor agonist that can increase platelet production and is approved for treatment of chronic ITP in adults. However, little is known about the efficacy and safety of romiplostim in pts ≥65 years (yrs).
Aims: To describe the efficacy and safety of romiplostim among pts ≥65 compared to pts <65 yrs.
Methods: For efficacy endpoints, data from 2 global phase 3 trials (2005–2006) and 1 Japanese phase 3 trial (2007–2009) of romiplostim in pts with ITP were analyzed separately. Efficacy endpoints included overall platelet response (≥1 weekly platelet count ≥50x10^9/L within the study treatment period) and number of weeks with a platelet response. For safety endpoints, data from 13 clinical studies of romiplostim in pts with ITP (2003–2009) were included. Data from pts given placebo or standard of care (SOC) were pooled, and pts who received placebo in the parent study and romiplostim in the extension study were included in both the placebo/SOC and romiplostim groups. Safety endpoints included rates of adverse events (AEs), serious AEs (SAEs), fatal events, and AEs of interest (ie, bleeding events, thromboembolic events, and bone marrow reticulin events). Results were adjusted for study duration and reported as rates per 100 pt-yrs.
Results: The efficacy analysis included 159 pts who were treated in randomized, controlled trials; 54 received placebo and 105 received romiplostim. Most were Caucasian (64.2%) or Japanese (21.4%). Mean (SD) age was 53 (15.7) yrs with 24.5% ≥65 yrs and 75.5% <65 yrs. In the romiplostim group, a trend towards higher overall platelet response rates was seen among pts ≥65 vs <65 yrs in the global studies (94.4% vs 86.2%) and the Japanese study (100% vs 93.3%). The mean (SD) number of weeks with platelet responses was similar among pts ≥65 vs <65 yrs in the global studies (7.2 [3.2] vs 6.7 [4.1]) and the Japanese study (9.9 [2.0] vs 9.3 [3.9]). In the placebo group from the global studies, the overall platelet response rate was lower among pts ≥65 vs <65 yrs (7.7% vs 20.7%), and the mean (SD) number of weeks with platelet responses was similar among pts ≥65 vs <65 yrs (0.4 [1.4] vs 0.6 [1.4]). Comparisons were difficult in the Japanese study because only 1 pt ≥65 yrs was included in the placebo group. The safety analysis included 718 pts. Most were Caucasian (80.9%) or Japanese (6.4%). Mean (SD) age was 52 (18.8) yrs with 27.3% ≥65 yrs and 72.7% <65 yrs. A total of 32 fatal events were seen; 2 pts had fatal bleeding events and 5 had fatal thromboembolic events. The incidences of AEs were similar among pts ≥65 vs <65 yrs in the romiplostim (92.4% vs 92.1%) and placebo/SOC groups (92.9% vs 93.8%). Duration-adjusted rates of overall AEs were similar among age groups, and rates of SAEs and fatal AEs were greater among pts ≥65 vs <65 yrs (Table 1). Of interest, pts ≥65 yrs had a lower rate of grade ≥3 bleeding events in both the romiplostim and placebo/SOC groups and a higher rate of thromboembolic events in the romiplostim group than pts <65 yrs (Table 1).
Summary / Conclusion: Though numerical differences were seen, key efficacy outcomes were similar among pts ≥65 and <65 yrs treated with romiplostim. Safety outcomes varied among age groups with pts ≥65 yrs having greater rates of SAEs and fatal events than pts <65 yrs, regardless of treatment.

Table 1.

	Placebo/SOC[a]		Romiplostim[a]	
	≥65 yrs N = 42	<65 yrs N = 96	≥65 yrs N = 172	<65 yrs N = 481
Any AE, n (r)	397 (1046.8)	874 (1212.0)	3143 (1307.0)	9218 (1353.6)
SAE, n (r)	56 (147.7)	51 (70.7)	249 (103.5)	350 (51.4)
Fatal Event, n (r)	5 (13.2)	3 (4.2)	14 (5.8)	10 (1.5)
Grade ≥3 Bleeding Event, n (r)	2 (5.3)	17 (23.6)	20 (8.3)	83 (12.2)
Thromboembolic Event, n (r)	1 (2.6)	5 (6.9)	24 (10.0)	45 (6.6)
Bone Marrow Reticulin Event, n (r)	0 (0.0)	0 (0.0)	3 (1.2)	9 (1.3)

[a]Pts who received placebo in the parent study and romiplostim in the extension study were included in both groups.
n = number of events.
r = study duration-adjusted event rate per 100 pt-yrs (n/pt-yr x 100).

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EFFICACY OF ENZYME REPLACEMENT THERAPY WITH VELAGLUCERASE ALFA IN PATIENTS WITH TYPE 1 GAUCHER DISEASE AND THROMBOCYTOPENIA OR SEVERE SPLENOMEGALY

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Background: The responses of type 1 Gaucher disease (GD)-related throm-

bocytopenia and splenomegaly to enzyme replacement therapy (ERT) are linked to their pretreatment severity.
Aims: To evaluate the efficacy of velaglucerase alfa in type 1 GD patients with thrombocytopenia or severe splenomegaly.
Methods: TKT032 and HGT-GCB-039 were parallel-group trials; eligible patients were ≥2 years old with untreated type 1 GD. In both trials, 1 treatment arm was allocated to velaglucerase alfa 60 U/kg ERT every other week (EOW). Patients completing either trial could enroll in a combined extension study, HGT-GCB-044. Informed consent was obtained from all adult patients and from the guardians of patients ≤17 years old.
Results: 27 type 1 GD patients received velaglucerase alfa 60 U/kg EOW in TKT032 or HGT-GCB-039 and HGT-GCB-044 over 24 months. 15/27 patients had a pretreatment (baseline) platelet count <100×10^9/L; 6 of these 15 had a platelet count <60×10^9/L. All 15 had an intact spleen. 6/27 patients had severe baseline splenomegaly (splenic volume >15 multiples of normal); all 6 had a platelet count <100×10^9/L. At 24 months, 14/15 (93%) patients had reached the platelet count therapeutic goal and 6/6 with severe baseline splenomegaly had reached the splenic goal. 5/6 (83%) patients with a baseline platelet count <60×10^9/L had a normal platelet count (≥120×10^9/L), including 2 with severe baseline splenomegaly (Table).
Summary / Conclusion: Clinically significant improvements in platelet count and splenic volume occurred in the first 24 months of velaglucerase alfa treatment among patients with type 1 GD and severe baseline splenomegaly and/or a platelet count <100×10^9/L (including those with a platelet count <60×10^9/L).

Table 1. Individual platelet and splenic responses: 24 months of study drug.

Baseline age, y	Gender	Platelet count, ×10^9/L				Splenic volume, multiples of normal			
		Baseline	12 months	24 months	24-month change, %	Baseline	12 months	24 months	24-month change, %
7	M	44	124	148	236	31.6*	11.1	5.8	–81.7
9	M	66	92	134	105	35.0*	11.0	6.3	–82.0
18	M	77	188	228	196	8.9	4.1	3.2	–63.5
19	F	47	150	181	285	6.4	2.7	2.2	–66.2
23	F	50	102	152	204	10.8	5.1	3.5	–67.4
24	M	50	62	147	197	26.4*	16.0	6.2	–76.7
25	F	68	85	151	122	33.3*	7.7	4.3	–87.0
27	M	89	190	176	99	9.5	4.3	3.7	–61.6
29	M	62	75	75†	21	36.9*	17.2	12.3	–66.7
29	F	44	85	222	405	11.2	3.8	2.6	–76.4
31	F	99	193	192	94	5.7	3.4	2.7	–52.4
36	F	99	101	163	66	7.2	4.3	3.5	–50.9
42	M	64	94	109	70	19.1*	10.3	7.1	–63.0
44	F	90	155	192	113	8.5	5.1	4.0	–52.7
58	M	50	92	112	124	12.0	4.1	3.0	–75.3

*Patients with spleens >15 multiples of normal in volume at baseline.
†Below goal.
Therapeutic goals by 2 years: splenic volume must decrease 50–60%; baseline platelet count 60–120×10^9/L must be ≥100×10^9/L; baseline platelet count <60×10^9/L must increase 2-fold.

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AN UNEXPECTED ROLE OF THE VON WILLEBRAND FACTOR-CLEAVING PROTEASE ADAMTS-13 DURING EXPERIMENTAL INFLAMMATION IN LUNGS

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Background: The metalloprotease ADAMTS-13 is a key factor for the development of thrombotic thrombocytopenic purpura (TTP). ADAMTS-13 cleaves ultra-large *von Willebrand* multimers into smaller fragments. The presence of ADAMTS-13 activity is essential for blood clotting homeostasis, since genetic deficiency or blockade by auto-antibodies can result in micro-thrombi formation and thrombocytopenia during TTP. Infection-associated inflammation has been described as a triggering factor for episodes of TTP in humans. Genetically targeted mice with deletion of ADAMTS-13 have only recently become available.
Aims: In this study, we sought to induce TTP-like symptoms in ADAMTS-13 deficient mice (ADAMTS-13-/-) by inducing acute experimental inflammation in mouse lungs. In addition, the dependency of the acute inflammatory response on the activity / presence of ADAMTS-13 was characterized.
Methods: Breeding colonies of ADAMTS-13-/- mice were housed and genotyped at our institution and phenotyping was accomplished using a fluorescent activity assay for ADAMTS-13 (FRETS-VWF73). Anaesthetized ADAMTS-13-/- mice and wild type mice were injected intra-tracheally with 40µg of LPS (from *E. coli*, O111:B4). Alternatively intra-pulmonal immune-complexes were induced by intra-tracheal injection of 125 µg of anti-bovine serum albumin antibody along with injection of intra-venous 1 mg bovine serum albumin. At the end of experiments broncho-alveolar lavage fluids and blood samples were harvested and analyzed using a Sysmex KX-21 N instrument or immunoassays.
Results: Intra-tracheal challenge with LPS did not induce thrombocytopenia in ADAMTS-13-/- mice or wild type mice after 8 hours. As expected, LPS intratracheally promoted the accumulation of myeloid cells (6-fold increase, mainly polymorphonuclear neutrophils) in the alveolar lung compartment as counted in broncho-alveolar lavage fluids of wild type mice. Surprisingly, the LPS-induced influx of polymorphonuclear neutrophils to the alveolar compartment was reduced by around 50% in ADAMTS-13-/- mice as compared to wild type mice (Figure 1, **P<0.01). In addition, circulating leukocytes in peripheral blood were substantially reduced in ADAMTS-13-/- mice but not wild type mice. When mice were subjected to immune-complex induced inflammation of lungs, significant lower numbers of leukocytes in broncho-alveolar lavage fluids (*P<0.05) and peripheral blood (**P<0.01) were observed in ADAMTS-13-/- mice as compared to wild type controls 8 hours after immune-complex inflammation. When the disruption of the alveolar/capillary barrier was assessed by detection of alveolar albumin concentrations in broncho-alveolar lavage fluids, no significant differences between ADAMTS-13 and wild type mice were observed in both models of lung inflammation. The molecular mechanisms underlying the observed results, including alterations of chemokine expression signatures, are currently under investigation in our laboratory.
ISummary / Conclusion: Collectively, these results reveal an unexpected role of ADAMTS-13 as a modulator of leukocyte migration in two models of acute lung inflammation. Our future studies will aim to evaluate, if targeting ADAMTS-13 activity may be a beneficial treatment strategy during acute lung diseases.

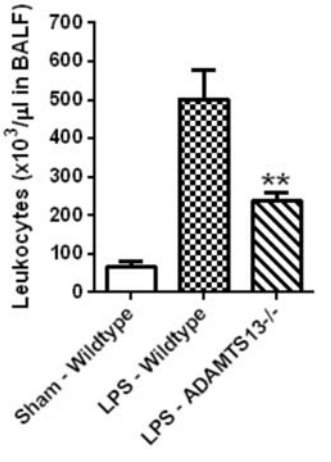

Figure 1. ADAMTS13-/- mice display reduction of leukocytes in bronchoalveolar lavage fluids following LPS-induced lung inflammation (LPS 40 mg/mouse, i.t.) 8h Sham mice received PBS i.t.)

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RITUXIMAB VERSUS SPLENECTOMY IN PERSISTENT OR CHRONIC ADULT PRIMARY IMMUNE THROMBOCYTOPENIA: AN ADJUSTED COMPARISON OF MORTALITY AND MORBIDITY

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Background: Splenectomy and rituximab are both recommended as second-line treatment in primary immune thrombocytopenia (ITP), while they have never been directly compared.
Aims: This study was aimed at conducting an adjusted comparison of efficacy and serious adverse outcomes (infection, bleeding and death) of splenectomy versus rituximab in persistent or chronic adult primary ITP patients.
Methods: Thanks to hospital databases, we built a retrospective monocentric cohort of 105 consecutive adult primary ITP patients exposed to either of these treatments. Outcomes were hospitalization for bleeding, overall mortality, reponse (no beleeding and platelet count >30 G/L) at 3 and 12 months, complete response (no bleeding and platelet count >100 G/L) at 3 and 12 months, loss of response and of complete response, hospitalization for infections. Outcomes were assessed by medical file reviewing and by phone interviews of the patients and their general pratictionners. Comparisons were made using Cox models. Risks were adjusted on gender, age-adjusted comorbidity Charlson score, disease duration, history of mucosal bleeding, treatments for ITP before splenectomy or rituximab.
Results: The cohort included 105 adult primary patients exposed either to splenectomy (n=62) either to rituximab as second-line treatments. Patients treated with rituximab were older and had more comorbidities than splenectomized patients. Mean follow-up were respectively 3 and 8.4 years. Response and complete response rates at 12 months were higher in the splenectomy group (87.9% *versus* 59.0% and 81.0% *versus* 35.9%, P<0.001). Splenectomy was independently associated to response at 12 months (OR 4.6, 95%CI[1.6-13.6]). Loss of response was higher in the rituximab group (P=0.02). In multivariate model, the risk of hospitalization for bleeding was associated with

a history of mucosal bleeding at diagnosis (HR 11.1, 95%CI[1.5-84.7]) and with age-adjusted Charlson score (for one point increase: HR 1.3, 95%CI[1.0-1.6]), but not with rituximab exposure (channeling bias). Two patients died by bleeding in the rituximab group and none in the splenectomy group (P=0.09). Overall mortality was increased in the rituximab group (P=0.01). In multivariate analysis, mortality was also associated to a high age-adjusted Charlson score (for one point increase: HR 2.2, 95%CI[1.6-3.0]). There was no difference between the two groups regarding hospitalizations for infection.
Summary / Conclusion: In a real life practice, older people are preferentially treated with rituximab. Age and comorbidities explain the poor prognosis of these patients. Nevertheless, splenectomy is an independant factor of reponse at 12 months and this study suggests that it should be proposed in elderly whenever possible. Prospective studies are mandatory to assess the benefit-to-risk ratio of both treatments in older people.

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EVALUATION OF THE IMMATURE PLATELET FRACTION IN THE DIAGNOSIS AND PROGNOSIS OF CHILDHOOD IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background: Idiopathic thrombocytopenic purpura (ITP) is an acquired immune-mediated disease in adults and children characterized by a transient or persistent decrease in the platelet count. A rapid assessment of platelet production would distinguish between thrombocytopenia due to bone marrow failure and thrombocytopenia due to increased peripheral platelet destruction. Immature platelet fraction (IPF%) is a measure of reticulated platelets (RPs), obtained from an automated hematology analyzer as one of the platelet parameters and represents the state of thrombopoiesis.
Aims: Therefore, we aimed to evaluate IPF as a diagnostic tool to differentiate between ITP and thrombocytopenia due to decreased platelets production and as a prognostic marker for severity of ITP patients.
Methods: Fifty patients with ITP were compared with 14 patients with hematological malignancies under chemotherapy representing a control group with thrombocytopenia due to bone marrow suppression and 30 age- and sex-matched healthy subjects served as healthy controls. Patients were studied stressing on bleeding manifestations, recent viral infection, history of drug intake, bleeding score, organomegaly/lymphadenopathy and therapy. Complete blood count (CBC) was performed using Sysmex XE-2100. The studied ITP Patients were classified into 2 subgroups: acute ITP with spontaneous resolution within 3 months from diagnosis and chronic ITP lasted ≥1 year from diagnosis.
Results: ITP patients had significantly lower hemoglobin, platelet count and plateletcrit (PCT) with higher mean platelet volume (MPV), platelet distribution width (PDW), platelet large cell ratio (P-LCR) and IPF compared with healthy controls. Similarly, MPV, PDW, P-LCR and IPF were significantly higher and PCT was significantly lower in ITP patients compared with patients having hematological malignancy under chemotherapy. However, hemoglobin level, WBC count and absolute lymphocyte count were significantly lower in those patients than ITP patients. Comparison between acute and chronic ITP showed that chronic ITP patients had significantly higher age with longer disease duration. Number of chronic ITP patients taking first line corticosteroids and second line IVIG, anti D, combined therapy or thrombopoietin was significantly increased compared with acute patients. Number of lines used and incidence of drug dependency were also elevated among chronic than acute ITP patients. Bleeding score and platelet count were negatively correlated with IPF. ITP patients taking second line IVIG, anti D, combined therapy or thrombopoietin had significantly lower IPF than untreated patients with those lines. IPF was positively correlated to number of lines used, MPV, PDW and P-LCR while negatively correlated to platelet count and PCT in ITP patients. Multiple regression analysis showed that platelet count, patient's condition, thrombopoietin intake and relapse were independently related to IPF. ROC curve analysis revealed that the cutoff value of IPF at 4.3% could be diagnostic for ITP patients with a sensitivity of 90%, specificity of 83%.
Summary / Conclusion: IPF is a rapid and inexpensive automated marker for thrombocytopenia and can be integrated as a standard parameter to evaluate the thrombopoietic state of the bone marrow. It may be considered as a diagnostic tool to differentiate between ITP and thrombocytopenia due to bone marrow pathology as well as a potential prognostic marker for disease severity in ITP patients.

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THALIDOMIDE CORRECTS IMPAIRED MESENCHYMAL STEM CELL FUNCTION IN INDUCING TOLEROGENIC DENDRITIC CELLS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: Thalidomide (THD) was first introduced as a non-barbiturate sedative-hypnotic drug in West Germany in the 1950s, but was withdrawn worldwide in 1961 due to its potential for teratogenesis, which resulted in 8000–10000 newborns with phocomelia malformation. However, it has since expanded its range of usage and was approved by the FDA in 1997 as an agent to treat erythema nodosum leprosum. Presently, thalidomide is used as an immunomodulatory agent to treat immune-mediated diseases. Immune thrombocytopenia (ITP) is an autoimmune disorder in which both T and B cells are involved. Recently a study found impaired proliferative and functional capacity of MSCs in patients with ITP.
Aims: The aim of this study was to explore whether THD could correct the defects of MSCs and to investigate its mechanism of inducing tolerance in ITP.
Methods: Thirty-three newly diagnosed primary ITP patients were enrolled and twelve samples of venous blood and nine samples of bone marrow from healthy donors were used as controls. MSC proliferation was determined by Cell Counting Kit-8 and cell-cycle analysis. The molecular changes in MSCs induced by THD were detected by microarray analysis and validated by quantitative real time polymerase chain reaction. Mixed lymphocyte reaction and cytokine production assays were performed to analyze the malfunction of MSCs in ITP patients. Interference with TGF-β inducible early-response gene 1 (TIEG1) small hairpin RNA (shRNA) (h) lentivirus was used to analyze the tolerogenic effects of dendritic cells (DCs) induced by MSCs. Phenotypic analysis of mature DCs after co-culture with MSCs was conducted by fluorescence-activated cell sorter (FACS)
Results: We demonstrated that MSCs in ITP had a reduced proliferative capacity and lost their immunosuppressive function, which could be corrected with THD treatment. According to the gene profile, the down-regulation of caspase 8, caspase10 and c-myc and up-regulation of oct 3/4 and TGF-β1 in MSCs may be associated with THD modulation. The inhibitory effects of MSCs on lymphocyte proliferation rely on the presence of DCs. The THD-modulated MSCs could regulate mature DCs by down-regulating the expression of costimulating factors and up-regulating IL-10 and TGF-β1 production. To study the functional alteration of DCs induced by MSCs, we sorted DCs after incubation with MSCs and performed T-lymphocyte reaction assays. Both the DCs induced by MSCs from healthy controls and those induced by THD-modulated MSCs from ITP patients suppressed T-cell proliferation activated by allogeneic mature DCs, whereas the DCs induced by unmodulated MSCs from ITP patients did not. When DCs were infected with TIEG1-interference lentivirus, the MSCs lost the ability to induce them into tolerogenic DCs. These results indicated that the modulatory effects of MSCs on DCs was dependent on the expression of TIEG1 in DCs.
Summary / Conclusion: The study demonstrates the inability of MSCs from ITP patients to induce mature DCs into a tolerogenic DC population. THD could restore the regulatory effect of MSCs on DCs. These findings will help us understand the pathogenesis of ITP, and further lay a foundation for a new regimen in the treatment of ITP.

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DISEASE PROGRESSION, TREATMENT PATTERNS, AND CO-MORBID BURDEN AMONG ADULT PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA (ITP): UPDATED RESULTS FROM THE UNITED KINGDOM ITP REGISTRY

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Background: Primary immune thrombocytopenia (ITP) is an autoimmune disorder that is characterized by decreased platelet count ($<100\times10^9$/L). Contemporary findings on the epidemiology of ITP and treatment patterns are limited. To address these gaps the United Kingdom ITP Registry (UKITP) took up an important task to recruit an adequate sample size of patients with ITP and is conducting extensive studies on the data and biological samples that it has collected to date.
Aims: We aim to study the disease's natural progression, including longitudinal platelet counts and bleeding events, evaluate treatment patterns, and measure the prevalence and incidence of at least 25 comorbid conditions in a primary ITP cohort. In this analysis, we estimated the baseline demographics, prevalence of comorbidities at diagnosis of ITP and the natural progression of platelet counts before and after presentation with ITP.
Methods: The registry, based at the Royal London Hospital, collects data and biological samples on ITP participants locally and externally through a network of 36 collaborating regional centres. We collect study-related data at diagnosis of ITP and throughout the clinical history of enrolees. Occurrences of comorbidities are followed throughout the lifetime of participants thus permitting the estimation of their prevalence and incidence. Historical platelet counts are also collected. We described the characteristics of the ITP cohort using stratified summary statistical analyses (inc. standard deviation). Mean platelet counts were estimated based on individual counts during specified time periods before and after ITP diagnosis date.
Results: Our sample consisted of 729 adults (18+ years) who had a mean age

of 47.4 (19.4) years at diagnosis and 52.8 (18.7) years at registration. Overall, more females than males (57% vs. 43%) were diagnosed with ITP; a pattern reversed among 70+ years old (N=94; 44% vs. 56%). The prevalence of at least 1 comorbid illness for 40+ years old was 37% (N=432) compared to 19.4% (N=263) among their younger counterparts. Before or at ITP diagnosis among those 40+ years, 3.7% had cataract, 4.4% had a myocardial infarction, 5.6% had osteoarthritis, and 4.9% had a solid tumour. The mean platelet count within ±7 days of ITP diagnosis was 40.2 (50.0) [N=423]. In a small proportion of patients platelet counts were available before the diagnosis of ITP was made, in the 2 months leading to the diagnosis a decline in mean platelet counts below 100 x10^9/L was noted, which subsequently rose sharply over this threshold during a shorter time following diagnosis (Figure 1). The rise in platelet counts post diagnosis in adults was treatment related. Next analyses will look at the platelet count drop reported here in context of the diagnostic process (e.g. eliminating other potential causes) and whether decision to treat were dependent on other clinical features (e.g. platelets count<50x10^9/L). Risk of experiencing certain comorbid illnesses will also be investigated, including the role of prevalent diseases and risk factors at baseline.

Summary / Conclusion: These results are preliminary to more thorough analyses currently being undertaken and patterns identified will be explained further with additional data. The UKITP registry presents a valuable source to study ITP and future ambitions include pheno-genotypic association studies with a view to predict disease severity and likely response to treatment (especially to novel therapeutic agents).

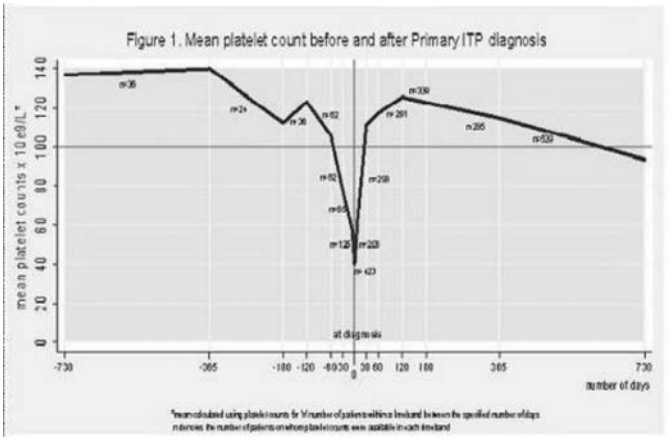

Figure 1.

P474

THROMBOEMBOLIC EVENTS IN PATIENTS WITH ADULT CHRONIC IMMUNE THROMBOCTOPENIA (ITP) TREATED WITH ELTROMBOPAG

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Background: Eltrombopag is a thrombopoietin receptor (TPO-R) agonist, which binds to the trans-membrane region of TPO-R and promotes platelet production by inducing differentiation and proliferation of megakaryocytes. Eltrombopag has been approved in Japan for adult chronic ITP patients who are intolerant to conventional therapy, show insufficient efficacy to other therapeutic options or have high risk for bleeding. The approved initial dose of eltrombopag is 12.5 mg/day (maximum dose; 50mg/day). Post marketing surveillance (PMS) is mandatory and all patients must be registered for collection of safety and efficacy information in Japan.

Aims: To investigate the relationship between thromboembolic events and eltrombopag treatment based on interim analysis of PMS data in Japan.

Methods: PMS data of patients who were treated with eltrombopag from December 2010 to June 2012 were analyzed. In these patients ertrombopag was taken for up to 6 months. This analysis was focused on the thromboembolic events.

Results: Data were collected from 683 patients (female: 65%). For those patients with information available, 43.3% of patients had ITP for more than 3 years, 14.2% for 1 to 3 years and 19.9% for less than 1 year. In 29.3% of patients platelet count was less than 10x10^9/L at baseline. Corticosteroid was taken concomitantly by 76% of patients during eltrombopag treatment. Eltrombopag increased the mean platelet count up to more than 50x10^9/L after 2 weeks (N=656) and reached to more than 90x10^9/L at 16 weeks (N=460) from the baseline (26x10^9/L). During the 6-month observation period, 25.5% of patients reported an adverse drug reaction (ADR). Thromboembolic events were reported in 29 patients (4.2%) (median age: 70 years, female 69%). Serious ADRs was reported in 45 (6.6%) of 683 patients. As serious ADRs thromboembolic events were reported in 24 patients (3.5%). Among 29 patients associated with thromboembolic events, arterial or venous thromboembolism was seen in 11 and 15 patients, respectively. Two patients had both arterial and venous thromboembolism. In another one patient site of thromboembolic event was unknown. Concomitant corticosteroid was used in 26 (89.7%) of these 29 patients. A past history of thromboembolism, the presence of anti-phospholipid syndrome (APS), or other reported risk factor such as obesity or long-term bedridden was a significant risk factor of thromboembolic events, but concomitant use of corticosteroid or positive anti-phospholipid antibody alone was not significant. Fourteen (48.3%) of 29 patients associated with arterial or venous thromboembolic event had these risk factors. Patients who experienced venous thromboembolic event had significantly more of these risk factors than those associated with arterial thromboembolic event (73.3% vs. 27.3%) (P=0.02). Eleven (37.9%) of 29 patients showed rapid increase of platelet count (above 3 times or more from the baseline and 50x10^9/L or more) within 2 weeks before thromboembolic event. Eight (27.6%) of 29 patients had neither past history of thromboembolic events nor rapid increase of platelet count. In 30% of patients with thromboembolic event, the event was observed at lower platelet count (less than 50x10^9/L).

Summary / Conclusion: Thromboembolic events (ADRs) in eltrombopag treated ITP patients were reported in 29 (4.2%) of 683 ITP patients in Japan. Past history of thromboembolism, the presence of APS, or other reported risk factor such as obesity or long-term bedridden was identified as a significant risk factor of thromboembolic events. ITP patients with venous thromboembolic event had risk factors of thromboembolic event more frequently than those with arterial thromboembolic event.

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MANAGEMENT OF ADULT CHRONIC IMMUNE THROMBOCYTOPENIA IN JAPAN: PATIENT AND HEMATOLOGIST PERSPECTIVES

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Background: Immune thrombocytopenic purpura (ITP) is an uncommon disorder and hematologists' experience is therefore limited. Although many treatments have been recommended for ITP, there are no evidence-based recommendations for when different treatments should be used, or even when any treatment should be used rather than managing a patient by observation alone. There is no absolute consensus on first line of therapy for treatment of ITO. The prognosis of individual patients with chronic refractory ITP is very variable. Patients may not always understand the disease and available treatments due to the generally mild nature of the disease

Aims: The purpose of this study is to examine the perspectives of Japanese hematologists and adult ITP patients about the disease and treatment.

Methods: A multicenter, questionnaire-based survey conducted between November 2012 and February 2013 in Japan. Both hematologists and their patients with ITP were invited to participate in this survey. The hematologists were mainly asked about their management of ITP. A survey questionnaire for patients asked about their attitudes towards applied treatments, the effects of treatments (effectiveness and safety), impacts on quality of life (QOL), and treatment satisfaction. Written informed consent was obtained from each patient. A response distribution was calculated for each item.

Results: 178 hematologists and 154 adult chronic ITP patients had completed the survey at the time of abstract submission. Regardless of the presence of bleeding symptoms, approximately 80% of hematologists started treatment based on platelet count. Treatment was considered when platelet counts are less than 20x10^9/L in patients without bleeding, and less than 30 ×10^9/L in patients with bleeding. However, in patients without bleeding symptoms whose platelet count had been 10x10^9/L, 11% of hematologists did not start their treatment. Ninety six percent of hematologists consider that platelet counts of 2 to 5x10^9/L are enough for ITP patients. Sixty nine percent of hematologists recognized that quality of life (QOL) has been disturbed in ITP patients. Corticosteroids was considered to be the most effective treatment (44.1%), but 99.4% of physicians expressed concern about its adverse events, especially hyperglycemia (40.9%). In the patient survey, 59.1% of participants were 60 years of age or older and the duration of disease was more than 10 years in 43% of patients. The minimum platelet counts were less than 10x10^9/L in 57% of patients. The most common symptom of ITP was fatigue (45%). Approximately 60% of patients reported at least one side effect associated with ITP treatment. Of ITP treatments, side-effects were most frequently associated with corticosteroid use (55%). Among these side effects moon face was considered as a lot of bother. 49.7% patients reported impairment of QOL impairment. No improvement of QOL was recognized in 33% of patients. For ITP patients acceptable platelet counts after ITP treatment were 50 to 100x10^9/L. Fifty one percent of patients still have feeling of vague fear to ITP.

Summary / Conclusion: This is the first survey to examine the perspectives of Japanese hematologists and adult ITP patients about ITP and treatment of the disease in Japan. Inability to balance the risk of severe bleeding against the

risk of side effects with ITP treatment is still a major cause for concern among Japanese hematologists. The gap about target platelet counts between hematologists and patients may not be well recognized. Improvement of QOL was not the major treatment target for hematologists, although the both hematologists and patients recognized QOL impairment. Awareness of the different opinions about ITP treatment between hematologists and patients may improve management decisions.

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D-DIMER LEVELS IN ACUTE IDIOPATHIC AND SECONDARY THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background: Prompt and accurate diagnosis of Thrombotic Thrombocytopenic Purpura (TTP) is crucial. Aggressive plasma exchange with FFP reduces an untreated mortality rate of 90% in autoimmune TTP but is ineffective in secondary TTP. Differentiating autoimmune TTP from similar disorders e.g. cancer associated TTP can be challenging.
Aims: We retrospectively reviewed our patients over the past 4 years to see if any laboratory parameters would allow differentiation of primary from secondary TTP.
Methods: Patients with suspected TTP presenting to the West of Scotland Apheresis unit (catchment population circa 3 million), presenting between April 2009 and January 2012 were included. Medical records were reviewed for the final diagnosis and laboratory parameters at presentation. Due to variation in methods between laboratories, D-Dimer levels were expressed as a multiple of the upper limit of normal. Comparison of groups was made by Mann-Whitney tests using Stata 11, Stata Corporation.
Results: 38 patients presented with a clinical picture suggesting TTP, of whom 3 did not have notes or test results available leaving 35 in the analysis group. Nine of these patients had acute idiopathic (autoimmune) TTP, and 8 had an underlying cause identified (malignancy n=5, HIV n=1, pregnancy related n=1, pancreatitis n=1). In 6 patients a definitive diagnosis was not reached and no underlying cause was identified. Twelve patients had haemolytic uraemic syndrome. The autoimmune TTP group had significantly lower levels of both ADAMTS13 (P=0.008, n=10) and D-dimer (P=0.009, n=15). Three of the six patients with D-Dimer levels greater than 5 times the upper limit of normal (approximately 1000 ng/ml) were found to have an underlying malignancy (Figure 1). Haemoglobin levels tended to be higher at presentation in the autoimmune TTP group (median 95 g/L) compared to all other cases (median 80g/l), but the difference was not significant (P=0.07, n=28). There was no difference in platelet count between the two groups (P=0.749, n=28), and no difference in body mass index (P=0.21, n=33).
Summary / Conclusion: D-Dimer levels are not commented on as a diagnostic test in the current British Committee for Standards in Haematology guideline. However, elevated levels appear to be highly indicative of the TTP being secondary. Unlike the ADAMTS13 assay, D-dimer levels are widely available, give a result within minutes and are inexpensive. Elevated D-dimer levels in patients presenting with TTP should prompt urgent search for an underlying cause and possibly delay second line therapy until this has been undertaken.

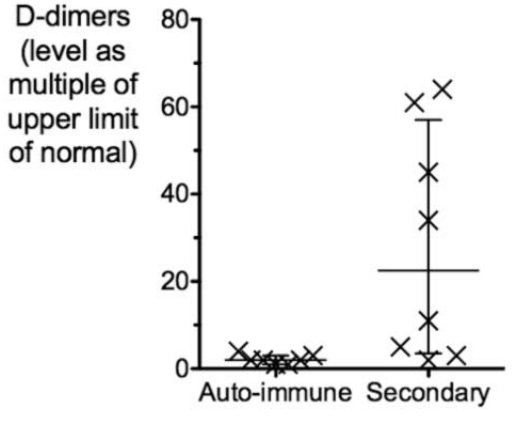

Figure 1.

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THROMBOCYTOPENIA IN PREGNANCY: OUR EXPERIENCE AT THE NATIONAL MATERNITY HOSPITAL

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Background: The National Maternity Hospital, Dublin is a tertiary referral centre, with approximately 11,000 deliveries per annum making it one of the biggest maternity hospitals in Europe. At booking and throughout the pregnancy, a low platelet count is not unusual. The main published causes of Thrombocytopenia are: Gestational Thrombocytopenia (GT, 70% of the cases), Preeclampsia/Eclampsia/HELLP syndrome (PEHS, 21%) Immune Thrombocytopenia Purpura (ITP3%) and other causes (6%).
Aims: To compare the published causes of Thrombocytopenia with our data at the NMH. To investigate the effect of age and ethnicity on the differential diagnosis.
Methods: TP is defined as a platelet count below the 2.5th percentile (116,000/μL). For GT diagnosis, the following were taken into account: a low platelet number, presentation of thrombocytopenia in the third trimester, in the absence of a bleeding history, and a mild thrombocytopenia (>70,000/μ L) as no specific diagnostic test can distinguish GT from mild ITP. For Preeclampsia/Eclampsia/HELLP Syndrome we considered the detection of low platelets in the third trimester, duration (days, weeks), recovery of platelet numbers (in the immediate postpartum period), haemolysis phenomena (rise of Bilirubin and LDH), schistocytosis (blood smear), elevated liver enzymes (AST, ALT), Hypoalbuminaemia, and correlated symptoms (rise in diastolic blood pressure, proteinuria, seizures, stomach symptoms, etc). For ITP diagnosis: a past history of persistent thrombocytopenia (plt <100,000/μL), bleeding symptoms and no recovery of platelet numbers after delivery, although the final diagnosis was made based on the exclusion of other possibilities. For diagnosis of TTP and HUS, symptoms at CNS or Kidneys respectively, were taken into account.
Results: A total of 11,538 mothers delivered at the NMH in 2011. 97.6% of these mothers had normal counts and 2.4% (281) presented at booking or thereafter with a platelet count ≤ 116.000/μL; with the latter rising to 4.65% with a cutoff point of 140.000/μL. In 57 cases (20.3 %) was not possible to reach a diagnosis due to insufficient data. In 224 cases (79.7 %) the cause of thrombocytopenia was defined. PEHS was dignosed in 93 cases (46%), GT 83 cases (41%), ITP 19 cases (9,4%) and thrombocitopenia related with delivery 7 cases (3,5%). There were no registered cases of TTP or HUS. The mean duration of thrombocytopenia in PEHS was 6.5 days reaching statistical difference compared with other causes. There was not difference between the mean age of mothers with normal platelet counts (31.91±5.20 years) and mothers with counts <116000/μL (32.91±5.12 years), The differential diagnosis was independent to the patient's age and ethnic origin. However the age in the Irish mothers with TP was greater than in the non Irish population (33.7±5.03 vs 30.59±4.79 years, p 0.00).
Summary / Conclusion: 7.6% of women during pregnancy present with mild thrombocytopenia (100,000/μL - 150,000/μL) and 65% of them will not be associated with any pathology. There is a disparity between our results and the published results. A possible reason for this difference is the lack of clarity with defining the cut-off point in the published literature. The main cause of TP at the NMH was Preeclampsia/Eclampsia/HELLP syndrome (46% vs 21% published), Gestational thrombocytopenia (41.1% vs 70% published), ITP (9.4% vs 3% published) and other causes (3.5 % vs 6% published) and we had no cases of HUS or TTP in 2011. In summary we had less cases of thrombocytopenia when compared with data being published so far elsewhere, and a different proportion of diagnoses that neither the age nor the ethnicity could explain.

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MONITORING ANTIPLATELET THERAPY : ACTIVITY OF ADENOSINE DIPHOSPHATE RECEPTOR INHIBITORS IN PATIENTS AFTER PERCUTANEOUS CORONARY INTERVENTION

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Background: Dual antiplatelet therapy with aspirin and adenosine diphosphate (ADP) receptor inhibitors (clopidogrel, prasugrel, ticagrelor etc.) represents standard prevention of thrombotic complications in patients undergoing percutaneous coronary intervention (PCI). However, resistant or low-responding patients are exposed to significantly increased risk of stent thrombosis, myocardial infarction and cardiac death. The most promising method for monitoring is vasodilator-stimulated phosphoprotein (VASP) assay. Based on flow cytometry, the assay is specifically aimed to determine activity of P2Y12 receptor (principle ADP receptor). On the other hand, results obtained by light transmission aggregometry (LTA) were proved to be valuable especially in association with clinical outcomes of the patients.
Aims: The main goal of our pivotal prospective study is to determine the diagnostic value of LTA and VASP assay.
Methods: Samples of venous blood from group of post-PCI patients were taken in three intervals: prior to PCI; 1day and 30 days after intervention. Further, samples undergo analysis by LTA (with induction by adenosin diphosphate and arachidonic acid) and VASP-P assay. Results will be correlated with treatment regimen, drug dose, clinical presentation and other selected attributes. Informed consent was obtained from all participants.
Results: Studied group (n=30) consisted of patients with acute coronary syndrome who underwent coronary intervention. Patients were in the age of 48 to 84 years. Benchmarks defining altered response to antiplatelet therapy were set at > 50% PRI for VASP-P assay, >50% for (ADP 10 µmol/l) and >20% (AA 0,5 µmol/L induction) for aggregometry assays. Seven patients experienced at least one of the severe complications : either intrastent thrombosis (n=3), myocardial infarction (n=4) or cardiac death (n=3). Five of these patients were detected as low-responders to ADP receptor inhibitor treatment.
Summary / Conclusion: Both LTA and VASP-P assays are clinically relevant diagnostic tests to identify patients not benefiting from antiplatelet therapy. However, challenge remains in standardising the benchmarks to distinguish poor responders.

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ANALYSIS OF RETICULATED PLATELETS IN THE ROUTINE CLINICAL PRACTICE USING THE CELL-DYN SAPPHIRE INSTRUMENT

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Background: Major breakthrough in the treatment of thrombocytopenic conditions, especially chronic immune thrombocytopenia (ITP), has been seen in the last few years with introduction of thrombopoietin (TPO) receptor agonists. However, many important issues still need to be addressed in the management of thrombocytopenias and lack of diagnostic tests as well as clinical tools to guide treatment are important issues to be resolved. Indeed, even in ITP the thrombocytopenia is a result of both depressed platelet production and increased platelet destruction. The bone marrow produces around $2x10^{10}$/L platelets per day. The newly released platelets contain RNA and are named reticulated platelets (retPLT). They provide information about the thrombopoietic activity in analogy with reticulocytes for eryhtropoietic activity. The flow cytometric analysis of retPLT is a non-standardized test hampered by variable assay conditions, arbitrary separation between positive and negative cells and poorly defined reference ranges. The Abbot CELL-DYN Sapphire instrument measures reticulated platelets as part of the reticulocyte assay, which is automated and fast, and uses the fluorescent dye (*CD4K530)*. Also, it utilizes fully standardized assay conditions and two-dimensional gating for background fluorescence correction.
Aims: To evaluate the CELL-DYN Sapphire instrument for analysis of retPLT in the routine clinical practice.
Methods: During 4 consecutive weeks all blood samples from the Hematology Department at Sahlgrenska University Hospital were assayed for retPLT using the CELL-DYN Sapphire instrument, in duplicate. A total of 1382 samples were analyzed, coming from more than 600 patients. Reticulated platelets were measured in CELL-DYN Sapphire as a part of the reticulocyte assay in a blood sample diluted with the proprietary fluorescent dye CD4K530, which stains RNA in reticulocytes and retPLT. Then the blood cells pass through a laser beam, where 0°, 7° and 90° light scatter and green fluorescence (FL1) are recorded. Subsequently a variable gate is applied for separating RBC and PLT. Within the PLT cluster, mature and reticulated PLT are distinguished by their fluorescence intensity, using the FL1 vs. 7° scatter plot with dynamic compensation for the size-dependent background fluorescence of PLT. retPLT are expressed as a percentage of the total PLT count.
Results: The highest retPLT percentages were seen in patients with platelet counts below $50x10^9$/L (Figure 1). Platelet counts and retPLT percentages, for a representative patient following high dose chemotheraphy, are shown in Figur 2. Further clinical details of the patient population will be presented. The imprecision (coefficient of variation, CV%) for the retPLT analysis ranged between 11-32%, being highest for patients with the lowest platelet counts (Figure 3).
Summary / Conclusion: Reticulated platelet fraction is a potentially useful diagnostic test that might distinguish between decreased platelet production and increased destruction. It might also predict platelet regeneration after high dose chemotherapy. Measurement of reticulated platelet fraction with fully automated methods overcomes tedious flow cytometry with its lack of standardization. Further clinical studies are needed to assess the value of retPLT by the CELL-DYN Sapphire instrument in thrombocytopenic conditions.

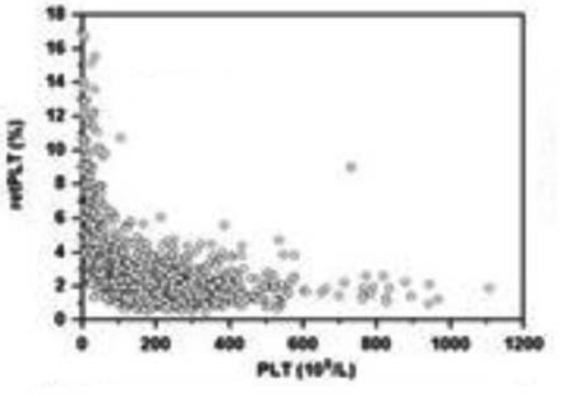
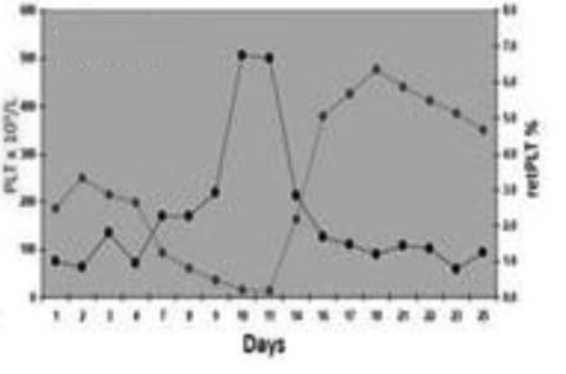


Figure 1.



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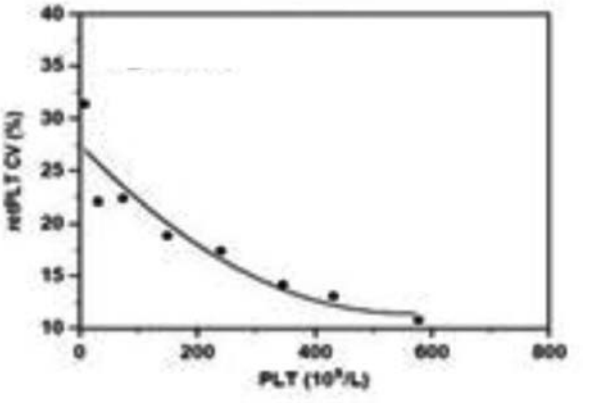

Figure 3.

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THE ROLE OF OXIDATIVE STRESS IN CHILDREN WITH ACUTE AND CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background: İdiopathic thrombocytopenic purpura (ITP) is an autoimmune disease but, every thrombocytopenia is not an autoimmune disease. Oxidative injury plays a pivotal role in pathogenesis of autoimmune diaseases resulting from DNA , protein and lipid oxidation.
Aims: To investigate whether oxidative damage is involved in acute and chronic idiopathic ITP in childhood.
Methods: Twenty-seven patients with a diagnosis of acute ITP and 27 with a diagnosis of chronic ITP were included in the study group. Thirty-one age and sex-matched healthy children constituted the control group. Cases were investigated in three groups; Group 1- chronic ITP cases, Group 2- acute ITP cases and Group 3- the control group. Total blood count, direct Coombs test, antinuclear antibodies, the oxidation products 8-OH deoxyguanosine (8-OHdG), protein carbonyl and malondialdehyde (MDA) and also total oxidative stress (TOS), total antioxidant capacity (TAC) and oxidant index (OI) for analysis of the oxidant/antioxidant balance were all investigated from specimens collected from peripheral blood in all three groups. OI value was obtained from the TAC/TOS ratio (OI=(TOS, µmol/L)/TAC, mmol trolox equivalent/lx100). Blood samples were collected following a one-month break in treatment from those patients in Group 1 receiving cyclosporine or other immunosuppressive drugs. Patients with a diagnosis of acute ITP were enrolled at time of diagnosis, before the administration of any drug. Data for the three groups were compared using SPSS 13.01.
Results: A statistically significant difference was determined in 8-OHdG, MDA, TAC and TOS levels between the three groups (P=0.000, P=0.000, P=0.004 and P=0.000, respectively). No significant difference was observed in protein carbonyl and OSI levels between the groups (P=0.208 and P=0.066, respectively). MDA, TAC and TOS levels in Group 1 were statistically higher compared to those in groups 2 and 3 (P=0.000, P=0.004 and P=0.000, respectively), and 8-OHdG levels in Group 2 were higher than those in groups 1 and 3 (P=0.000). 8-OHdG levels were higher in Group 2 compared to Group 1 (P=0.013), while 8-OHdG, MDA and TOS levels in Group 2 were higher than those in Group 3 (P=0.013, P=0.000 and P=0.000, respectively); MDA, TAC and TOS levels in Group 1 were again higher than those in Group 3 (P=0.000, P=0.004 and P=0.000, respectively).
Summary / Conclusion: Both oxidant stress and total antioxidant response as a response to this rise in acute and chronic ITP patients. 8-OHdG, MDA and TAC which shows the antioxidant defense system and TOS were higher in acute and chronic ITP patients compared to the control group. Protein carbonyl exhibited no statistically significant difference among the groups, suggesting that oxidative damage occurring in ITP affects the lipid in thrombocytes and DNA before the protein oxidation stage. In conclusion, we think that oxidative damage is involved in the pathogenesis of acute and chronic ITP.

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PILOT OBSERVATIONAL STUDY OF ROTATIONAL THROMBOELASTOMETRY (ROTEM) TO PREDICT BLEEDING RISK IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES (ATHENA STUDY)

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Background: Previous studies have shown that the platelet count is a poor predictor of bleeding in severely thrombocytopenic patients with hematological malignancies. Rotational thromboelastometry (ROTEM) detects thrombocytopenia but is also sensitive to functional alterations of platelets and soluble coagulation proteins which could contribute to bleeding in severe thrombocytopenia.
Aims: The aim of this study was to evaluate whether ROTEM parameters of extrinsically activated coagulation [EXTEM (clotting time (CT), clot formation time (CFT), maximum clot firmness (MCF), maximum lysis (ML))], intrinsically activated coagulation [INTEM (CT, CFT, MCF, ML] and the fibrinogen contribution to coagulation [FIBTEM (test based on EXTEM but contains cytochalasin D to inhibit platelets) (MCF, ML)] were better at predicting bleeding than the platelet count.
Methods: Prospective cohort study of adults with a hematological disorder undergoing intensive chemotherapy or stem cell transplant (ISRCTN81226121) at two UK centers (Bristol and Oxford) between September 2010 and September 2012. The study inclusion criterion was development of thrombocytopenia (platelet count ≤50x10^9/L). From the start of thrombocytopenia, the participants underwent daily formalized bleeding assessments until platelet count recovery, hospital discharge, death, or for up to 30 days. Bleeding was graded by the WHO score. Alongside routine tests, additional venous blood samples were collected into EDTA and citrate collection tubes three times a week for detailed analysis of platelet and ROTEM parameters. The data were analyzed using a generalized linear model with binomial family and logit link function clustered on patient identity to account for repeated measures.
Results: All 50 participants were followed up until study completion. The baseline characteristics were: mean age 51.0 years; male (33/50); diagnosis (leukemia 16/50; lymphoma 14/50; myeloma 9/50; other 11/50); treatment (chemotherapy 4/50; allograft 33/50; autograft 13/50). Bleeding symptom data were available for 99.7% of study days. The participants had a median 3 days of bleeding (any severity) (interquartile range (IQR) 0-6); and median 11 days (IQR 8-16) with platelet count ≤50x10^9/L. 30.6% of patients had at least one episode of WHO grade 2 or above bleeding during the study period. The unadjusted odds ratio (OR) for bleeding the following day when the platelet count was ≤50x10^9/L was 0.98 (95% confidence interval (CI) 0.97-1.00; P=0.03). The unadjusted OR for bleeding based on the ROTEM parameters (EXTEM MCF and INTEM MCF) were close to statistical significance. However, this effect was lost when the results were adjusted for the platelet count (Table 1). The unadjusted OR for WHO grade 2 or above bleeding the following day when the platelet count was ≤50x10^9/L was 0.98 (95% CI 0.96 to 1.00; P=0.055). The unadjusted OR for bleeding based on the ROTEM parameters (EXTEM MCF and INTEM MCF) were statistically significant but this effect was lost when the results were adjusted for the platelet count (Table 1).
The unadjusted OR for any bleeding or WHO grade 2 or above bleeding based on FIBTEM MCF were not significant.
Summary / Conclusion: This preliminary study does not suggest that ROTEM has any greater predictive value than the platelet count for any bleeding or WHO grade 2 or above bleeding. The statistically significant OR for some of the ROTEM parameters are likely to be explained by a direct effect of platelet count on the ROTEM assay endpoints.

Table 1.

	Unadjusted Odds Ratio	95% CI	P value	Odds Ratio adjusted for plt count	95% CI	P value
Any Bleeding						
Plt count	0.98	0.97 to 1.00	**0.03**			
ROTEM MCF						
EXTEM	0.97	0.95 to 1.00	0.058	0.98	0.94 to 1.03	0.405
INTEM	0.98	0.95 to 1.00	0.069	0.98	0.94 to 1.03	0.523
FIBTEM	1.01	0.97 to 1.05	0.50	1.02	0.98 to 1.06	0.346
WHO Grade 2 or above bleeding						
Plt count	0.98	0.96 to 1.00	0.055			
ROTEM MCF						
EXTEM	0.93	0.88 to 0.97	**0.001**	0.94	0.89 to 1.00	0.07
INTEM	0.93	0.89 to 0.98	**0.004**	0.95	0.89 to 1.01	0.214
FIBTEM	0.95	0.88 to 1.02	0.132	0.95	0.88 to 1.03	0.183

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AUTOCRINE AMPLIFICATION OF INTEGRIN AIIBB3 ACTIVATION AND PLATELET ADHESIVE RESPONSES BY DEOXYRIBOSE-1-PHOSPHATE

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Background: Platelet activation is a complex series of events originating from the stimulation of surface receptors by biochemical signals linked to vascular damage, such as the exposure of the subendothelial matrix and the generation of thrombin in the coagulation cascade. Besides responding to their microenvironment and to the biochemical messages released by other circulating or vascular cells, platelets also release their own rich array of extracellular signals. Amongst the small molecules released by platelets, thromboxane A_2 (TXA_2), adenosine diphosphate (ADP), epinephrine, and serotonin (5-HT) are key autocrine regulators of platelet responses. Recently, we utilised gas-chromatography mass spectrometry (GC-MS) to identify new molecules released by platelets. The pro-angiogenic metabolite deoxyribose-1-phosphate (dRP) was detected in micromolar concentrations in stimulated platelet supernatants.
Aims: In light of the ability of dRP to stimulate the generation of reactive oxygen species (ROS) in different cell types and the pro-aggregatory role of ROS in platelets, we investigated whether platelet-derived dRP plays any autocrine role in the regulation of the redox balance, the intracellular signalling, or the functional responses of platelets.
Methods: The experiments were performed on human platelets from healthy donors or mouse platelets, either wild type or transgenic characterised by genetic deletion of thymidine phosphorylase and uridine phosphorylase ($TP^{-/-}$ $UP^{-/-}$), which results in impaired dRP release upon platelet activation. The effects of deprivation of endogenous dRP (transgenic platelets) or addition of exogenous dRP were tested on washed platelet aggregation (by turbidimetry), integrin αIIbβ3 activation (by flow cytometry), washed platelet static adhesion (by phase contrast imaging), whole blood thrombus formation (by microfluidics and time-lapsed epifluorescence microscopy), ROS accumulation (by live platelet confocal imaging), and signal transduction pathway (by immunoblot).
Results: The addition of exogenous dRP to human platelets significantly increased platelet aggregation and integrin αIIbβ3 activation in response to thrombin. In parallel, genetically modified platelets with double genetic deletion of thymidine phosphorylase and uridine phosphorylase were characterised by reduced release of dRP, impaired aggregation and decreased integrin αIIbβ3 activation in response to thrombin. *In vitro* platelet adhesion onto fibrinogen and collagen under physiological flow conditions was potentiated by treatment of human platelets with exogenous dRP and impaired in transgenic platelets with reduced dRP release. Human and mouse platelets responded to dRP treatment with a sizeable increase in ROS generation and the pre-treament with the antioxidant apocynin abolished the effect of dRP on aggregation and integrin activation. Experiments directly assessing the activation of the small G protein Rap1b and protein kinase C suggested that dRP increases the basal levels of activity of these two pivotal platelet-activating pathways in a redox-dependent manner.
Summary / Conclusion: Taken together, we present evidence that dRP is a novel autocrine amplifier of platelet activation, which acts on platelet redox levels and modulates integrin αIIbβ3 inside-out signalling.

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INCREASED CIRCULATING MICROPARTICLES COEXPRESSING ENDOTHELIAL AND PLATELET MARKERS IN ISCHEMIC CEREBRAL STROKE

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Background: Cellular microparticles (MPs) are submicron plasma membrane derived vesicles shed into the circulation by a variety of blood cells and vascular cells during cellular activation and apoptosis. Currently no practical, rapid and sensitive test is available for the diagnosis of acute ischemic stroke. Current knowledge from earlier studies on MPs suggests that they represent reliable biomarkers as they are cell specific and released early in the pathophysiological cascade of the disease.
Aims: To study the potential use of circulating microparticles assay as biomarkers in the prediction and early diagnosis of thrombotic stroke.
Methods: This study included 20 patients with acute cerebrovascular ischemic infarction confirmed by neuroimaging as well as 20 matched healthy controls. Full evaluation of clinical, radiological and laboratory data was done. Peripheral blood endothelial, platelet, erythrocyte and monocyte microparticles were measured by flowcytometry using their corresponding monoclonal antibodies (anti CD 62 E, anti CD 61 P, anti CD235 and anti CD 14) respectively. An informed written consent was obtained from all patients or their legally authorized relatives as well as the healthy controls prior to their enrolment. Approval of the Institutional Ethical Review Board was obtained.
Results: A significantly higher CD 235 and highly significantly elevated CD 61P and CD 14 were observed in stroke patients compared to controls where platelet derived microparticles PMPs were the most commonly occurring (28.8%). Co-expression of CD61P and CD62E was a common feature in stroke patients (39.5%) which was found to be highly significant when compared to controls. A cutoff value for the co-expression of CD 61P and CD 62 E as a marker of thrombotic stroke was suggested to be 13.5% using ROC curve statistical method. This co-expression was higher among stroke patients with diabetes mellitus and cardiac disease while CD61P expression was significantly higher in diabetic patients when compared to non-diabetics. A significant higher expression of CD 61P and CD 235 was found in patients not receiving anticoagulation at the time of sampling when compared to controls.The MPs of erythrocyte origin was present in 19.2% of patients while those of endothelial origin were the least occurring (2%).
Summary / Conclusion: The higher levels of CD 61P, CD 62 E, CD 14 and co-expression of CD 61P and 62E suggests that the systemic endothelial, platelet and inflammatory cell activation increases the risk for cerebrovascular morbidities especially in patients with diabetes mellitus and history of cardiac disease. MPs co-expressing CD62E and CD 61P can be used as a test for the early diagnosis of thrombotic stroke with high sensitivity and specificity. Establishing a cutoff value for co-expression of CD62E and CD61P in stroke patients can contribute to the clinical applications as using MP assay in diagnosis of thrombotic propensity, monitoring of anticoagulant therapy, and detection of risk of stroke and ischemic heart disease in high risk patients.

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LOW MOLECULAR WEIGHT HEPARIN (LMWH) FOR PRIMARY THROMBOPROPHYLAXIS OF AMBULATORY PATIENTS WITH SOLID CANCER –SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Background: Patients receiving chemotherapy for cancer are at increased risk for venous thromboembolism (VTE). The role of low molecular weight heparin (LMWH) as antithrombotic prophylaxis has been appraised in several studies, yielding conflicting results in terms of efficacy and safety. Currently, prophylaxis is not routinely recommended for ambulatory patients receiving chemotherapy.
Aims: We performed a meta-analysis of all randomized controlled trials (RCTs) which evaluated the addition of LMWH as primary thromboprophylaxis in ambulatory patients with solid malignancies while receiving chemotherapy, in order to assess reduction of VTE.
Methods: A comprehensive search of The Cochrane Library, MEDLINE, conference proceedings and references was conducted until December2012. Two reviewers appraised the quality of trials and extracted data. We assessed both efficacy outcomes and safety outcomes. Outcomes assessed were: symptomatic VTE, pulmonary emboli (PE), any VTE, symptomatic DVT. In addition we assessed mortality and adverse events. For dichotomous data, relative risks (RR) with 95% confidence intervals (CIs) were estimated and pooled.
Results: Nine trials conducted between the years 2004 and 2012 met the inclusion criteria, and randomized a total of 6691 patients. The LMWH used in these trials included: nadroparin (3 trials), certoparin (2 trials), semuloparin (1 trial) and dalteparin (3 trials). Patients included in the trials had locally advanced or metastatic cancer. Cancer types included lung, breast, pancreas, stomach, bladder, colorectal and other. Primary prophylaxis with LMWH significantly reduced the rate of symptomatic VTEs (RR 0.47, 95% CI 0.32 to 0.69, 8 trials), the rate of PE (RR 0.51, 95% CI (0.30 to 0.86, 6 trials), the rate of symptomatic DVT (RR 0.35, 95% CI 0.21 to 0.60, 6 trials), and the rate of any VTE (RR 0.58, 95% CI 0.43-0.77, 8 trials). Meta-analysis of the sixtrials which reported mortaliy outcomes, revealed no statistically significant benefit for LMWH in 1 year mortality rates (RR 0.93, 95% CI(0.83 to1.04). There was no significant increase in major bleeding events in the LMWH arm (RR 1.36, 95% CI 0.91-2.04, 8 trials).
Summary / Conclusion: LMWH reduced the incidence of VTE in ambulatory patients receiving chemotherapy for cancer, with no apparent increase in major bleeding. Nevertheless, LMWH had no significant effect on survival outcomes. Future studies should delineate which specific cancer populations may benefit the most.

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PLATELET COUNTS DECREASE WITH INCREASING LUPUS-LIKE ANTICOAGULANT RATIOS, IRRESPECTIVE OF ANTIPHOSPHOLIPID SYNDROME CRITERIA

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Background: Thrombocytopenia has been described in association with clinically-expressed antiphospholipid syndrome (APS), in up to a quarter of patients, in whom it could be due to immune phenomena, consumptive thrombocytopenia or both. In contrast, the relationship between reduced platelet counts and the presence of lupus anticoagulant (LA) irrespective of APS criteria, has been less consensual, with some authors relating incidences of up to a third of patients, and others finding it a rare event.
Aims: To clarify the relationship between the behaviour of platelet counts and the quantification of LA, in a large cohort of LA-positive patients, irrespective of the presence of clinical criteria for APS.
Methods: We reviewed all requests received in the coagulation sector of our laboratory from 01-01-2000 to 12-31-2012, selecting the 7974 patients with LA determinations. Results were considered Negative when the Lupus Ratio was under 1.2; positive results were further divided into Low (1.20-1.99), Moderate (2.00-2.99) and High Positive (3.00 and over). Platelet counts were compared across these four groups. Results for activated partial thromboplastin time (aPTT) and prothrombin time (PT) were also compared over the four groups, as a control.
Results: LA ratios varied between 0.50 and 5.25, with a median of 1.06, being Positive in 13.7% (1095) of patients with LA determinations. Mean platelet counts were 235.3±102.3 G/L in Negative LA and decreased with increasing ratios of LA - 225.5±99.0 in Low, 195.4±81.3 in Moderate and 157.6±113.8 in High Positive results (P<0.001). Thrombocytopenia, defined as platelet counts under 150 G/L, was present in 13.6% of Negative patients and increased to 16.7% of Low, 28.0% of Moderate and 50.0% of High Positive patients (P<0.001). There were no statistical differences between Positive and Negative patients in mean platelet volume (9.2±1.4 and 9.1±1.3 fL, respectively) or platelet distribution width (16.5±3.0% and 16.5±3.1%, respectively).
As expected, and by definition, there was a significant correlation between increasing ratios of LA and increasing aPTT ratios (r^2 = 0.365). The mean aPTT increased with increasing positivity, being 29.8±8.0s in Negative LA, 41.5±18.7s in Low, 81.8±37.3s in Moderate and 94.6±34.0s in High (P<0.001), corresponding to aPTT ratios of 1.0±0.3, 1.5±0.6, 2.8±1.3 and 3.4±1.2, respectively (P<0.001). The mean PT was 15.4±4.7s in Negative LA (an increase of 1.9±4.7s over the control), and increased with increasing ratios of LA - 16.8±6.6s in Low, 20.4±9.0s in Moderate and 20.1±10.2s in High (P<0.001), corresponding to increases of 3.3±6.6s, 6.7±9.0s and 6.4±10.3s over the control, respectively (P<0.001).
Summary / Conclusion: In a large cohort of nearly 8000 patients with LA determinations, 14% of whom were positive for the presence of LA, we found that there was a consistent decrease in the mean platelet count, from Negative to High Positive lupus ratios, with a difference of nearly 80 G/L of platelets between the two extremes. The incidence of thrombocytopenia likewise increased significantly through the LA groups, rising from 14% of LA-Negative subjects to 50% of High Positive patients. We suggest that decreasing platelets are a feature of the presence of lupus-like anticoagulants, even in the absence of APS. We also show that overt thrombocytopenia is not the only manifestation of the impact of LA on platelet counts, with a consistent decrease being noted even before the threshold for thrombocytopenia is reached. Our results on aPTT were consistent with the published literature and with the known laboratory behaviour of lupus-like anticoagulants, pointing to the validity of our LA results. The described association between the intensity of platelet reduction and the severity of positivity for LA could help explain the opposing results reported by different authors, if distinct LA cut-off values are used, or APS-negative LA-positive patients are excluded.

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REDOX-DEPENDENT ANGIOGENESIS BY NUCLEOSIDE DERIVATIVES OF DEOXYRIBOSE-1-PHOSPHATE

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Background: Reactive oxygen species (ROS) are associated with multiple cellular functions such as cell proliferation, differentiation and apoptosis. Signal transduction by ROS, 'redox signaling' in angiogenesis is an important and emerging topic in cardiovascular sciences. ROS mediate vascular endothelial cell (EC) proliferation and migration, but the molecular mechanism underlying this phenomenon remains largely unclear. Recently we investigated the pro-angiogenic effect of deoxyribose-1-phosphate (dRP) released by stimulated platelets, which was shown to induce an increase in EC motility *in vitro* and a strong angiogenic response *in vivo*.
Aims: In this study, we investigated the role of ROS generation in the angiogenic response mediated by dRP.
Methods: ROS generation was investigated in human umbilical vein endothelial cells (HUVECs) by live cell confocal imaging post stimulation with dRP or its derivatives, deoxyribose-5-phospate (dR5P), glyceraldehyde-3-phosphate (G3P), ribose-1-phosphate (R1P) and deoxyribose (dR). The protein expression of heme oxygenase-1 (HO-1) was investigated, which is known to be upregulated under oxidative stress conditions. We also analysed the protein expression of integrin β3 to investigate the link between dRP-dependent increase in endothelial ROS generation and cell motility. Endothelial cell motility *in vitro* was tested using monolayer repair scratch healing assay. The expression of critical pro-angiogenic molecules such as vascular endothelial growth factor (VEGF), interleukin 8 (IL-8), and stromal cell-derived factor 1α (SDF-1α) in HUVECs treated with dRP was tested by immunoblot.
Results: The addition of exogenous dRP decisively increased HUVEC ROS generation. Similarly, the dRP derivatives dR5P, G3P, and R1P also induced ROS accumulation in HUVECs, whereas dR did not. As expected, the redox stress marker HO-1 was shown to be upregulated by dRP and its derivatives. Interestingly, integrin β3 was shown to be upregulated by HUVEC treatment with dRP and its phorphorylated derivatives (i.e. except dR), as demonstrated by immunoblot experiments in the presence of the ROS scavenger N-acetylcysteine (NAC) and the antioxidant apocynin. In parallel, only the phosphorylated derivatives of dRP appeared to increase HUVEC cell motility in scratch-healing assay. The causative link between integrin β3 upregulation and increased HUVEC motility was proved using integrin-specific inhibitory antbodies.
Summary / Conclusion: In summary, this study demonstrates a link between dRP-dependent increase in endothelial ROS generation, the upregulation of integrin β3 and the increase in endothelial cell motility and angiogenic activity. The paracrine regulation of endothelial cell motility by platelet-derived dRP is likely to play an important role in the stimulation of postnatal angiogenesis and tissue repair.

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THE JAK2 V617F MUTATION IN PATIENTS WITH CEREBRAL VENOUS THROMBOSIS IS ASSOCIATED WITH AN INCREASED RISK OF RECURRENCE

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Background: Cerebral venous thrombosis (CVT) is a rare and severe event. Recently a part of patients has been reported to carry the molecular marker of myeloproliferative neoplasm (MPN) JAK2V617F. Little is known about the follow-up of CVT patients.
Aims: To investigate in a cohort of patients with CVT the rate of recurrent venous thromboembolism and the risk for recurrence associated with either thrombophilia and the JAK2V617F mutation
Methods: We investigated an ambispective cohort of 114 CVT patients; 25 (M/F 18/7) were aged <18 years (median5, range 0-13). The 89 adults (M/F 16/73) had a median age of 35 (range 18-82). Patients with cancer were excluded. Inherited thrombophilia, antiphospholipids, and JAK2V617F were searched in all patients. A follow-up >6 months after CVT was recorded in 72 patients (60 adults), being prospective in 50 of them (31 adults). The probability of recurrence was estimated by the Kaplan–Meier method.
Results: In pediatric patients the leading causes of CVT were loco-regional infections (n=13, 52%). Thrombophilia was diagnosed in 5 (20%); no child had JAK2V617F. In adults CVT was associated with oral contraceptives or pregnancy in 35 (48% of females) and 16 (22% of females), respectively, with other risk circumstances in 13 (14% of cases), and was unprovoked in 24 (27%). Thrombophilia was diagnosed in 27 (30%). Eight patients had JAK2V617F (9%); only one CVT was unprovoked. Four of them had diagnosis of MPN at the CVT event. In the 60 adult patients with an evaluable follow-up (median 2.3 years, range 0.5-32.6, total 336) the incidence of recurrent venous thrombosis was 3.1% pt-years (11 events in 10 patients: three CVT, six DVT- two in the same patient -, two splanchnic thromboses). Recurrences occurred in one of 31 reference patients without either thrombophilia and JAK2V617F (3.2%), three of 21 patients with thrombophilia (14.3%), and six of seven patients with JAK2V617 (86%). No increase in risk was associated with thrombophilia (hazard ratio, HR, 1.11, 95%CI 0.18-6.76); in contrast, the overall HR ratio for recurrence associated with JAK2V617F was 4.75 (95%CI 1.81-35.77). The incidence of recurrence was 9.1% pt-years in the JAK2V617F-positive patients and 1.8% pt-years in those JAK2V617F-negative.
Summary / Conclusion: In CVT patients the overall risk of recurrence is low and is not associated with thrombophilia. However, JAK2V617F is associated with an exceedingly high risk of recurrence, suggesting need for long-term anticoagulation.

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RETROSPECTIVE THREE YEAR AUDIT COMPARING HOSPITAL ACQUIRED THROMBOSIS RATES FOR UNPLANNED AND ELECTIVE ADMISSIONS

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Background: Since 2010, data has been collected on both total venous thromboembolism (VTE) and hospital acquired thrombosis (HAT) events within Derriford Hospital, using the radiological reporting system (CRIS). Root cause analysis is then undertaken reviewing a number of standards, including appropriate VTE prevention. Feedback in real-time on HAT events is then shared with the speciality involved, including whether adequate prevention was used. HAT data has been scrutinised by speciality against the number of admissions to produce information about the incidence of HAT within disciplines and to observe trends.
Aims: Having identified HAT outcome data and incidence by speciality, type of admission (elective or unplanned), was reviewed to see if it had any impact on HAT metrics. Four main surgical specialities; orthopaedic, neurosurgery, cardiac surgery and general surgery, were selected over three years, to determine whether the type of admission influenced the number of HAT events.
Methods: HAT data from 2010-2012 was used to identify events from the four specialty areas investigated. These events were cross-checked with the hospital patient information system to determine whether the admission was elective or unplanned presentation. Information was then produced on the annual number of admissions by speciality, with separate totals for both elective and unplanned. Data was then analysed using a statistical package 'R' reviewing HAT by percentage of admissions for each speciality, by year and for both elective and emergency, to provide outcome data.
Results: Table 1 identifies data for the four specialties showing an increase in HAT for unplanned compared to elective admissions for three of the four specialties. In general surgery, no difference is seen between elective and unplanned HAT probably because 50% of surgical unplanned admissions are discharged without surgery, therefore no analysis for this group was possible. In the three remaining specialities where almost all elective and unplanned patients have an operation, chi squared test was used to assess the total incidence of HAT for all admissions, showing these were statistically similar for every year (x^2=0.1575, p value 0.9243). Comparing elective and unplanned admissions using The Cochran-Mantel-Haenszel test for conditional independence, this showed a statistically significant difference in the incidence of HAT over the three years (P-value 1.533e-11, 95% CI 0.238 – 0.459 for the null hypothesis). Furthermore, HAT rates are significantly different between elective and unplanned admissions according to a chi-squared test of proportions (X^2= 50.6538, p-value 1.102e-12, 95% CI -0.008717488 - -0.004133094 for the null hypothesis). A Fishers exact test gives the same result. [odds ratio (0.3307654)]. These analyses all demonstrate that VTE is significantly higher in the unplanned over elective surgical admission groups.
Summary / Conclusion: Outcome data demonstrates statistically significant increase in HAT for unplanned admissions (2-3 fold), compared to elective admissions in orthopaedic, neurosurgery and cardiac surgery. Elective patients are usually more fit and often attend preoperative clinics where VTE information is given. The importance of VTE risk assessment, prophylaxis and education is important for all surgical patients but particularly emergency patients as the risk of HAT is higher than elective admissions and been shown to remain elevated for several months post discharge.

Table 1. HAT by speciality 2010-2012.

		ELECTIVE		UNPLANNED	
Surgical Speciality	Year	Total HAT/ Total Admissions	HAT %	Total HAT/ Total Admissions	HAT %
Cardiac	2010	2/821	0.24	2/248	0.81
Cardiac	2011	4/921	0.43	2/234	0.85
Cardiac	2012	3/983	0.31	2/236	0.85
Neuro	2010	8/1725	0.46	10/596	1.68
Neuro	2011	8/1502	0.53	10/596	1.68
Neuro	2012	4/1604	0.25	6/646	0.93
General	2010	14/5391	0.26	18/7266	0.25
General	2011	17/4953	0.34	14/7099	0.2
General	2012	10/5349	0.19	18/7536	0.24
Orthopaedic	2010	16/5665	0.28	15/1962	0.76
Orthopaedic	2011	13/5014	0.26	15/2011	0.76
Orthopaedic	2012	16/4846	0.33	20/1859	1.08

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ADVANTAGES AND DISADVANTAGES OF INCORPORATING C-REACTIVE PROTEIN LEVELS, AGE AND D-DIMER LEVELS IN DIAGNOSING PULMONARY EMBOLISM

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Background: Over the past several years the number of performed radiological scans to diagnose pulmonary embolism (PE) increased dramatically, while the incidence of PE hardly increased. This resulted in an increased number of patients exposed to radiation and higher costs. Recently, the use of an age-adjusted D-dimer cut-off value was suggested to increase specificity of D-dimer testing.
Aims: This study investigated the impact of incorporating age and levels of C-reactive protein (CRP) and D-dimer on the sensitivity and specificity to diagnose PE.
Methods: This observational study (2004-2007) included all consecutive patients suspected for PE presenting on the Emergency Department and with simultaneously measured levels of CRP and D-dimer. Only patients who received pulmonary CT angiography were further analyzed. We investigated the correlation between age, CRP, D-dimer and the effect of using an age-adjusted cut-off values for D-dimer [age in years/100 mg/L] for patients ≥50 years. Moreover, the predictive value of these parameters for PE was studied.
Results: Of 4609 patients suspected for PE, 1164 patients got radiological imaging. PE was demonstrated in 309 patients (26.5%). Correlation between D-dimer and CRP levels was 18% (P<0.001) i.e. 18% of variation in D-dimer can be explained by variation in CRP. Interestingly, D-dimer levels were positively correlated with age (8%, P<0.01), but only in patients >50 years and independent of presence of PE. Multivariate regression analysis showed significant contribution of age, D-dimer levels and age*D-dimer (i.e. age-adjusted D-dimer) in the prediction of PE, while CRP did not. Compared to the conventional cut-off level, using an age-adjusted D-dimer cut-off value in patients ≥50 years the negative predictive value decreased from 96% to 93%, while specificity increased from 37% to 50%. In patients ≥70 years this effect was even stronger.
Summary / Conclusion: In the prediction of PE, age and dimer levels are relevant, while CRP levels are not. Using an age-adjusted D-dimer cut-off in older patients modestly reduces the negative predictive value, but dramatically increases the specificity of D-dimer testing, thus reducing the need of performing CT scans.

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EVALUATING ACQUIRED DISORDERS OF PROTEIN C SYSTEM IN PATIENTS ON VITAMIN K ANTAGONISTS TREATMENT BY THE CALIBRATED AUTOMATED THROMBOGRAM

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Background: There is a theoretical concern that abrupt vitamin K antagonists (VKA) discontinuation may result in a temporary hypercoagulable state due to imbalance in the rates of normalization of activity of the vitamin K dependant coagulation factors on the one hand and the natural anticoagulants protein C (PC) and protein S on the other hand. Results of the studies concerning this problem are inconsistent. The Calibrated Automated Thrombogram (CAT) is now considered to better correlate with patients phenotype compared to traditional coagulation tests. When thrombomodulin (TM) is used thrombin generation (TG) becomes sensitive to all disorders of the PC system.
Aims: Aim of our study was to determine how PC system is affected by VKA treatment at different dose regimens (INR range 0,9-3,4).
Methods: TG was measured according to Hemker *et al.* with fluorogenic assay, at 5 pM TF and 4 μM phospholipids in platelet poor plasma (PPP) with PPP plasma+/-TM reagent (Thrombinoscope BV, Maastricht, The Netherlands). The procedure was carried out on an automated fluorometer (Fluoroscan Ascent, Thermolab system, Finland). Thrombin generation curves were calculated using the Thrombinoscope software (Thrombinoscope BV, The Netherlands). The study involved 43 patients with venous thrombosis on VKA in the extended-treatment phase (M/F 23/20, mean age 54,6±15,8 yrs, duration of VKA 1,5-4 yrs), among them 19 patients with therapeutic INR range of2,0 to3,0 and 24 patients at low intensity treatment before the discontinuation of VKA's therapy with INR range of 0,9 to1,8. Control group (n=28) was age matched. STATISTICA 6.1 was used.
Results: From four parameters derived from the thrombin generation curves: Lag time (min), endogenous thrombin potential ETP (nMmin), peak height for thrombin (nM), time to peak (min) only ETP and peak height for thrombin (PHT) seemed important to evaluate disorders of PC system. When the PC pathway was challenged by adding TM to plasma both ETP and PHT were markedly reduced. Normal ranges of reduce defined as 5-95th percentile of ↓ETP calcu-

lated as (ETP in PPP_{-TM} minus ETP in PPP_{+TM})/ETP in PPP_{-TM}×100% and ↓PHT calculated as (PHT in PPP_{-TM} minus PHT in PPP_{+TM})/PHT in PPP_{-TM}×100% in controls were 21-62% and 14-51% respectively. Interquartile range of ↓ETP and ↓PHT in controls was 35-54% and 24-43% respectively. Significant inverse correlation with INR was found both for ↓ETP and ↓PHT (R =-0,72 and R =-0,52, respectively, P<0,05). Values below 21% for ↓ETP and 14% for ↓PHT (i.e. abnormal) were found in 79% of patients with INR 1,9-3,4 (15 persons from 19) and 8% (2 patients from 24) for ↓ETP and 4% (1 patient from 24) for ↓PHT in those with INR 0,9-1,8. Importantly in 26% of cases (5 patients from those 19 with INR range 1,9-3,4) PC system was markedly affected - ↓ETP below 10% and ↓PHT below 5%.

Summary / Conclusion: When TG is measured both with and without TM it is possible to evaluate the function of PC system in the presence of antithrombotic therapy. The results of our study suggest that patients with therapeutic INR range of2,0 to3,0 are at greater risk of rebound phenomenon (i.e. temporary hypercoagulable state) associated with abrupt discontinuation of VKA than low intensity VKA patients and patients on gradual tapering of the dose to discontinuation. Our data support the positions that certain patients could benefit from dose adaptation under CAT controlling and that in thrombophilia patients gradual tapering of the dose to discontinuation could be preferable.

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CONTROL OF VITAMIN-K ANTAGONIST TREATMENT BY MEASURING THROMBIN GENERATION IN WHOLE BLOOD – EFFECT OF THROMBOMODULIN

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Background: Whole blood thrombin generation (TG) testing has been recently introduced. If it can be used for evaluating the effect of antithrombotic treatment this would be a starting point for the development of point of care and home control of vitamin K antagonist (VKA) treatment. The antithrombotic action of VKA treatment results from the fact that its effect on factors II, VII, IX and X overrules the concomitant decreases of the natural anticoagulants protein C and S. In clotting tests the effect on anticoagulant factors goes unobserved, but not in TG experiments. We therefore also investigated the effect of addition of soluble thrombomodulin (TM).

Aims: To compare, in samples from patients taking VKA, TG in whole blood to TG in plasma and to investigate the effect of activation of the APC system with TM in both types of test and to relate the results to the degree of anticoagulation as assessed by the International Normalized Ratio (INR).

Methods: In blood samples from 105 consenting patients on VKA treatment (mainly because of atrial fibrillation) TG was measured with calibrated automated thrombinography (CAT) in the absence and presence of exogenous TM. This was performed in whole blood, platelet rich and platelet poor plasma (PRP, PPP). The INR was determined in PPP of the same patients.

Results: Endogenous thrombin potential (ETP), peak height, lag time and time to peak (ttpeak) measured in whole blood were all significantly correlated with the ones determined in plasma (P<0.01). The concentration dependent parameters of TG (ETP and peak) from whole blood, PRP and PPP of the patients correlated significantly to the inverse of the INR (P<0.01). The time dependent TG parameters (lag time and ttpeak) correlated linearly with the INR (P<0.01). In the majority of the patients, addition of TM (20 nM) to a level that reduced the ETP to around 50% in normal plasma, caused less inhibition in plasma from the patients: 29.8±12.8% in whole blood, 28.5±19.8% in PRP and 39.6 ±12.0% in PPP. This effect was virtually independent of the INR (1.1-5.9).

Summary / Conclusion: This study demonstrates that whole blood thrombin generation can be considered as a reliable tool for determining the anticoagulant activity of vitamin K antagonists. VKA therapy induces TM –resistance when assessed by TG in whole blood as well as in plasma. This effect appears to be independent of the level of anticoagulation.

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THE ROLE OF ANTICOAGULANT THERAPIES ON COAGULOPATHY (DISSEMINATED INTRAVASCULAR COAGULATION) IN PATIENTS WITH HEMOLYTIC–UREMIC SYNDROME AFTER INFECTION WITH ENTEROHEMORRHAGIC ESCHERICHIA COLI O111.

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Background: Enterohemorrhagic Escherichia coli (EHEC) is a major cause of hemolytic uremic syndrome (HUS) and its outbreaks sometimes occur. In 2011, EHEC O111 outbreaks occurred in Japan. A multicenter survey revealed that EHEC-HUS developed in 32 out of 71 patients, resulted in poor prognosis in spite of intensive care. The pathogenesis of HUS is thought to be microangiopathies, but the optimal management using anticoagulant agents and transfusions remains to be established.

Aims: To assess the effectiveness of anticoagulants in hospitalized EHEC-HUS patients.

Methods: We retrospectively reviewed medical records of all hospitalized HUS patients over the outbreak period. Patients' characteristics, therapies and outcome were analyzed. Anticoagulants were as follows: antithrombin (AT), low molecular weight heparin (LMWH), protease inhibitors (PI) and recombinant human thrombomodulin alpha (TM). To clarify which factors were responsible for the outcome, we compared clinical data of survival patients with that of dead patients. Statistical analyses were performed using JMP 10.0.0 (SAS Institute Inc.).

Results: Confirmed and probable EHEC infections were found in 71 out of 181 suspected patients. 32 out of 71 patients diagnosed as clinical HUS [11 males, 21 females; median age at diagnosis 19.9 years (range, 1-70 years)]. Seventeen out of 25 evaluable cases met the overt DIC criteria of International Society on Thrombosis Haemostasis. 26 out of 32 patients (81.2%) received anticoagulants therapies solely or in combination; 5 patients were treated with AT, 5 with LMWH, 20 with PI, and 12 with TM. 23 patients (71.9%) also underwent plasmapheresis at least once. Blood transfusions were performed as necessary. HUS-related encephalopathy was developed in 20 patients (62.5%). 5 patients (15.6%) died with disease; all of them were suffered from encephalopathy. Early/concomitant onset of encephalopathy was associated with poor prognosis. We then assessed the relationships between clinical interventions and outcomes. Univariate analyses showed that platelet counts and several biomarkers for coagulopathy seemed to be improved after administration of anticoagulants and blood transfusions. Decrease of plasma FDP level was associated with treatment of TM (paired t-test; P=0.039). Decrease of serum creatinine level was associated with treatment of TM (P=0.0013). However, in terms of prognosis, no interventions including anticoagulants were associated with survival and duration of inpatient care. Hemorrhagic and thrombotic events were not increased in usage of anticoagulants.

Summary / Conclusion: Although management for EHEC-HUS using anticoagulants was likely to improve laboratory abnormalities, no prognostic advantage was found in our retrospective cohort. TM is a candidate drug to relieve HUS, but it is clear that further investigations are needed.

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UNEXPECTED FATAL PULMONARY EMBOLISM FOUND AT FORENSIC AUTOPSIES

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Background: The most threatening complications of venous thromboembolism are non-fatal and fatal pulmonary embolism (PE). During treatment of a verified deep vein thrombosis or PE the rate of fatal PE is infrequent ranging from about 0.5% to 1.5%, respectively. If a PE is left untreated the mortality is high reaching above 30%. In some cases the first and only objective finding of pulmonary embolism is through an autopsy. The data on unexpected fatal PE mostly found at autopsies are sparse.

Aims: To investigate patients with fatal unsuspected PE diagnosed at forensic autopsies. We wanted to study extension of PE, possible source of emboli as well as co-morbidity and possible risk factors.

Methods: We studied all fatal PE found as the primary cause of death at forensic autopsies during a seventeen year period between 1992 and 2008 at the Department of Forensic Medicine in Gothenburg, Sweden. We searched data from forensic reports, police reports and medical records at local hospitals.

Results: During this 17 yrs. period 150 (94 men and 56 female) out of 15028 autopsies (1 per 100) had a fatal PE as primary cause of death corresponding to less than 1 fatal PE per 100000 persons per year in the underlying population of about 1.5 million people from the region of Västra Götaland, Sweden. The mean age of fatal PE was 63±16 yrs (range 19-93 years) and no difference between male and female. Place of death was in 78% (n:116) at home, 17% (n:26) during hospitalizations, 4% outdoors and 1% at elderly residences. Of those 26 who died of fatal PE during hospitalization the causes of hospitalization were multi-trauma (9), postoperative (3), psychiatric diseases (4), acute dyspnea (2), chest pain (1), suspected PE (1), post-abortion (1) and unknown causes (5). Of all fatal emboli, 147 (98%) had massive extension or a saddle emboli. In 62% (n:93) thrombotic residuals could be found in the legs as a possible source of PE. Of possible thromboembolic risk factors, 25% (n:37) were obese with BMI>30. About 10% had cancer. Psychiatric diseases could be found in 29% (n:44) and alcohol abuse was found in 32% (n:48) being significantly more common in males (47% vs 13%, P<0.0001). In 5% (n:8) abuse of drugs and in 1% (n:2) narcotics were used. Of cardiovascular diseases 15% (n:23) had severe coronary atherosclerosis. In 25% (n:37) the patient had before death complained of syncope (n:10), dyspnea (n:15), chest pain (n:7) or pain in a leg (n:5), but only 9 of these patients had asked for medical attention.

Summary / Conclusion: Among 150 fatal PE found at forensic autopsies, besides hospitalizations of various causes, alcohol abuse (especially among men) and psychiatric diseases might be important risk factors for sudden unexpected death as well as overweight in the form of obesity. Even though one out of four fatal PE had shown symptoms that might recall PE it remains unclear why only a few of them asked for medical advice and how physicians consider the probability of thromboembolism.

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INCIDENCE OF VENOUS THROMBOEMBOLISM FOLLOWING ABDOMINAL CANCER SURGERY IN KOREA

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Background: Cancer and major surgery are well known risk factors of venous thromboembolism (VTE) in Western populations. They have developed several guidelines of thromboprophylaxis for cancer patients who undergoing major surgery. However, there are few epidemiologic studies regarding post operative VTE in Asian cancer patients.
Aims: We performed this study to evaluate the incidence of VTE after general abdominal surgeries for the frequent cancers in Korea.
Methods: The VTE patients after major abdominal cancer surgeries from 2007 to 2011 were retrospectively identified by both diagnostic codes and prescription codes of heparin or low-molecular-weight heparin, using nationwide data from Health Insurance Review and Assessment Service.
Results: Of the 87403 patients who underwent stomach cancer surgery in Korea between 2007 and 2011, 409 patients (0.47%) developed VTE. Of those 409 patients, 306 (0.35%) had deep vein thrombosis (DVT) and 103 (0.12%) had pulmonary embolism (PE) with or without DVT. Multivariate analysis showed advanced age was a risk factor for developing VTE after stomach cancer surgery. Of the 73961 colorectal cancer surgeries, VTE, DVT, and PE cases represented 1235 (1.67%), 1111 (1.50%), and 125 (0.17%), respectively. Laparoscopic surgery significantly increased the risk of VTE after colorectal cancer surgery. The incidence of VTE, DVT and PE after 24026 hepatobiliary cancer surgeries were 0.48%, 0.31% and 0.17%, respectively. Age and female sex were risk factors of VTE after hepatobiliary cancer surgery. The incidence of VTE, DVT and PE after pancreatic cancer surgery were 1.42%, 1.11% and 0.31%, respectively.
Summary / Conclusion: This epidemiologic study demonstrates the lower incidence of VTE after abdominal cancer surgery in Korean populations compared with Western populations. Our study warrants further prospective investigations on the incidence of VTE in abdominal cancer patients with operable disease of different ethnic groups to propose optimal thromboprophylaxis in Asian cancer patients.

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ANTITHROMBOTIC PROPHYLAXIS IN PREGNANT WOMEN WITH ANTITHROMBIN CONGENITAL DEFICIENCY IS EFFECTIVE IN PREVENTING ADVERSE OUTCOMES

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Background: Type I (quantitative) or II (qualitative) antithrombin (AT) deficiency is caused by more than 200 mutations of AT gene. Type II deficiency is subclassified according to dysfunction of the reactive site (RS) or heparin binding site (HBS) or pleiotropic effects (PE). Type II HBS is associated with a low thrombotic risk.
Aims: To investigate the rate of thrombotic and obstetric complications in pregnant women with AT congenital deficiency and the effect of antithrombotic prophylaxis on the outcome of pregnancy.
Methods: We investigated in 32 women with congenital AT deficiency the outcome of their pregnancies with or without antithrombotic prophylaxis. Twenty-two women had type I deficiency and 10 type II (6 HBS, 2 RS, 2 PE); 24 were probands, and eight were relatives of eight probands.Nine women (none with HBS deficiency) had VTE prior to the first pregnancy. The outcomes were venous thromboembolism (VTE) or obstetric complications (OC) (early/late fetal loss, intrauterine growth restriction (IUGR), preeclampsia). Heparin prophylaxis was administered as therapeutic unfractionated or low molecular weight heparin (UFH or LMWH) in 21 women with type I or II (RS or PE) deficiency, as a sequence therapeutic UFH-warfarin-UFH in five women with type I deficiency and as prophylactic LMWH in all women with HBS deficiency.
Results: We analyzed ambispectively 69 pregnancies. Forty-seven non-HBS pregnancies (19 women) were not prophylaxed, and 53% were complicated: seven by VTE (four antepartum), 18 by OC, one was interrupted. Fifteen non-HBS pregnancies (11 women) received intensive antithrombotic prophylaxis, and IUGR occurred in one (relative risk 0.12, 95%CI 0.01-0.84, vs. no prophylaxis). Thus, intensive prophylaxis reduced by 88% the risk of pregnancy complications. Four HBS pregnancies in four women were conventionally prophylaxed and uneventful; finally, two HBS women had three pregnancies not prophylaxed, with OC in one pregnancy.
Summary / Conclusion: Use of therapeutic dosages of heparin in pregnant women with AT deficiency is effective in preventing adverse outcomes; prophylactic dosages of LMWH seem enough in the low-risk HBS deficiency.

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HIGH PREVALENCE OF PROTEIN S DEFICIENCY IN THAI POPULATION

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Background: Factor V Leiden and prothrombin mutations were the most prevalent hereditary thrombophilia in Western patients, while protein S and protein C deficiencies are more common among Asian venous thromboembolism (VTE). In Caucasian population, the prevalence of protein S or protein C deficiencies was approximately 0.2%. Preliminary data suggested that the frequency in Asian population was higher. However, all previous reports in Asians did not repeat the tests to confirm the persistently deficient states.
Aims: The study aimed to investigate the prevalence of protein S deficiency in general Thai population.
Methods: Free protein S antigen and protein C activity were assayed in healthy Thai volunteers in Bangkok and nearby provinces. Subjects with low protein S or protein C were called to repeat the tests and investigate to exclude the acquired causes of deficiencies, i.e. hormonal uses, vitamin K deficiency or antagonist, liver dysfunction, autoimmune diseases or HIV infection.
Results: There were 5244 subjects participating in the study. The mean age was 44.4±13.8 years and 40.9% were male. The numbers of low protein S and protein C on the first tests were 254/5243 (4.84%) and 56/5234 (1.07%), respectively. For protein S, a volunteer with hormonal use and the other with HIV infection were excluded. Fifty-one had persistently low free protein S levels. The lowest estimated prevalence of protein S deficiency was 0.97% (51/5243, 95% CI 0.71-1.23%). The uses of sex-adjusted normal values gave similar results. The mean age was 42 years and 36 (71.6%) were type III protein S deficiency. All cases had no thrombosis, but 5 had history of miscarriages and 2 had family history of thrombosis. For protein C, 2 volunteers with abnormal liver function tests were excluded. Sixteen had repeatedly low protein C activity. Therefore, the lowest estimated prevalence of protein C deficiency was 0.31% (16/5234, 95% CI 0.17- 0.45%).
Summary / Conclusion: Protein S deficiency is more common in Thai population compared with Caucasians. This may explain the different risks for VTE among races. The heritability and clinical significance remain to be determined.

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MANAGEMENT OF EXCESSIVE ANTICOAGULATION IN PATIENTS TREATED WITH ACENOCOUMAROL, A VITAMIN K ANTAGONIST.

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Background: Excessive anticoagulation is frequently seen in patients treated with vitamin K antagonists (VKA) drugs. The management of this situation is controversial concerning the administration and amount of the natural antidote, vitamin K (VK).
Aims: Evaluate the efficacy of VK therapy in terms of both, correction of INR and percentage of subjects reaching a therapeutic range in in-patients with an excessive anticoagulation by acenocoumarol.
Methods: We performed a retrospective study in 801 patients treated with acenocoumarol who had 852 episodes of excessive anticoagulation in absence of bleeding. INR ranging 4-5 have been managed with withholding a dose or, reducing acenocoumarol amount. The choice in patients with an INR ranging from 5-6, 6-7 or 7-8 was to withhold one dose of acenocoumarol, or to administer, in addition a small dose (1 mg) of vitamin K. Those patients with an INR between 8 to10 were managed by withholding one dose of acenocoumarol and some of them also received 1 or 2 mg or vitamin K. If the INR was above 10, the VKA was discontinued for one or two days, and some of these patients were treated with 2 mg of VK.
Results: In patients with INR ranging 4 to5, the withholding of a dose (n=77) achieved a higher decrease in the INR at 24 hr (3.1±0.9) compared with the reduction of one dose of acenocumarol (3.5±0.9, n=92, P<0.001), but with this approach, more patients had INR below therapeutic range (P=0.008). There were no differences between both choices at 72 hr. The reduction of INR, measured at 24 or 72 hr, in those patients treated with 1 mg of VK was not influenced by the route, either oral or subcutaneous. Forty five out of 196 patients with an

INR ranging from 5 to 6 were treated with 1 mg VK and their INR at 24 hr (2.8±1.2) was lower (P<0.05) compared with those patients that did not receive VK (3.2±1.1). However, more patients (24.4% vs 7.3%) were below therapeutic range at this time (P=0.003). No differences were observed at 72 hr. In addition to withhold one dose of VKA, 1 mg of VK was given to 90 out of 230 patients with INR ranging between 6 to 7. This practice achieved a lower INR at 24hr (3.1±1.4 vs 3.9±1.5, P<0.0001) and 72 hr (2.2±0.9 vs 2.8±1.2, P<0.001). Nevertheless, VK administration was associated to more patients with an INR below the recommended range at 24 hr (20.0% vs. 3.6%, P=0.001) and 72 hr (47.8% vs. 21.7%, P=0.001). In patients with an INR from 7 to 8, the withdrawal of one dose of AVK plus the administration of 1 mg of VK (53 out 105) resulted in a lower INR at 24 hr (3.5±1.6 vs 4.0±1.4, P<0.0001), and also at 72 hr (2.2±0.9 vs 3.4±1.5, P<0.001), compared to those patients managed with the withdrawal of one dose of acenocoumarol. In addition, VK administration was associated with fewer patients with INR above 4 at 72 hr (P=0.01). Patients with INR ranging 8-10 treated with, either 1 or 2 mg of VK in addition to withdraw one dose of AVK, reached similar reductions of their INR at 24hr (3.6±1.7 vs 3.8±2.0) and 72 hr (2.5±1.1 vs 2.2±0.8), but improved the reduction obtained at 24 hr with respect those subjects who did not receive VK (5.3±2.4, P<0.001). The number of patients reaching an INR within the therapeutic range at 24 hr was also similar with either dose of VK and improved the group that was not treated with VK (P=0.003). In addition to withhold one dose of acenocoumarol, 26 out of 52 patients with an INR above 10, were given 2 mg of VK. This therapy resulted in a higher reduction of the INR at 24 hr (5.6±2.7 vs 7.3±3.0, P<0.05) and also, fewer patients maintained an INR above 4 (P=0.003). However, the INR was similar in both groups after 72 hr, probably due to the fact that, in 14 patients who were not treated with VK, the acenocumarol had to be withdrawn for two days.

Summary / Conclusion: The current work suggests that the management of overanticoagulation by acenocoumarol requires different strategies including the extent of INR prolongation but also the individual risk of bleeding and thrombosis.

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GENETIC POLYMORPHISMS RELATED TO HEMOSTASIS AND THEIR ASSOCIATION WITH PRIMARY RECURRENT PREGNANCY LOSS

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Background: Pregnancy is characterized by a hypercoagulable state in which levels of some coagulation factors and fibrinolytic inhibitors are increased, whereas levels of the natural anticoagulant free protein S are decreased. Single nucleotide polymorphisms (SNPs) can be associated with alterations in the concentrations of factors, and may be related to the gain of function and formation of thrombus in the placenta.

Aims: The aim of this study was to investigate the association between SNPs in genes of proteins related to coagulation (FI, FII and FV), fibrinolysis (PAI1 and TAFI) and generation of thrombin (PC, AT, TM and TFPI) and risk of RPL.

Methods: This is a case-control study that included 117 unrelated non-pregnant women with three or more consecutive losses prior to 20 weeks of pregnancy without a previous history of carrying a fetus to viability (primary RPL), who were evaluated in the Prenatal Care Unit at the Hospital of the University of Sao Paulo. The control group was comprised of 264 unrelated healthy fertile women recruited among users of Public Health Service or employees of the University of Sao Paulo, who had at least two term deliveries and without known pregnancy losses as well as known medical history of thrombosis, diabetes, hypertension, cancer and autoimmune disorders. Real Time PCR, from Applied Biosystems, was performed using validated TaqMan SNP genotyping assays to determine polymorphisms in fibrinogen beta chain (*FGB* 455G>A, rs1800790 and *FGB* 148C>T, rs1800787), protein C (*PROC* 2418A>G,rs1799809 and *PROC* 2405C>T, rs1799808), antithrombin (*SERPINC1* 786G>A, rs2227589), thrombomodulin (*THBD* 1418C>T, rs1042579), TFPI (*TFPI* T-287C, rs10931292, *TFPI* T-33C, rs8176592 and *TFPI* C-399T, rs10153820) and TAFI (*CBP2* 505G>A, rs3742264) genes. SNPs in factor V (Factor V Leiden, *F5* 1691G>A, rs6025), prothrombin (*F2* 20210 G>A, rs179963) and PAI1 (*PAI1* 4G/5G rs1799889) genes were determined by polymerase chain reaction followed by enzymatic restriction (PCR-RFLP). A model of multivariate forward conditional logistic regression for dependent variable to have RPL was carried out including as independent variables the genotypes for 13 SNPs. The reference for each SNP was: GG + GA genotypes for *FGB* 455G>A, CC genotype for *FGB* 148C>T, AA genotype for *PROC* 2418A>G, CC genotype for *PROC* 2405C>T, GG genotype for *SERPINC1* 786G>A, CC genotype for *THBD* 1418C>T, TT genotype for *TFPI* T-287C, TT for genotype *TFPI* T-33C, CC for *TFPI* C-399T and GG genotype for CBP2 505G>A.

Results: In a forward conditional logistic regression, 2 SNPs related to thrombin generation were selected in model (Table 1). The SNPs in genes related to coagulation and fibrinolysis were not associated with RPL.

Summary / Conclusion: Our data indicate that the variant of *FGB* 148C>T, *THBD* 1418C>T and *CBP2* 505G>A SNPs are associated with higher risk of having RPL, while the variant of TFPI T-287C SNP has a protection effect.

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Table 1. Forward conditional logistic regression for dependent variable to have RPL.

Independent variables	OR	95% CI	P value
FGB 148C>T	2.06	1.20 – 3.55	0.009
THBD 1418C>T	1.75	1.01 – 3.06	0.048
TFPI T-287C	0.41	0.20 – 0.83	0.014
CBP2 505G>A	2.43	1.37 – 4.32	0.002

The independent variables included in the model were the genotypes for *FGB* 455G>A (reference: GG + GA genotypes), *FGB* 148C>T (reference: CC genotype), *PROC* 2418A>G (reference: AA genotype), *PROC* 2405C>T (reference: CC genotype), *SERPINC1* 786G>A (reference: GG genotype), *THBD* 1418C>T (reference: CC genotype), *TFPI* T-287C (reference: TT genotype), *TFPI* T-33C (reference: TT genotype), *TFPI* C-399T (reference: CC genotype) and *CBP2* 505G>A (reference: GG genotype).

P500

HIGH ON-TREATMENT PLATELET REACTIVITY: NOT AN ISOLATED PROBLEM

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Background: High on-treatment platelet reactivity is associated with increased risk of repeated acute coronary syndrome and mortality. Unfortunately, there is a lack of evidence about association with other hemostasis system abnormities. Such knowledge is necessary for effective management of patients with poor response to antiplatelet drugs.

Aims: The aim of the study was to assess the relationship between high on-treatment platelet reactivity and other hemostasis abnormities in patients with acute coronary syndromes.

Methods: A total of 72 patients (57 men, 15 women, average age 64.1±10.4 years) with acute myocardial infarction treated by percutaneous coronary intervention were enrolled in the study. In these patients the response to aspirin and clopidogrel was assessed by impedance aggregometry. Simultaneously NT-proBNP, TNF-α, of markers of primary hemostasis (platelet count, mean platelet volume, von Willebrand factor, P-selectin), coagulation (prothrombin time, activated partial tromboplastin time, fibrinogen, D-dimers, factor VIII, tissue microparticles), fibrinolysis (plasminogen, tissue plasmin activator, plasminogen inhibitor activator1, thrombin activated fibrinolysis inhibitor) were assessed.

Results: In patients with poor response to aspirin, we documented increased level of von Willebrand factor (380.6±35.3 % vs. 201.8±76.6% P<0.001), factor VIII (408.1±201.2% vs. 227.6±92.0 %P<0.01), fibrinogen (6.4±1.6 g/L vs. 4.8±0.8 g/L P<0.001) plasminogen inhibitor activator 1 (26.7±4.3 µg/L vs. 15.9±9.7 µg/L P<0.05), NT-proBNP (631.9±732.4 vs. 262.2±423.7, P<0.05) and TNFα (10.5±5,2 vs. 6.0±5.4 P<0.05).

Summary / Conclusion: Poor response to aspirin is associated with severe disturbances in the whole hemostasis system. It might be related to heart failur and immune system activation as well. Detailed description of these changes is essential for improvement of acute coronary syndrome treatment.On the other side, we did not find any factor associated with poor response to clopidogrel.

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P501

EVALUATION OF PIVKA-II AS A PREDICTOR MARKER FOR PORTAL VEIN THROMBOSIS IN HEPATOCELLULAR CARCINOMA PATIENTS

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Background: Hepatocellular carcinoma (HCC) is the sixth most common malignancy and the third most common cause of cancer-related death worldwide. While the survival of patients with most malignancies has improved over the last decade, 5-year survival of patients with HCC has remained less than 10% . Presence of portal vein thrombosis hinders prognosis and limits therapeutic options of management in HCC. Des-gamma carboxyprothrombin (DCP), also known as protein induced by vitamin K absence/antagonist-II (PIVKA-II), is an abnormal form of the coagulation protein, prothrombin .
Aims: The aim of our study was to evaluate the value of PIVKA-II measurement as a predictor of portal vein thrombosis among other factors when HCC on liver cirrhosis is diagnosed with trans-abdominal ultrasound.
Methods: The present study included fifty newly diagnosed patients (37 males and 13females) with proved histopathological diagnosis of HCC who were diagnosed at Hepatology Unit at Ain Shams University hospital. Patients were subjected to history taking, physical examination and radiological assessment with abdominal ultrasonography and computerized tomography. The following laboratory investigations were done, -Complete blood picture (CBC),Prothrombin time (PT) and international normalized ratio (INR).,Liver function tests (ALT, AST, ALP, serum albumin and serum bilirubin) :,Kidney function tests (serum creatinine).,Alpha feto protein (AFP) determination (using AFP Mab ELISA Kit by EQUIPAK).and Serum PIVKA-II level determination using micro cup type enzyme immunoassay test kit (Eitest PIVKA-II kit by sanko junyako – Tokyo Japan)
Results: In the present study, a statistically higher PIVKA-II (z=2.575; P=0.010) has been found in patients with PVT on comparison with patients without PVT. PIVKA-II levels were 97.0mAu/ml±81.3 (median=88.4), with a range from 0.8 to 266.3 for patients with PVT and 40.2mAu/ml±40.6 (median=39.2), with a range from 0.2 to 146.3 for patients without PVT. For determination of the best PIVKA-II cut-off value that best predicts PVT, a receiver operating characteristics curve (ROC curve) has been plotted. The best cut-off value of PIVKA-II that best predicts the PVT was 100 mAU/mL. AT this cut-off point the diagnostic performance of PIVKA-II was 74.0% with 47.6% sensitivity and 93.1% specificity with a highly significant association between the studied marker and PVT.
Summary / Conclusion: serum PIVKA II levels >100mAu/ml is a predictor for development of PVT in patients with HCC and which influences the therapeutic options available for the patients

P502

HIGH INCIDENCE OF VENOUS THROMBOEMBOLISM AND ITS PROGNOSTIC IMPLICATION IN PATIENTS WITH SMALL CELL LUNG CANCER

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Background: Recent studies reported that cisplatin is associated with an increased risk of venous thromboembolism (VTE) in patients with advanced solid tumors compared to non-cisplatin-based chemotherapy. Small-cell lung cancer (SCLC) is highly responsive to platinum-based combination chemotherapy; cisplatin in combination with etoposide or irinotecan is the most commonly used regimen. Therefore, we can assume that the risk of VTE in SCLC is quite high.
Aims: This study aims to determine the incidence, risk factors and prognostic implication of VTE in patients with SCLC. We also evaluated the association between cisplatin-based chemotherapy for the treatment of SCLC and the risk of VTE.
Methods: Between January 2006 and June 2010 we consecutively enrolled 221 patients diagnosed with SCLC at Seoul National University Hospital. We retrieved all reports of radiologic or nuclear medicine studies performed on each patient from the electronic medical record. All reports were converted to Microsoft Office Excel files and identified for VTE cases by search function. Two independent investigators double-checked the diagnoses of VTE by reviewing respective records. In order to minimize the observed confounding bias, we used propensity score-matching to compare overall survival between patients with and without VTE.
Results: The 6-month and 2-year cumulative incidence of VTE were 8.2% and 11.8%, respectively. The 2-year cumulative incidences of VTE were 5.1% in limited-stage and 17.6% in extensive-stage. The only independent predictor of VTE was the extensive-stage (vs. limited-stage) (HR 3.9; 95% CI 1.12–13.59; P=0.033). Among a total of 18 cases of VTE, 15 (83.3%) were developed in the first 6 months after cancer diagnosis. About 80% of patients had received cisplatin-based chemotherapy and all 13 cases of VTE developed during chemotherapy had occurred after the exposure to cisplatin. Cisplatin-based chemotherapy increased the risk of VTE approximately 2-times higher than non-cisplatin based chemotherapy (HR 2.11; 95% CI 0.27–16.17; P=0.47), which were not statistically significant. The development of VTE was not associated with an increased risk of death in overall (HR 1.48; 95% CI 0.91–2.41; P=0.18) and propensity-matched (HR 1.43; 95% CI 0.71–2.89; P=0.32) cohort.
Summary / Conclusion: Our study shows that the risk of VTE in patients with SCLC is quite high. The exposure to cisplatin could not influence meaningful differences in the incidence of VTE according to the exposure status. Instead, cisplatin-based chemotherapy may increase the overall incidence of VTE in SCLC. A significant association between VTE development and decreased survival was not found in patients with SCLC.

Health economics

P503

PERIPHERALLY INSERTED CENTRAL CATHETERS (PICCS) CAN BE SUCCESSFULLY UTILIZED IN HAEMATOLOGICAL PATIENTS RECEIVING INTENSIVE CHEMOTHERAPY OR ALLOGENEIC/AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: Patients with haematological disorders frequently require the insertion of medium or long-term central venous catheters (CVCs) for stem-cell transplantation, the administration of chemotherapy, or transfusions. Although peripherally inserted central catheters (PICCs) have been in use for many years, little data exist on their use in patients receiving intensive chemotherapy and blood progenitor cell transplantation.
Aims: Here, we carried out a clinical prospective investigation to determine the efficacy of these interventions in reducing the rate of PICC-related complications (thrombotic events, exit site infection and other complications requiring early removal of PICCs); the studied population included hematology patients receiving intensive chemotherapy compared to allogeneic/autologous stem cell transplant recipients.
Methods: Evidence-based interventions were implemented in our department from November 2009 to July 2012, and include: 1.An high level nurse education program for correct practices and prevention of catheter-associated complications. was developed for PICC nursing team; 2) The use of ultrasound guide for the insertion of the tip of PICCs, thanks to a special operator training; 3) Bedside placement and confirmed PICC tip placement by chest radiography after removal of the guidewire and before the securing of the catheter; 4) Maintenance of maximum sterile barrier precautions during PICC insertion and aftercare; 5) chlorhexidine preparation, replace 10% povidone iodine for skin antisepsis; 6) adoption of PICC patient nurse archive, including the information of weekly PICC line review at our department for each patient.

Table 1. Outcomes according to underlying disease and relative adhibition.

	Chemotherapy		ASCT		Statistics					Odds Ratio	
Diagnosis	% Definite CRBSI	% Thrombosis	% Definite CRBSI	% Thrombosis	Definite CRBSI (%)	Definite CRBSI per 1000 PICC days	Thrombosis (%)	Thrombosis per 1000 PICC days	Range (days)	Means (days)	OR CRBSI	OR Thrombosis
Lymphoma (n. 182))	0,69	4,16	2,63	2,63	1,10	0.10	3,85	0,34	4-336	146,3	3,86	0,62
Acute Leukemia (n 120))	0.95	4.76	0	13.33	0,83	0.07	5,83	0,46	99-432	213.8	0	3.07
Others (n. 62)	0	2.32	0	0	0,00	0	1,61	0,19	6-287	79.2	0	0
Overall	0.68	4,11	1.39	4,17	0,82	0.07	4,12	0,36	0-482	112,94	2,042	1,014

Results: Three hundred sixty-four (364) PICCs were in place in 299 patients for a total of 41.111 PICC days (range, 1-482 days; mean 112,94 days); 292 were inserted in patients receiving conventional chemotherapies, and 72 in patients undergoing allogeneic or autologous hematopoietic stem cell transplantation (SCT). Sixty-six (60) PICCs were inserted during severe thrombocytopenia (platelets <50x10^9/L), seventy (70) during severe neutropenia (neutrophils <0.5x10^9/L) and thirty-eight (38) during antithrombotic prophylaxis. Predominantly, patients had Lymphoma (50%). The rate of major complication was very low: 15 thrombotic complications PICC-related (4%; 0.36 per 1,000 CVC days), and 3 CRBSI (0,8%; 0.07 per 1,000 CVC days) during neutropenia. Mechanical complications occurred in 52 catheters, and were accidental dislodgement (30), catheter break (3), catheter inadequate (19); other reasons for catheter removal were completion of therapy (137), lumen occlusion (19) and death (58). Interesting, taking in account the underlying disease, lymphoma and leukemia patients have, respectively, an increased risk of developing a CRBSI and a thrombotic PICCs-complication when submitted to hematopoietic stem cell transplantation (SCT) (Table 1). However, compared with allogeneic/autologous stem cell transplant group, the intensive chemotherapy group was associated with a marginally lower incidence of CRBSI complication rate (0.6 % vs 1.0 %, 0.10 vs 0.60 per 1,000 CVC days) [odds ratio (OR)2,042]; no relevant differences in terms of thrombotic complications between the two cohorts (4.11 % vs 4.17%), 0.29 vs 0.39 per 1,000 CVC days) [odds ratio (OR) 1.014].
Summary / Conclusion: Our findings suggest, therefore, PICC devices are a viable and safe option for management of the haematology patients receiving intensive chemotherapy and even in patients particularly prone to infective and thrombotic complications such as patients receiving blood stem cell transplantation.

P504

PICC INSERTION AND MANAGEMENT IN HEMATOLOGY PATIENTS: 5 YEARS PROSPECTIVE STUDY THE CAGLIARI'S DEPARTMENT OF HAEMATOLOGY

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Background: Central Venous Catheter (CVC) is crucial for a haematology patients appropriate clinical management (chemotherapy, supportive treatment, aphaeresis procedure, hematopoietic stem cell transplantation). However these devices can cause clinical complications either related to disease itself or to catheter features.
Aims: The purpose of our study was to evaluate if introduction of Peripheral Insertion Central Venous Catheter (PICC) in clinical practice is feasible and could simplify patients management.
Methods: In our Medical Oncology Department, a PICC team, consisting by an hematology physician and two dedicated nurses, has been formed. Since the start of the experience a prospective study was started to evaluate complication rate and usefulness of PICC device in hematology practice. Inclusion criteria included inpatient and outpatient who needed program of chemotherapy, support or hematopoietic stem cell transplantation, regardless the underlined hematological disease or white cells and platelets counts. All patients were submitted to a preliminary evaluation of arms vascular anatomy by ultrasonography. All implantation procedures has been done under ultrasound guide with radiographic control after insertion. Probability of catheter life was calculated with Kaplan-Meier method. Univariable analysis was performed with the method of log-rank test.
Results: From March 2007 to July 2012 1060 consecutive PICC device have been implanted by our PICC team. Of those 321 (30%) consecutive PICC have been implanted in hematology patients. This analysis is restricted to the 305 hematology patients. There were 165 male and 140 females. Median age was 46 years, range 16-94. With regard to disease, 108 patients (35,4%) had Hodgkin Lymphoma, 61 (20%) non Hodgkin lymphoma, 48 (15.7%) acute lymphoblastic leukemia, 44 (14.2%) multiple myeloma, 36 (11.8%) acute myelogenous leukemia, and 8 (2.6 %) other hematological disease. Forty-two PICC (13.8%) have been used for autologous stem cell transplantation and 1 (0.3%) for allogenic transplantation. Catheter insertion was successful in 305 instances (95%), in 16 instances (5%) PICC insertion was not possible**.** At the time of this analysis 23 out of 305 PICC (7,5%) are still *in situ* and in use and 282 (61%) have been removed. Reason for removal was end of therapy in 186 instances (61%), accidental withdrawal in 27 (8.8%), patient death in 36 (11,8%) and catheter related complication in 33 (10,8%). Catheter related complications were the following: 2 (0.65%) catheter ruptures , 6 (1.96%) malfunctioning, 15 (4.9%) occlusions, 2 (0.65%) delayed abnormal dislocations, 6 (1.96%) suspected PICC-related sepsis, 1 (0.3%) local infection and 1 (0.3%) patient poor compliance. Only 1 episode of confirmed PICC-related septicemia (0.3% - 0.02/1000 days/PICC) was recorded and E.Coli was isolated. There were only 3 cases (0.98%) of symptomatic PICC-related thrombotic complications without the need forremoval.PICC median life was 131 days (1-722) for a total of 45,674 days of implanted PICC. Kaplan-Meier probability of catheter life, censored forremoval by the end of therapy and the patient'sdeath, was 80% at day 180 and 51% at day 455. Neither disease nor white cells or platelets count had influenced on catheter life.
Summary / Conclusion: These data encourage the use of PICC in the hematological patient because of insertion easiness, duration of life and low rate complication.

P505

A NEW SCORE TO DETERMINE THE PROBABILITY OF FINDING AN HLA IDENTICAL UNRELATED DONOR: A VALIDATED EFFICIENT TIME AND COST SAVING METHOD

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Background: We recently demonstrated that a delayed time to find an unrelated HLA compatible donor and also to proceed to allogeneic-HSCT can worsen the transplantation outcome (Michallet *et al*. ASH 2010). The French transplant registry has recently developed a software called "Easy Match" that predicts the number of HLA compatible donors for a given patient. The goal of our study is to validate this new program comparatively to a known score method

(Tiercy *et al. BMT 2007, 40; 515-522*) and to improve the cost and search delay.
Aims: To accelerate the process of donor search using the results of EasyMatch program and define a new score for donor finding probability, in order to be time- and cost-efficient.
Methods: For the first step, we retrospectively analyzed 217 patients transplanted between 2009 and 2011 after finding an unrelated donor (identical or not) or cord blood units. We used the EasyMatch software which realized a "qualitative" analysis that consisted on checking that each HLA recipient phenotype was found among all possible pair wise combinations of 2 haplotypes of the different sets of haplotypes. Various "quantitative" analyses calculated the likelihood associated to each recipient phenotype for a given set of HLA genes, in a given population, at low versus high resolution typing. The EasyMatch software gave for each patient a number of potential donors sharing the same phenotype as the patient. We validated the probability given by the EasyMatch program with the known published score method.
For the second step, we used the new defined score prospectively for 62 new patients, directing the search in donor or cord blood files as stipulated by the probability to find a suitable 10/10 donor. We, therefore, analysed the number of complementary typing asked for each group of patients (before and after new score), and the delay between registration of the patient and identification of the donor or cord blood.
Results: Our 217 patients were classified in 3 different categories according to the combined results of the EasyMatch software and the HLA score (Tiercy): - score 0 with low probability to find a suitable 10/10 donor (96% of transplantations performed with a non 10/10 donor in our study). The choice of the source will be defined considering the HLA characteristics of the recipient; in case of class I rare allele or rare HLA-BC linkage disequilibrium, a cord blood unit will be easier and more rapidly available. A complementary help should be given by an associated analysis with 4-digit haplotypes using the HaploStats program (http://www.haplostats.org/home.do). - score 1 with a median probability to identify a suitable 10/10 donor (67% of transplantations performed with a non 10/10 donor in our study). - score 2 with high probability to find a suitable 10/10 donor (95% of transplantations with a 10/10 donor in our study)
The number of supplementary HLA typing and the delay to identify a suitable donor or cord blood was significantly reduced when using the new score, in each of the three categories (Table 1).
Summary / Conclusion: The EasyMatch program provides us (1) easy tracking of mismatches (2) estimation of the number of potential donors (3) selection of population following ethnic origin of patients and a high prediction when number of potential donors is higher than 5 or <1. Its use could be improved when coupled with a second scoring system. In conclusion, the use of this new scoring system allows time and cost spare. In case of low chance to find a donor, physicians can switch to searching for unrelated HLA mismatched donor or a CBU, or to use another alternative treatment in order to keep an optimal result.

Table 1.

Score		Before score	After score	p
0	Median nb of supplementary typing Delay (days)	n=75 7.8 144	n=31 6.5 37	<0.001
1	Median nb of supplementary typing Delay (days)	n=39 7.2 61	n=12 4.75 28	0.003
2	Median nb of supplementary typing Delay (days)	n=103 2.5 46	n=19 1.74 30	0.05

P506

ESTIMATES OF BURDEN OF DISEASE ASSOCIATED WITH MANAGEMENT OF ACUTE MYELOID LEUKEMIA IN UK AND US

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Background: Acute Myeloid Leukemia is the most common leukemia whose incidence increases with age. It accounts for approximately 1.2% of cancer deaths in the United States and the incidence is expected to rise as the population ages (Burnett, 2011). The median age at diagnosis is 65 years, with almost half of the cases occurring in individuals over 60 years (Redaelli, 2004). Although relatively uncommon, AML generates high resource use and total burden. Previous studies of the burden of disease (BoD) are rather outdated. Additionally, no current estimates have been published for the UK and US that incorporate the costs associated with basic disease management for this group of patients.
Aims: To estimate the economic burden of AML in the UK and US.
Methods: Initially, a systematic literature review was conducted in order to understand the standard types of AML treatment . Secondly, economic costs were estimated per course of treatment per individual patient which included intensive induction therapy (IT), consolidation therapy (CT), including use of stem cell transplantation (SCT), supportive treatment during CR, and salvage therapy for those with AML relapse. The cost calculation combined all available treatment UK- and US-specific costs estimates, such as drugs, hospitalization, support staff, diagnostics and transfusion. The total economic burden was calculated combining cost per patient with epidemiology data. Incidence rates for the US and UK and treatment outcome probabilities were calculated from the Surveillance Epidemiology and End Results (SEER), Eurostat and peer reviewed literature. Unit costs were identified for UK and US using publicly available databases. Calculations were conducted for younger (<65) and older (>65) patients given differences in incidence rates identified between these groups.
Results: In 2011, the total cost of treatment with standard induction chemotherapy was £29,858($35,539) in UK and $ 241,427 in US while for best supportive care (BSC)£24,154($28,750) and $ 187,315, respectively per patient. The total economic burden of AML treatment ranged from £13 M for population aged >65 and £38 M for age<65 in the UK and approx. $0.5 billion and 1.5 billion in US, respectively. Hospitalization was the major component of the burden (66% to 92%)in induction, consolidation, and relapse, while for BSC, these were transfusion costs in UK (65%) and drugs costs in US (63%). Cost of CR consists primarily of laboratory monitoring and supportive care. The financial burden after relapse was high, comparing to the cost of being followed in CR stage, e.g. cost of relapse in UK in BSCwas£683and£6,387 in chemotherapy vs. £4,097in CR. Respectively in US relapse cost equaled$2,477 for BSC and $56,588 for chemotherapy vs. $14,861of CR.
Summary / Conclusion: Despite the variety of therapies which are available for AML patients, the prognosis of AML is still relatively poor, especially among older patients where the risk for relapse is large. Moreover, there is a shortage of the costs and cost-effectiveness of the different stages of AML therapy. The economic burden of AML treatment is very high. Furthermore, the disease cost increase when relapse occurs. Therefore, there is a need for new therapies which will sustain CR status. Due to the different AML patient profiles, it is highly important to present the treatment consequences in the groups which are the most susceptible.

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TIME AND RESOURCE SAVINGS WITH RITUXIMAB SUBCUTANEOUS (SC) INJECTION VS. RITUXIMAB INTRAVENOUS (IV) INFUSION: FIRST RESULTS FROM A TIME-AND-MOTION STUDY (T&M)

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Background: Rituximab (RTX) is used as a standard treatment for indolent non-Hodgkin lymphoma (iNHL). As an alternative to intravenous (IV) infusion, a fixed dose, subcutaneous (SC) formulation for RTX is currently being developed. While a first IV infusion typically lasts up to 4 hours, and any subsequent infusion more than 2 hours, results from SABRINA showed that SC administration enables the delivery of RTX over approximately 5 min without compromising RTX's proven efficacy and safety. With a switch to SC, it is expected that significant healthcare professional (HCP) time would be saved.
Aims: To quantify resource utilization in terms of active HCP time (i.e. time actively dedicated to a patient) and chair time related to RTX SC injection vs. RTX IV infusion in the treatment of patients with iNHL; to estimate potential time savings with a conversion from IV to SC (per administration session, per patient treatment course).
Methods: This is a multi-national, multi-centre, prospective, observational T&M study. Data associated with RTX SC injections are being collected alongside the MO25455 trial, while data for RTX IV infusions are collected per real-world practice during the same data collection period. Following interviews with a nurse and pharmacy member in each centre, generic case report forms (CRFs) for IV, SC, and pharmacy processes were tailored to reflect local site practices. Adequate "start" and "stop" descriptions for pre-specified tasks were defined to ensure accurate and unbiased T&M data collection. Trained observers measured the duration that HCPs are actively completing tasks using stop watch. Infusion chair time was captured based on the time of day the patient entered and exited the chair for RTX infusion/injection. This is a descriptive study with convenience-based sample sizes ranging between 20-40 observations per process per site. While data collection is ongoing (final data expected during Q3 2013), first results for HCP time in treatment room and chair time in 4 countries (Italy [IT], Russia [RU], Slovenia [SL], and United Kingdom [UK]) and 9 sites are presented in this abstract (no pharmacy HCP data are yet available for the

UK). Per country, estimated time for a single IV vs. SC process (pooled across sites) is calculated as the sum of individual mean task times.
Results: Differences in active HCP time (and % reduction) are 7.3 min in SL (IV 15.0 vs. SC 7.7; -49%), 11.8 min in UK (IV 26.0 vs. SC 14.2; -45%), 16.3 min in IT (IV 39.8 vs. SC 23.5; -41%), and 17.1 min in RU (IV 26.5 vs. SC 9.4; -65%). Data on active HCP time in pharmacy (IT, RU, SL) show similar relative reductions in the range of -37% (SL) to -65% (RU), while 82% (RU) to 86% (IT) of time savings are achieved in the treatment room. Differences in mean chair time are 131 min in SL (IV 201 min vs. SC 70 min; -65%), 176 min in UK (IV 195 min vs. SC 19 min; -90%), 225 min in IT (IV 339 min vs. SC 114 min; -66%), and 262 min in RU (IV 278 min vs. SC 15 min; -94%). Simulating these findings for a hypothetical center treating 50 patients for 9 sessions annually (6 induction, 3 maintenance) resulted in total chair time savings between 109 (SL) and 219 eight-hour days (RU).
Summary / Conclusion: Preliminary results indicate that a switch from IV infusion to RTX SC may lead to a substantial reduction in administration chair time as well as in active HCP time. Consequently, time freed up could be invested in other patient care activities, or increase the number of patients that could be treated, hence increasing the centre's overall efficiency.

P508

TREATMENT PERSISTENCE WITH AZACITDINE (VIDAZA) IS ASSOCIATED WITH LOWER INPATIENT HOSPITALIZATION COSTS OVER TIME AMONG MDS PATIENTS

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Background: In the US, understanding the costs associated with Myelodysplastic Syndromes (MDS) is challenging given that multiple channels including pharmacy, ambulatory, and inpatient hospitalization (IPH) settings make up the total expenses to manage patients. Recent studies suggest that MDS patients under active medical management experience fewer cytopenia-related medical problems compared to untreated, transfusion dependent (TD) patients who require more medical treatments, often for recurrent infections and bleeding complications. It is clear that persistence of drug therapy is essential to achieve optimal clinical responses for MDS patients and we sought to determine if continued therapy also optimized costs related to the disease.
Aims: To evaluate the relationship between treatment persistence with AZA and health care costs encountered for patients with MDS.
Methods: Commercial and Medicare Advantage enrollees with a diagnosis of high grade MDS (ICD-9, 238.73) who initiated AZA with pharmacy and medical benefits in the prior 6 months and who had a variable follow up period from initiation of AZA to disenrollment or end of study were identified in a US health plan claims database (1/1/2007-6/30/2010). The number of AZA "cycles" was calculated by dividing the total number of AZA administrations by 7 days, with a sensitivity analysis for 5 day administration, - commonly utilized in the "real-world". Persistence was defined as the number of cycles of AZA. Eligible patientshad to have at least 2 AZA cycles. An independent analysis identified health care costs for the same patients during periods of transfusion-dependence (TD) - defined as periods in which they received 2 transfusions in an 8 week period and did not receive erythropoietin-stimulating agents (ESAs) or AZA. Average Per Patient Per Month (PPPM) costs were examined among patients with various lengths of TD periods, up to 1 year. Linear models were used to examine the relationship between persistence on AZA and PPPM health care costs. Healthcare costs included both payer and patient paid amounts under the medical and pharmacy benefit. Medical costs were further broken out into IPHs, ambulatory, and other costs captured. Several sensitivity analyses were performed to confirm the robustness of the results such as excluding patients with IPH prior to AZA initiation, and including patients with <2 cycles of AZA.
Results: The baseline cost breakdown for MDS patients (n=225) who were transfusion dependent and not receiving treatment are outlined in Figure 1. Interestingly, the largest proportion of the medical costs for TD patients comes from IPHs. In fact, the PPPM IPH costs among TD periods account for approximately 65-75% of total health care costs - even at one year of their diagnosis. A similar analysis was done for patients completing at least 2 cycles of AZA (n=100) which suggested that the proportion of cost related to IPHs was closer to 40%. This cohort averaged 6.3 cycles (median=5) with 24% of patients completing at least 8 cycles. Importantly, completion of every additional AZA cycle (baseline 7day analysis) was found to be associated with, on average, a 6% decrease in medical care costs (P=0.005) driven largely by an 18% decrease in IPH costs (P<0.001) due to fewer medical events. Even a single additional AZA cycle was found to be associated with 5% lower total health care cost (P=0.006). These results also hold in the sensitivity analyses. As expected, an examination of medical needs of both TD and AZA treated patients led infections as a frequent driver of IPHs.
Summary / Conclusion: Patients who persist with AZA therapy have lower PPPM medical costs, driven by decreased expenditures on IPHs. This is consistent with results identified in the AZA-001 clinical trial in which patients receiving AZA experienced reduced IPHs driven by less transfusions and need for IV antibiotics, antifungals, and antivirals. These lower overall costs offset the expected increase in continuing therapy based on the cost of drug alone. Improving duration of therapy of AZA may not only optimize clinical outcomes but may decrease cumulative costs of care among high risk MDS patients.

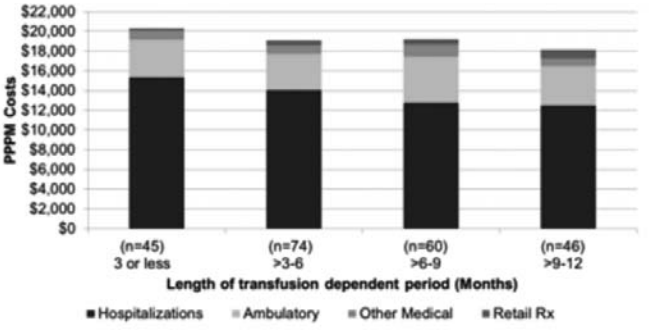

Figure 1. Average monthly hospitalization costs remain steady during TD periods of up to 12 months.

P510

HEALTH CARE COSTS IN NEWLY-DIAGNOSED PERSONS WITH MYELOFIBROSIS WITH MYELOID METAPLASIA: DATA FROM A US COMMERCIAL AND MANAGED MEDICARE POPULATION

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Background: Myelofibrosis with myeloid metaplasia (MF) is a myeloproliferative neoplasm characterized by bone marrow fibrosis and abnormal blood cell production. Median age of diagnosis is about 65 y and median survival, 5-6 y. There are diverse treatment options but none are curative. Little is known about overall health care utilization and costs in persons with MF.
Aims: Describe the economic burden of MF from a payer perspective.
Methods: US health plan data were assessed between 01 July 2002 to 30 June 2012 to identify enrollees newly diagnosed with MF (ICD-9-CM diagnosis codes 238.76), which includes idiopathic myelofibrosis, myelofibrosis with myeloid metaplasia, and primary myelofibrosis. Enrollees were required to be continuously enrolled in a commercial or Medicare Advantage plan with a medical and pharmacy benefit for six months before diagnosis and were followed until they dis-enrolled from the health plan or until the end of the study period (30 June 2012). Enrollees <18 y were excluded. Health care costs included all reimbursed amounts to the provider from the health plan and enrollee (e.g., co-payments, co-insurance). Costs were captured in the six months before diagnosis of MF and the year following diagnosis. Cost analyses were conducted from the payer perspective.
Results: 365 newly diagnosed enrollees with MF persons were identified. Average of duration of follow-up was 26±21 mo. (range: 1- 69 mo) with 120 (33%) were enrolled <1 y. Median age was 66 y (range, 19 - 87 y). The majority of people were 65 y or older (196, 54%) with 134 enrollees (37%) age 44- 64 y. Most people age ≥ 65 y were Medicare enrollees (60%), however, there were substantial numbers of older people with commercial insurance (40%).
Total health care costs in the 6 mo before diagnosis of MF averaged $2,525 ± 5,261. In the year after diagnosis, total health care costs average $10,523 ± 51,654 even though 33% of the persons were enrolled less than the entire year (disenrollment may be due to a change in insurer or death). Approximately 81% of costs were for medical services with a smaller fraction (19%) for drugs dispensed via retail pharmacies. However, mean costs of subjects with evidence of anemia treatment (i.e., RBC transfusions or ESA use), are 85% greater than among those without evidence of anemia treatment (p-value < 0.001).
Summary / Conclusion: Total health care costs for persons with MF in the US, enrolled in a commercial or Medicare Advantage health plan, increase following diagnosis. Average health care costs among those with evidence of anemia are significantly greater than those without evidence of anemia. Treatments that control anemia have the potential to reduce costs in this MF population.

P511

THE IMPACT OF UNRESTRICTED FUNDING ON HAEMATO-ONCOLOGY TREATMENT CHOICE

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Background: In England funding for treatment of haematological cancers is routinely available for nationally approved treatments, often costing < £30 000 per QoL adjusted year, supplemented by geographically variable locally agreed treatments, often at a multi-hospital network level, usually of older and cheaper treatments, such as R-CHOP for high grade B-Cell lymphoma. Following concerns about inferior survival rates, in October 2010 the UK government made additional funding for anti-cancer treatments available to 10 regions in England (The Cancer Drugs Fund). Uniquely amongst these the South-West region, with a population of approximately 6 million served by 18 hospitals grouped into 5 networks, did not set any limits on drug choice or treatment cost for which clinicians could request funding. All treatments supported by the local hospital multidisciplinary team and for which adequate supportive evidence was supplied were to be funded until no funds remained. The adequacy of supportive evidence for applications was reviewed by a panel of same specialty senior doctors.
Aims: What treatments haematologists seek when funding is unrestricted. The level of evidence informing treatment choice. How well patients match the evidence used to treatment choices. Funding utilisation variability between haematology treatment centres
Methods: Funding applications between October 2010 and September 2012 were reviewed. Demographic, treatment line, regimen/drug requested survival from funding approval date, requesting site and expenditure were extracted. 50applications were randomly selected and the supporting evidence reviewed and quality rated using the GRADE system.
Results: There were 596 applications for haematology and 1772 for oncology patients in this time. Complete data was available for 346 haematology applications, the rest containing some missing fields. In both specialties applications for men: women ratio was 2:1. Haematologists were significantly less likely than oncologists to seek treatment at 5th line and beyond (Chi squared, P<0.05). 10% of haematology treatment requests were based on data published in abstract only and only 15% on meta-analyses or phase III randomised control trials, the rest were based on case series, unrandomised phase II studies or opinion pieces. Only 5% of patients did not match the supporting evidence. The haematology commonest drugs requested were Rituximab (61% applications), Bendamustine (26%, 19% together with Rituximab), Bortezomib (11%, 6% with rituximab), lenalidomide (5%), ofatumumab (2%) and brentuximab (2%). There were up to 3 fold differences in funding uptake per population served both between networks and between individual haematology services of comparable treatment capability within and across those networks.
At an average funding request per patient of £13 043 80% of haematology patients survived more than 300 days from funding approval compared to 54% of oncology patients at an average of £15800.
Summary / Conclusion: Removal of funding restrictions on haemato-oncology treatments show that haematologists are much less likely to pursue therapy beyond 4th line than oncologists though survival for haematology patients is greater at lower cost, potentially reflecting more effective therapy and/or greater use of palliative care. Many chemotherapy choices are based on evidence rated as low or very low quality, and twice as many men receive treatment as women. 62% of expenditure was on only 2 drugs while differences in funding uptake suggest large differences in practice between similar haematology services.

P512

PATIENT SAFETY AND CLINICAL EFFICIENCY GAINS FOLLOWING IMPLEMENTATION OF AN INTEGRATED HEALTH INFORMATION SYSTEM IN EAST LONDON.

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Background: Digitisation of care records and pathways is a global ambition in healthcare designed to prevent patient harm and to harness the efficiency savings that result from advanced use of data and information systems. We report on our experience of using an integrated health information system (Cerner Millennium) in the clinical haematology department at the Royal London hospital, which is part of Barts NHS Healthcare Trust, a large academic health science system in East London.
Aims: To evaluate how the Electronic Health Record (EHR) supports efficient service provision in the clinical haematology department: referrals and outpatients, clinical handover, day care admissions and episode coding for health analysis and billing.
Methods: Quantitative and qualitative data sets were collected by use of database analytical tools, semi-structured observation of clinical interactions and review of ICD 10 coding used to generate Health Related Groups (HRGs) data for billing in the NHS.
Results: *Referrals:* 55% of patients attending the department from general practitioners were referred using the national Choose and Book system, an electronic referral system supplied to all English GPs. These referrals were reviewed online by 5 senior haematologists. Accepted referral letters appear automatically in the EHR. Inappropriate referrals were directed to other clinicians or returned back to the referring GP. *Outpatients:* 66% of patients attending the department had at least one SNOMED clinical term added to the EHR at the point of first attendance. All patients with haemostasis or haemoglobin disorders are fully SNOMED termed and these data are used to ensure that the correct treatment protocols are attached to every patient record. These data are actively used by non-specialist clinicians in the emergency department with 96% of patients reviewed and treated within 4 hours of arrival. *Handover:* Patient EHRs are projected to the whole team at least once weekly. 66% of patients had documented care pathways – all patients with haemophilia and sickle cell disease had EHR related protocols. Review of online laboratory data resulted in case management changes in 3% of patients. Images were reviewed in 25% of handovers and handover to specialist nurses for extended follow-up was agreed in 19% of cases. *Day unit/ambulatory care:* Over a three month period, 431 blood tests were performed with all results reviewed, validated and management plans documented in EHR. A total of 288 transfusions were administered with detailed haemovigillance data recorded electronically. 132 venesections, 111 haemophillia treatments and 68 infusions (parenteral iron/immunomodulatory therapy) were administered and management documented. 100% of nursing notes, management plans and physician reviews are documented in the patients' electronic health record. *Coding:* SNOMED Clinical terms entered at the point of care by clinicians are reviewed by the hospital coding team and used to develop depth of coding ICD 10 which improves public health data and income recovery through billing. The average number of diagnoses coded per Clinical Haematology episode is 3.7, which is above the peer average of 3.2.
Summary / Conclusion: Full implementation of an electronic system has significant benefits for patients and service provision. Haematology care depends heavily on decisions based on laboratory data and precise communication of care plans, we believe that the EHR is an invaluable tool in delivering efficient, cost-effective and safe patient care.

P513

PHARMACOLOGICAL DRUG EXPENDITURE OF FIRST-LINE INDOLENT NON-HODGKIN LYMPHOMAS IN SPAIN

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Background: Non-Hodgkin lymphomas (NHL) are a group of different hematological neoplasms which are the sixth most common cancer in many developed countries. Indolent Non-Hodgkin lymphomas (iNHL) are a subtype of NHL characterized by a chronic relapsing-remitting disease course, with patients usually exposed to several successive treatment courses. These indolent subtypes represent 40% of all NHL. Patients with iNHL in advanced stages and high-tumour burden usually receive front-line chemoimmunotherapy, Rituximab (Rtx) + chemotherapy, during the so called induction phase, followed by maintenance therapy with Rtx in patients who achieve at least a partial response at the end of the induction phase, as it is recommended by several Clinical Guidelines.
Aims: Analyze the different therapies used and the drug expenditure associated to the first line treatment phase of iNHL in Spain (considering induction plus maintenance phases).
Methods: Based on recent market research data (IMS Oncology Analyzer™, 2nd quarter wave in 2012) a total year population of 3,906 iNHL patients were estimated under induction treatment. An extrapolation of 2 previous years data was used to estimate the patients under maintenance therapy for the same time period, assuming that only those iNHL patients with a follicular lymphoma (FL) who would have achieved, at least, a partial response during the induction phase would receive maintenance therapy. Ninety percent of the patients would persist on maintenance therapy during the first year, and 80% during the second year. Cost analysis was carried out from the perspective of the Spanish Health System. Pharmaceutical expenditure on cytostatic agents and Rtx was analyzed, excluding other medical costs. The drug costs were based on the official ex-factory prices (EFP), discounting the price reduction established in 2010 by the Spanish Government. A deterministic sensitivity analysis (SA) was performed, switching the most relevant variables included in the analysis, to confirm the results robustness.
Results: Drug treatment expenditure in 1st line iNHL is above € 53.6 Mio/year in Spain. Almost 2/3 of this expenditure would be consumed during the induction phase and about €20 Mio/year would represent the expenditure dedicated to maintenance therapy with Rtx in FL responder patients. During the induction phase, the chemoimmunotherapies most widely used by far were R-CHOP (42%) and R-CVP (25%), although other 40 different therapies were identified. Rtx treatment cost also represents more than 90% of the pharmaceutical expenditure during this induction phase.

Summary / Conclusion: First line treatment of iNHL has not a very high economic impact cost in Spain, compared to other cancer pathologies. The main drug costs are based on the wide Rtx utilization. Nevertheless, Rtx treatment has also undoubtely represented a very relevant turning point in the outcomes of this pathology, with a remarkable therapeutic clinical improvement in terms of increasing response rates and prolonging the progression-free survival time in patients affected by this pathology.

P514

A SYSTEMATIC REVIEW OF ECONOMIC ANALYSES, UTILITY, RESOURCE USE, AND COST ESTIMATES OF FIRST-LINE TREATMENTS FOR CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Fludarabine/cyclophosphamide/rituximab (FCR) is the standard of care as first line treatment for healthy, fit patients with chronic lymphocytic leukemia (CLL). Patients with CLL are often elderly and many suffer from comorbidities which tend to make them inappropriate for fludarabine-based therapies. A number of therapeutic options are available for these medically less-fit or 'slow-go' patients.
Aims: The objective of the systematic review was to identify published economic evaluations of first-line treatments of CLL. The review also identified published estimates of utility, resource use, and costs that could be relevant to the economic evaluation of these first-line treatments for CLL.
Methods: The following electronic databases were searched for publications from the last 15 years (without limitations on language): MEDLINE, MEDLINE In-Process, EconLit, Embase, BIOSIS, and the Cochrane Library. Reference lists of relevant papers were searched manually. Conference proceedings from January 2010 to 2012 also were searched. Articles were screened for relevance by two independent researchers using predefined inclusion and exclusion criteria. Titles and abstracts were first screened (level 1) for relevance, and then full-texts were obtained and screened (level 2) for those articles that had appeared relevant at level 1.
Results: A total of 51 articles were identified from level 1 screening, 19 of which were deemed relevant after level 2. These related to 18 unique studies, five of which were economic analyses. Of these five, four studies were in fludarabine-ineligible patients; three reported cost-effectiveness estimates for bendamustine (B) and one reported estimates for chlorambucil (Chl) monotherapy. Incremental cost-effectiveness ratio estimates reported for B versus Chl monotherapy were £11,960 per quality-adjusted life-year (QALY) [United Kingdom, year 2009] (Woods *et al*., 2012; Napp Pharmaceuticals, 2010), £10,621 per QALY [Scotland, year 2010] (Napp Pharmaceuticals, 2011), and $50,763 per QALY [United States, year 2009] (Malin *et al*., 2010).
Four utility studies were identified from level 2 screening. Beusterien *et al*. (2010) elicited utility values for CLL health-state descriptions from United Kingdom general population samples using the standard gamble technique. Ferguson *et al*. (2008) and Tolley *et al*. (2012) elicited utility values for CLL health-state descriptions from United Kingdom general population samples using the time trade-off technique. Estimates reported by Beusterien *et al*. (2010) were substantially higher than those reported by Ferguson *et al*. (2008) and Tolley *et al*. (2012). Utility weights referenced to Knauf *et al*. (2009) were mapped from European Organisation for Research and Treatment of Cancer's Quality of Life Questionnaire-Core 30 data to the EuroQol EQ-5D.
Data from nine resource use and cost studies were extracted, three of which studied patients receiving fludarabine. Resources included drugs, hospitalizations, physician visits, administrative costs, adverse events, tests and procedures, and productivity losses. The majority of the studies were performed in the United States.
Summary / Conclusion: In summary, there is increased interest in the economic evidence regarding the cost-effectiveness of frontline interventions aimed at patients with CLL. However, there is wide variation in the methods used, limiting the impact of these analyses. The quality of economic evaluations should be increased to inform better the decision makers and clinicians.

P515

A COST-BENEFIT ASSESSMENT OF TREATMENT (TX) FOR NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM) PATIENTS: AN EFFICIENCY FRONTIER APPROACH

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Background: Access to cancer Tx can be constrained in certain countries due to cost-effectiveness requirements. The efficiency frontier (EF) shows the trade-off between costs and benefits of available Tx for a particular therapeutic area and identifies the regimens which provide the most value for a given level of cost.
Aims: An EF approach was used to conduct a cost-benefit analysis of four Tx in NDMM stem cell ineligible patients ≤75 years which included: melphalan and prednisone (MP), MP with lenalidomide as induction therapy followed by lenalidomide as maintenance therapy (MPR-R), MP with bortezomib (VMP), MP with thalidomide (MPT).
Methods: A cost-effectiveness model was developed to perform this assessment from a US payer perspective. Time to progression (TTP), adverse events, QALYs, life years (LYs) and costs for 1st-line Tx were estimated using a Markov approach, while a Tx algorithm was used to estimate post-progression survival, QALYs, and costs for subsequent Tx. 75% and 25% of the MPR-R treated patients and 25% and 75% of the patients receiving other regimens were assumed to receive bortezomib- and lenalidomide-based 2nd-line Tx, respectively. TTP for all the 1st-line Tx were estimated with data from the MM-015 trial and a network meta-analysis of published studies. TTP data for subsequent lines of Tx were obtained from a post-hoc analysis of MM-015 trial and other published studies. Utility values for different health status were drawn by mapping QLQ-C30 data collected from MM-015 onto EQ-5D scores. Costs (in 2013 USD), including those of treatments, adverse events, and routine monitoring, were obtained from various public databases. All costs and outcomes were discounted at 3% per annum.
Results: MPR-R resulted in more discounted LYs and QALYs per patient than MP, VMP and MPT. MPR-R also incurred the lowest lifetime Tx cost per patient next to MP. VMP and MPT were dominated by MPR-R. MP and MPR-R were the only two Tx defining the EF for QALYs (Figure 1) and LYs. The likelihood of MP, MPR-R, VMP, MPT being on the EF based on QALYs were 97%, 96%, 19%, and 3%, respectively. Similar results were observed for LYs.
Summary / Conclusion: The analysis suggests that for NDMM patients ≤75 years who are ineligible for stem cell transplantation, MPR-R is the most efficient among all analyzed treatments having a greater clinical benefit than MP.

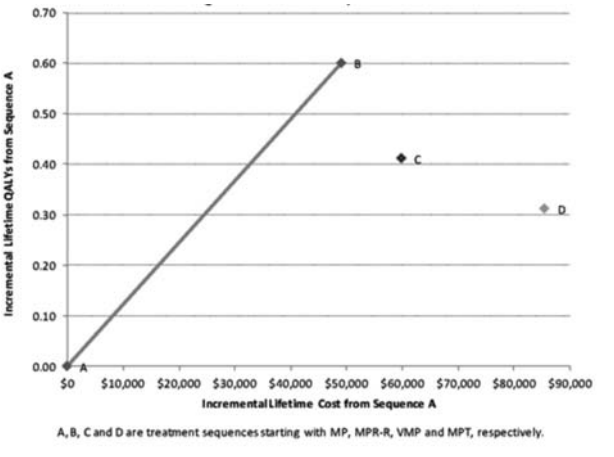

Figure 1. NDMM efficacy frontier.

P516

EVALUATION OF A NOVEL STRATEGY OF TRIAGE IN THE HEMATOLOGY AMBULATORY SETTING

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Background: Demand for specialty assessment and care often exceeds the capacity to provide it. In 2010, at our academic referral centre, the wait-time for patients referred with non-urgent hematologic problems exceeded 6 months and, considering the rate of growth in requests for consultation, some patients may never have been seen. We sought to change our triage system to better manage the limited capacity of hematologists to see new patients. In 2010, 444 letters were written by a hematologist at Capital District Health Authority in Halifax, NS to referring practitioners. These letters, written in response to requests for consultation from practitioners, reviewed the patient's hematologic abnormality (for example, leukopenia), suggested possible causes of the abnormality, and provided a recommended course of action for follow-up. Patients were then managed by their referring practitioner based on the recommendations in the letter and not assessed by the specialist.
Aims: The objectives of this study were to 1) characterize the cohort of patients for whom written recommendations were provided and 2) assess whether the written recommendations were a helpful and satisfactory alternative for the referring practitioner.
Methods: The study was conducted at a teaching hospital in Halifax, NS, Canada. All patients referred to the hematology clinic in 2010 who received written recommendations were included in the study. Follow-up was conducted 1 year after the date of written recommendations. All practitioners who were sent recommendation letters received a practitioner satisfaction survey comprised of 7 "yes or no" questions.
Results: 444 patients were managed by written recommendations [male 49% (n=220); females 51% (n=224)]. Common reasons for referral to the hematology clinic were mild leukopenia (17%; WBC between $3.0x10^9$/L and the lower limit of normal), mild to moderate thrombocytopenia (21%; platelet count between $51,000x10^9$/L and the lower limit of normal) and mild anemia (22%; hemoglobin between 100 g/L and the lower limit of normal). The average wait-time for providing written recommendations was 151 days (range: 0-623). No further follow-up was recommended for 22% of patients for whom written recommendations were written. At 1 year follow-up, 13% (n=58) had complete resolution of the abnormality for which they were referred and in 45% (n=201), the abnormality had remained stable. There was a single hematological-related death within the year follow-up in a patient who had declined further medical investigations and intervention. In 1% of patients (n=4), the patient's hematological abnormality worsened over the 1 year of follow-up. Of the 203 referring practitioners who responded to the satisfaction survey, 95% were pleased to receive recommendations in this manner and 90% said they would be satisfied with written recommendations in the future.
Summary / Conclusion: Our review of the practice of providing written recommendations to referring practitioners in response to a request for consultation suggests that there is a group of patients with hematologic abnormalities for whom this practice is safe and reasonable. In the year of follow-up, the majority of these abnormalities either resolved or remained stable. The positive response from the practitioners who were surveyed suggests that this endeavor has been well received. Future work will be undertaken to determine whether patients are satisfied with this approach and whether this leads to benefits in continuing health education as measured by a decrease in requests for referral.

Table 1. 444 patients were followed one year after the writing of written recommendations. The majority of patients were found to either not require follow-up, had their abnormality resolve or remained stable. A small portion of patients had the abnormality become worse and were subsequently seen in person. One patient died within 1 year of receiving written recommendations.

Patient Outcome	Number of Patients
Did not require follow-up	96 (22%)
Abnormality resolved	58 (13%)
Abnormality remained stable	201 (45%)
Abnormality was worse	4 (1%)
Lab values were not available at follow-up	70 (16%)
Another referral sent by referring practitioner and patient seen in clinic	12 (3%)
Patient was seen by Hematology	19 (4%)
Other treatment was recommended	9 (2%)
Patient was admitted	9 (2%)
Death	1 (0.2%)

P517

THE COST OF TREATING CHRONIC MYELOID LEUKEMIA (CML) AND THE COST OF ACHIEVING 1 MAJOR MOLECULAR RESPONSE (MMR) UNDER DIFFERENT TKIS IN GREECE

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Background: Innovative treatments have transformed CML into a chronic disease mostly managed in outpatient care.
Aims: This study aimed to estimate the cost of treating patients with CML including cost of adverse events and the cost for achieving one Major Molecular Response (MMR) under different Tyrosine Kinase Inhibitors (TKIs) in Greece.
Methods: A retrospective study was performed in the Heamatology Department of Evangelismos General Hospital of Athens. Study sample consisted of all patients (n=79) who visited the hematology outpatient unit of the hospital from January 2007 to December 2011. Cost analysis was based on patient's direct cost, including cost of personnel, supplies, medication, laboratory and imaging tests, infrastructure and various other on-site costs. Mean annual cost per patient was elaborated separately in outpatient and inpatient care. Inpatient costs mostly refer to the treatment of adverse events. Cost analysis is based on each year's NHS prices transformed in 2013 values and the National Health System (NHS) perspective has been used. The results of the costing analysis were used to estimate the cost of achieving 1 MMR under different TKIs in Greece. The MMR and AE rates were taken from the CML-CP frontline trials –DASISION (dasatinib 100mg QD vs. imatinib 400mg QD) and ENESTnd (nilotinib 300mg BID vs imatinib 400mg QD). Patient's number needed to treat (NNT) was calculated as the inverse of the MMR rate by 12 months (1/MMR) per. Costs of adverse events were multiplied by the incidence rates reported in the trials.
Results: The mean annual cost per patient in chronic phase of Myeloid Leukemia treated in outpatient care is estimated at €22.972. The most frequent adverse events managed were anemia (n=7, 9% of patients) and neutropenia (n=12, 15% of patients). Mean hospitalization cost per patient due to the treatment of adverse events is as follows: for anemia €2.193 and neutropenia of €1.312. The cost of treating anemia corresponds to 9.5% of the overall cost of treatment and the cost of neutropenia about 5,7%. Annual cost of newly diagnosed patients in 2011 was estimated at €14.493, 41% lower than the respective cost of non-newly diagnosed patients the same year (€24.558). The medication and hospitalization cost in order to perform a transplantation has been estimated at €22.955, without taking into consideration the cost of graft. The number needed to treat with nilotinib was 51% lower than the imatinib NNT in ENESTnd (1.8 vs. 3.7) and the dasatinib NNT was 39% lower that the IM NNT in DASISION (2.2 vs. 3.6). Annual cost of nilotinib including cost of AEs is estimated at €27.339, dasatinib €35.566 and of imatinib €21.782. The cost of achieving 1 MMR is €49.707 for nilotinib, €77.317 for dasatinib and €80.675 for imatinib. Therefore, the cost of achieving 1 MMR with nilotinib is lower by 35,71% vs. dasatinib and 38% vs. imatinib.
Summary / Conclusion: Innovative treatments for CML have resulted in treating patients in outpatient care with lower adverse events and consequently lower cost. The NNT findings and the differential cost of managing AEs in each treatment from this evaluation suggests that nilotinib provides better clinical outcomes and would result in lower costs for hematologic AE management from the perspective of the Greek NHS.

P518

A CALL FOR A STANDARDISED MEASUREMENT OF HAEMOGLOBIN IN THE REPUBLIC OF IRELAND

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Background: Diagnosis of anaemia is considered when the haemoglobin level is below the lower limit of the reference range used by a laboratory and erythrocytosis is diagnosed when the level exceeds the upper limit. Variation in the level of the upper and lower limit will affect diagnosis of these conditions and referral rates.
Aims: To assess and quantify the variation in haemoglobin reference ranges for adults in Irish laboratories in the context of findings on haemoglobin concentrations from a large, nationally representative sample of Irish adults.
Methods: Haemoglobin reference ranges used in laboratories in Ireland for adult men and women were obtained via phone contact, email correspondence or laboratory/hospital websites. Data was also obtained on the type of analyser used in each laboratory and on the source of the reference ranges. Haemoglobin levels were measured in a sample of 1,133 men and women aged ≥45 years, participants in the 2007 Irish National Survey of Lifestyle Attitudes and Nutrition (SLAN 2007).
Results: Haemoglobin is tested for both men and women in 53 laboratories and for women alone in 5 laboratories. In adult men, the lower limit varied from 13-14 g/dL and the upper limit from 16.7-18.5 g/dL. In adult women, the lower limit varied from 11-12.5g/dL and the upper limit from 14.8-16.5g/dL. At least 16 different analysers were used across the laboratories. Standard haematology textbooks were the source of reference ranges in at least 6 laboratories.
Of the representative sample from SLAN 2007, 546 (48.2%) were men and 587 (51.8%) were women. Biomnis laboratory reference range was used as the "gold" standard using Sysmex XE 2100 analyser (13.5-17.2 g/dL in adult men, 11.3-15.2g/dL in adult women). A suggested reference range was extrapolated from the SLAN data using the mean (15.2 g/dL for men, 13.9 g/dL for women) and standard deviations (1.2 g/dL and 1g/dL). For men, this is 12.9-17.7 g/dL and 11.9-15.9 g/dL for women. Applying the spread of reference ranges to the SLAN population, diagnosis of anaemia for men would range from 2.8% to 8.5% for a lower limit of 13-14g/dL. Diagnosis of erythrocytosis would range from 6.8% to 0.04% for an upper limit of 16.7-18.5g/dL. For women, diagnosis of anaemia would range from 0.7% to 7.3% for a lower limit of 11-12.5 g/dL and for diagnosis of erythrocytosis from 13.7% to 1.0% for an upper limit of 14.8-16.5 g/dL.
Summary / Conclusion: There is wide variation in lower and upper haemoglobin limits, with an almost 2 g/dL difference at the upper limit in both genders. A variety of analysers are used in the laboratories and the sources of the reference ranges vary from samples of local healthy volunteers to standard haematology textbooks. On comparing the ranges to the SLAN data, there was substantial variation in the proportion of the population diagnosed with anaemia or erythrocytosis. Given that a diagnosis of anaemia often generates referrals for resource intensive investigations such as endoscopy, overdiagnosis has significant health and resource implications. Similarly, underdiagnosis due to an inappropriate lower limit is a matter of concern. Our data highlights the need to standardise haemoglobin reference ranges in laboratories throughout the Republic of Ireland.
We would like to acknowledge the SLAN 2007 Consortium led by Professor Hannah McGee.

P519

DEMAND MANAGEMENT IN A UNIVERSITY LABORATORY IN THE WEST OF IRELAND; THE FINANCIAL BENEFIT OF DISTRIBUTION OF LOCAL GUIDELINES FOR VITAMIN B12 AND FOLATE TESTING.

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Background: Laboratory medicine services are critical to delivering high quality patient care. Despite diminishing resources, the demand for laboratory services is rising exponentially. Balancing clinicians' access to investigations and a laboratory's ability to manage its demands is vital, so that limited resources can be used to deliver high quality patient care. A review of ordering practices of general practitioners (GPs) using the laboratory at Galway University Hospital (GUH) was performed. There was a 185% increase in Vitamin B12 and folate testing between 2004 and 2011 in GUH. As these were invariably requested in association with a ferritin level, this equated in 2011 to €376,522 in assay cost alone. There was no appropriate indication for testing identifiable on the majority of requests. While the majority of GPs knew the appropriate indications for B12 and folate testing when surveyed, this was not reflected in their practice[1]. Evidence based guidelines are critical for achieving best practice in medicine. The most recent international guidelines for Vitamin B12 testing are the 1994 British committee for standards in haematology (BCSH) guidelines[2]. Education of clinicians requesting investigations is imperative to bring about a change of practice[3].
Aims: 1.) To distribute local guidelines to all general practitioners using the laboratory at GUH. 2.) To evaluate the effect of distribution of these guidelines.
Methods: In August 2012 local guidelines for Vitamin B12 testing were distributed to all general practitioners using the laboratory at GUH. 200 random request forms were audited in October 2012 to ascertain if the distribution of guidelines had resulted in a change of practice. The monthly requests for Vitamin B12 from 2011 and 2013 were compared.
Results: B12 and folate testing fell from 96,544 in 2011 to 87,411 in 2013. This decrease is visible following two points of communication with GPs; distribution of a survey on indications for B12 testing and distribution of local guidelines for B12 testing. Fatigue and routine screening as clinical indications for B12 testing fell from 27% to 16.2%, and 17% to 8.3% respectively.
Summary / Conclusion: Distribution of guidelines for Vitamin B12 and folate testing has led to a reduction in inappropriate testing. This equates to an estimated saving of €35,620 - €52,767 in our institution in 2012. Education of clinicians and involvement of stakeholders in policy changes are effective ways to bring about a change in clinical practice.

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SIMULTANEOUS SESSION I

Chronic lymphocytic leukemia - Microenvironmental interactions

S520

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

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Background: CC-292 is an oral, highly selective, small-molecule covalent inhibitor of BTK, a kinase that plays an important role in the biology of B-cell malignancies, such as CLL and B-NHL.
Aims: To investigate safety, dose limiting toxicities (DLT), and clinical activity of CC-292 monotherapy in patients (pts) with relapsed or refractory (R/R) CLL and B-NHL.
Methods: Cohorts of 3 to12 eligible pts with R/R CLL or B-NHL after ≥1 prior therapy received CC-292 at doses of 125, 250, 400, 625, 750, and 1000 mg QD or 375 and 500 mg BID. An expansion cohort of CLL pts at 750 mg QD was also tested. All pts received continuous dosing in 28-day cycles until progressive disease or intolerable toxicity. Clinical activity in CLL and NHL pts was investigator assessed per the 2008 IWCLL criteria and updated IWG Criteria for Malignant Lymphoma, respectively. Preliminary safety and clinical activity for all pts enrolled as of cutoff date Feb 22, 2013 are reported.
Results: We evaluated 86 pts (23 B-NHL, 6 Waldenstrom's Macroglobulinemia, 57 CLL/SLL) across all doses for safety, including 25 expansion cohort CLL pts who received 750 mg QD. Median age was 65 (29-89) and median prior therapies was 3 (1-12). Of the 57 CLL pts, 39 (68.4%) had at least 1 high risk factor, including 30 (52.6%) with unmutated *IGHV*, and 26 (45.6%) with del11q22 (12 pts), del17p (14 pts), or both (2 pts). Median time on therapy for all pts is 144 days (13-515). 3 DLTs have been reported and include thrombocytopenia (400mg), pneumonitis (1000 mg) and altered mental status (500 mg BID). The MTD has not been reached. Most frequent treatment emergent AEs in ≥10% of pts regardless of causality were diarrhea, fatigue, thrombocytopenia, neutropenia, nausea, headache, muscle spasm, respiratory infections, cough, anemia, abdominal pain, supraventricular arrhythmias, vascular disorders, peripheral edema, and dysgeusia; the majority of which were Gr1 and Gr2. All 17 efficacy-evaluable B-NHL pts reached stable disease (SD); 7 continue to show SD (mean treatment duration 226.8 days [64-522]): 2 DLBCL, 2 FL, 2 MCL, 1 NMZL. 1/17 pts reached partial response (PR): a marginal zone lymphoma pt who started at 250mg and escalated sequentially up to 750 mg QD, achieving a PR at Cycle 16. Of 50 efficacy-evaluable CLL pts, 17 (34%) achieved PR. Of those, ALC increases occurred in 10 pts but resolved in 5 pts. Of 57 CLL pts enrolled, 53 were evaluable for ≥11 lymph node assessment; 24 (45%) showed lymph node reduction of ≥50% as best response. While cohort numbers are small, and many pts in early treatment cycles, ORR was 31% at 750 mg QD, 50% at 1000mg QD, and 66.7% at 375mg BID. Most pts at 500 mg BID are not yet eligible for response evaluation.10 PRs were sustained through at least 2 efficacy evaluations and 10 had poor risk factors, including unmutated *IGHV* (8), del11q (3) and/or del17p (2). The median duration of treatment is ongoing at 176 days (16-473), and 2 CLL pts have been on treatment for over 15 cycles, both initiating treatment at 400 mg QD and experiencing nodal reduction of 32% and 27% respectively.
Summary / Conclusion: This dose-finding study demonstrates that CC-292 is generally well tolerated at doses ranging from 125-1000mg QD, and 375 and 500 mg BID. 17 CLL pts and 1 B-NHL pt achieved PR at doses of 750 mg QD, 1000 mg QD, 375 mg BID and 500 mg BID. Significant nodal reduction and PRs were seen in these heavily pre-treated CLL and B-NHL pts.

S521

SILENCING OF BRUTON'S TYROSINE KINASE BY RNA INTERFERENCE INDUCES APOPTOSIS IN PRIMARY CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background: Signals propagated through the B cell receptor (BCR) are believed to play a key role in the initiation, maintenance and evolution of chronic lymphocytic leukemia (CLL). Drugs that can disrupt these signals have recently emerged as potential therapeutic agents in CLL and several of them have shown considerable activity in early clinical trials. Particularly promising responses have been obtained with ibrutinib, an orally bioavailable inhibitor of BTK. However, as ibrutinib can potentially inhibit other targets in addition to BTK, it remains unclear whether inhibition of this kinase is responsible for the clinical responses.
Aims: To determine whether BTK is an important therapeutic target in CLL.
Methods: Silencing of BTK in primary CLL cells was done by RNA interference and nucleofection. Leukemic cell viability was analyzed by Annexin V/PI staining. Downstream signaling events and expression of antiapoptotic proteins were evaluated by immunoblotting or ELISA.
Results: BTK was efficiently silenced in primary CLL cells (n=31) by RNA interference, resulting in 60-90% reduction in protein levels compared to CLL cells nucleofected with control siRNA. Silencing of BTK accelerated apoptosis in all of the investigated cases (% viable cells nucleofected with control siRNA: 44±15, % viable cells nucleofected with BTK siRNA: 31±16, P<0.001). Induction of apoptosis by BTK silencing was observed across various prognostic groups and was independent of IGHV mutation status, ZAP-70 and CD38 expression. To determine the mechanism responsible for apoptosis induction, we evaluated expression of the antiapoptotic proteins Mcl-1, Bcl-2 and Bcl-xL in samples transfected with control or BTK-specific siRNA. Silencing of BTK resulted in reduced Mcl-1 expression, whereas the levels of Bcl-2 and Bcl-xL remained unchanged. The reduced Mcl-1 expression was in part a consequence of caspase-mediated cleavage and in part a direct effect of BTK silencing, as evidenced by the partial restoration of Mcl-1 expression in siBTK-transfected CLL cells pretreated with the caspase 3 inhibitor Z-VAD. BTK silencing also reduced the levels of phospho-PLCγ2, phospho-CARD11 and nuclear NF-κB p50, suggesting that BTK is a component of the pathway responsible for the constitutive activation of NF-κB, a hallmark of CLL. Interestingly, BCR engagement with anti-IgM induced only a marginal rise in phospho-PLCγ2 and phospho-CARD11 levels, whereas the rise in phospho-AKT and phospho-ERK levels was only marginally affected by BTK knockdown, altogether suggesting that BTK does not play a major role in transducing signals induced by acute engagement of the BCR.
Summary / Conclusion: This study shows that BTK expression is required for CLL cell survival, confirming this kinase as an important therapeutic target in CLL. An unexpected finding was the greater impact of BTK silencing on the activity of downstream signaling pathways in unstimulated than anti-IgM-stimulated CLL cells. These findings suggest that BTK is primarily involved in transducing signals from the BCR and/or other receptors that do not depend on external antigen, and provide the rationale for exploring combinations of BTK inhibitors with other agents that target the BCR pathway.

S522

ENDOTHELIN-1/ETA RECEPTOR SIGNALING MEDIATES SURVIVAL, DRUG-RESISTANCE AND MICROENVIRONMENTAL INTERACTIONS OF CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background: In tissue microenvironments, chronic lymphocytic leukemia (CLL) cells interact with different accessory cells that provide signals for survival, proliferation and drug-resistance. The endothelin axis, comprising endothelins (ET-1, ET-2 and ET-3) and their receptors (ETAR and ETBR), has recently emerged as relevant player in tumor growth and metastasis. Several small antagonists of ET-1 receptors are currently undergoing clinical trials as novel agents in cancer therapy.
Aims: Here, we investigated whether ET-1/ETAR signaling pathway may represent a novel pathway involved in CLL maintenance and progression.
Methods: CLL cells isolated from untreated patients were cultured either in presence or absence of endothelial cell (HUVEC) layers. Apoptosis was evaluated by flow cytometry using Annexin V/PI staining. ET-1 and ETAR expression in CLL cells was evaluated by RT-PCR, ELISA, flow cytometry and immunohistochemical staining of CLL-infiltrated lymph nodes. Phosphorylation of Akt and Erk was evaluated by western blot.
Results: CLL cells circulating in peripheral blood and infiltrating tissues secreted ET-1 and expressed ETAR on cellular surface. When CLL cells were cultured for 72 hours alone in complete medium, the addition of recombinant ET-1 peptide at 1 nM, 10 nM and 100 nM determined a 4.1-, 5.9- and 6.7-fold increase in CLL survival relative to controls respectively (P<0.05). After CLL co-cultures (n=30) on endothelial cells for 72 hours, CLL cell-derived ET-1 mRNA levels increased from 5.1±1.2 (control cultures) to 189.1±43.2 Arbitrary Units (P<0.05). Consistently, we also found a huge increase in ET-1 secretion from 0.6±0.1 pg/mL in CLL alone to 51.6±0.5 pg/mL in co-culture (n=26, P<0.05, Mann-Whitney test). Then, CLL cells were cultured on HUVEC cell layer in presence or absence of the ETAR antagonist BQ-123. BQ-123 significantly neutralized the pro-survival effect of ET-1 secretion in co-culture: we found a 2.5-fold increase of CLL viability in co-culture, and a 30% reduction by pretreating cells with 0.1 μM BQ-123 (n=11, 72h, P<0.05). Consistently, BQ-123 inhibited ET-1-mediated Akt and Erk phosphorylation. Then, we evaluated whether ET-1 could protect CLL cells from Fludarabine-induced apoptosis. We found a significant inhibition of apoptosis in presence of ET-1 both in CLL cultured alone and in CLL in co-culture on endothelial layer (n=8, P=0.003 and P=0.012 respectively). Again, treatment with BQ-123 was able to restore CLL sensitivity to Fludarabine-mediated apoptosis. Furthermore, we measured the levels of ET-1 precursor (big ET-1 peptide) in plasma samples collected from a multicentric cohort of CLL patients (n=101) using an ELISA method. Big ET-1 levels ranged from 0.47 pg/mL to 20.40 pg/mL (median= 3.88). Higher levels of big ET-1 were detected in patients with advanced Binet stage (P=0.01), high β2 microglobulin (P<0.0001), unmutated IGHV status (P=0.033), and intermediate/high FISH risk (P=0.001). Patients with ET-1 precursor levels higher than an established cut off of 5.4 pg/mL showed shorter time to first treatment (TTFT), as compared to CLL with low big ET-1 levels (5-years TTFT, 50% vs. 80%, P=0.01).
Summary / Conclusion: Collectively, our data describe for the first time a role of ET-1/ETAR signaling in CLL pathobiology. ET-1/ETAR axis is involved in CLL prolonged survival and in CLL interaction with endothelial cells suggesting that ET-1 may contribute to establish a nursing and protective niche in infiltrated tissues.

S523

PROTEOMIC PROFILING OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS: IDENTIFICATION OF THYMOSIN BETA 4 (TB4) AS A DIFFERENTIALLY REGULATED PROTEIN

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Background: Tβ4 is a ubiquitously expressed G-actin binding and sequestering protein. In different cell contexts, Tβ4 promotes tissue repair processes and differentiation of stem/progenitor cells, cell migration, and angiogenesis. In this respect, no data are available in CLL, a disease characterized by a pathological expansion of leukemic B cells and by a remarkably heterogeneous clinical course.
Aims: In the present study, modern proteomic MALDI-TOF MS profiling analysis was applied to identify low-molecular weight peptides as new molecular markers potentially playing a role in CLL pathogenesis.
Methods: Highly purified B cells from peripheral blood samples ofuntreated Binet-A CLL patients (N=100, clinicaltrials.gov NCT00917540) and 17 healthy controls were subjected to cell lysis and analyzed using Voyager De PRO MALDI-TOF mass spectrometry (PerSeptiveBiosystem). Differentially expressed m/z ion signals in the CLL proteome compared to controls were evaluated by the Olex data-mining method. Ion signals, protein spots and control peptides were characterized using on-line bio-informatic tools and databases (UniProtKB/Swiss-Prot Swiss-Prot). Gene and miRNA expression data were obtained using Affymetrix HG-U133A GeneChip Arrays (Affymetrix) from 217 and 221 CLL patients, respectively. GEP analysis was also carried out in 6 cases of normal B-lymphocyte subpopulations purified by FACS sorting based on the expression of IgD versus CD38. Non abutting gates separated naïve B-cells (N) (IgDbrightCD38$^-$CD27$^-$); marginal zone-like B-cells (IgDlowIgMbright CD38$^-$); germinal center B cells (IgD$^-$CD38$^+$) and switched memory B cells (IgD$^-$IgM$^-$CD38$^-$).
Results: MALDI-TOF MS profiling was detected 17 statistically significant differentially expressed ion signals in CLL compared to controls. Applying Olex data mining, 2 ion signals (4963.7 m/z, and 7345.3 m/z) corresponding to 5.0 and 7.3 kDa proteins, respectively could correctly differentiate normal (F-measure=0.79) from CLL cases (F-measure=0.96). The 5.0 kDa protein was characterized as Tβ4 while the 7.3-kDa protein remains to be identified. CLL was strongly associated with a down-regulation of Tβ4 [%Relative peak area normal vs CLL, 49.36±14.46 vs 34.50±12.52; mean±SD, p<.00001]. Moreover, Tβ4 expression was unchanged in CLL cases stratified according to CD38 and ZAP-70 expression, or *IGHV* mutational status. To validate the results of the MALDI-TOF analysis, an independent GEP analysis comparing CLLs and the different normal B-cell subpopulations also confirmed TMSB4X mRNA down-regulation in CLL (3604±1244 vs 5715±1004, respectively; mean±SD; P<0.001). miRNA microarray analysis identified 19 miRNA which significantly anti-correlated with TMSB4X gene expression. Of these, miR-532-3p showed the highest significance. BJAB cells transfected for 48h with miR-532-3p mimic or inhibitor (50 nM) resulted in an up-regulation of TMSB4X gene expression by miR-532-3p inhibitor. A similar up-regulation of Tb4 protein was observed in HeLa cells, which express high levels of miR-532-3p and low levels of Tβ4 protein, after exposure to miR-532-3p inhibitor.
Summary / Conclusion: Tβ4 mRNA and protein are both upregulated in CLL compared with normal B cells. Preliminary data indicate that inversely correlated miRNA expressed in CLLs may control Tb4 expression both at gene and protein levels. Further functional studies aimed to the demonstration that endogenous suppression of NF-kB activity by Tβ4 may mimic its potential utility as a therapeutic drug should confirm the potential relevance of Tβ4 modulation in CLL.

S524

B CELL RECEPTOR STEREOTYPY MAKES A CLINICAL DIFFERENCE IN CLL: REVELATIONS FROM A MULTI-INSTITUTIONAL SERIES OF 4615 CASES

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Background: In CLL, ~30% of all cases, carry quasi-identical, stereotyped VH CDR3 sequences within their B cell receptors (BcR) and can be clustered to different subsets defined by distinct VH CDR3 sequence motifs. Increasing evidence suggests that patients belonging to the same subset may share similar clinico-biological features and, perhaps, outcome. Nevertheless, even the largest VH CDR3-defined subsets account for only up to 3% of the cases with available IGHV-D-J sequence information, clearly indicating that for meaningful conclusions to be reached, reliable subset assignment and large patient cohorts are imperative.
Aims: We investigated the clinical implications of BcR stereotypy identified in a series of 4615 patients with CLL, consolidated in the context of a multi-institutional collaboration.
Methods: Subset identification was based on our previously reported approach (Blood 2012;119:4467), and associations were sought for major subsets which collectively accounted for 727 cases, namely: (i) subsets with unmutated IGHV genes (U-CLL): #1, n=129; #3, n=47; #5, n=24; #6, n=47; #7, n=78; #8, n=33; #31, n=19; #59, n=19; (ii) subsets with mutated IGHV genes (M-CLL): #4, n=61; #16, n=19; #77, n=23; #148, n=49; #201, n=21; and, (iii) subset #2 (IGHV3-21, variable mutational status), n=158.
Results: Regarding age at diagnosis, (i) M-CLL subsets #4, #16 and #201 (all utilizing the IGHV4-34 gene), #77, and #148 as well as U-CLL subsets #3, #5 and #7 (all utilizing the IGHV1-69 gene) and #31 contained significantly younger patients (P<0.001) compared to subsets #1, #2, #6, #8 and #59. While, these findings confirm and significantly extend previous reports by us and others about subset #4 they provide novel information about all other subsets. Significantly (p<0.001) different gender distributions were identified, with male:female ratios ranging from greater than 4.0 for U-CLL subsets #3 and #5 (both IGHV1-69) and #31 (IGHV3-48) to less than 0.8 for U-CLL subset #8 (IGHV4-39) and M-CLL subset #201 (IGHV4-34). Among the most populated subsets, strikingly different (P<0.01) profiles of genomic aberrations (hierarchical model) were identified, exemplified by: (i) diminished frequency of del(17p) in subset #2 versus all others, especially subsets #1 and #8; (ii) significantly higher frequency of del(11q) in subsets #3, #5 and #7 (all IGHV1-69, range 37% to 44.4%) versus all others; (iii) significantly higher frequency of trisomy 12 in subset #8 (69.2%) versus all others; (iv) homogeneous profile of subset #4 that is characterized by high frequency of del(13q) and very low frequency of other aberrations extending to complete absence of del(17p). We assessed time-to-first-treatment (TTFT) and noted significantly shorter TTFT in subset #2 vs. M-CLL subsets vs. U-CLL subsets and also vs. U-CLL as a whole (p<0.01), thus corroborating previous observations about the adverse prognosis of subset #2. Among U-CLL subsets in general, significantly worse outcomes were found for subsets #31 and #59 (P=0.06 and P=0.005, respectively). Focusing on U-CLL subsets utilizing the IGHV1-69 gene (subsets #3, 5, 6, 7, 59), significantly shorter TTFT was identified for subset #59 (P=0.03). M-CLL subsets in general had significantly longer TTFT compared to U-CLL subsets (p<0.05). Among M-CLL subsets, a trend for longer TTFT was seen for subset #148 (P=0.06).
Summary / Conclusion: In conclusion, in the largest series analyzed thus far, we provide results strongly supporting the concept that the molecular classification of CLL into stereotyped subsets is biologically and clinically relevant.

Hodgkin Lymphoma

S525

TEMPORAL TRENDS IN MORTALITY FROM DISEASES OF THE CIRCULATORY SYSTEM AFTER TREATMENT FOR HODGKIN LYMPHOMA – A POPULATION-BASED COHORT STUDY IN SWEDEN (1973-2006)

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Background: Hodgkin lymphoma survival in Sweden has improved dramatically over the past 40 years, but little is known about the extent to which efforts aimed at reducing long-term treatment-related mortality have contributed to the improved prognosis.
Aims: To estimate the contribution of treatment-related mortality due to diseases of the circulatory system (DCS) to temporal trends in excess Hodgkin lymphoma mortality among Swedish patients.
Methods: We used population-based data from the national Swedish cancer register. Flexible parametric survival models were used to estimate excess mortality among 5,462 patients diagnosed at ages 19 to 80 between 1973 and 2006. The total excess mortality experienced by the patients was partitioned into component parts in order to isolate the excess DCS mortality (assumed to be caused by the treatment) from the remaining excess mortality (such as that from the underlying disease, second malignancies and infections). In addition we utilized recent advances in statistical methodology to estimate excess mortality in the presence of competing causes of death. These models were used to predict the long-term risk for patients diagnosed in the modern era to die from treatment-related DCS.
Results: Excess DCS mortality within 20-years after diagnosis has decreased continually since the mid 1980´s and is expected to further decrease among patients diagnosed in the modern era. Age at diagnosis and sex were important predictors for excess DCS mortality, with advanced age and male sex being associated with higher excess DCS mortality. However, when accounting for competing causes of death, we found that excess DCS mortality constitutes a relatively small proportion of the overall mortality among Hodgkin lymphoma patients in Sweden.
Summary / Conclusion: Excess DCS mortality is no longer a common source of mortality among Swedish HL patients. The main causes of death among long-term survivors today are deaths from other causes than Hodgkin lymphoma, although other (non-DCS) excess mortality also persists as long as 20 years after diagnosis, particularly among older patients.

S526

LONG-TERM EFFECTS OF AUTOLOGOUS STEM-CELL TRANSPLANTATION FOR FIRST RELAPSED OR REFRACTORY HODGKIN LYMPHOMA: AN UPDATED ANALYSIS OF THE PROSPECTIVE LYSA/SFGM-TC H96 TRIAL

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Background: There is a lack of long-term prospective data assessing efficacy and toxicity of autologous stem-cell transplantation (ASCT) for first relapsed or refractory Hodgkin lymphoma (HL).
Aims: H96 trial is one of the largest prospective studies on ASCT for first relapsed or refractory HL. The first analysis was published after a median follow-up of 51 months (Morschhauser F, J Clin Oncol 2008). Here we present an updated analysis after a median follow-up of 10.4 years.
Methods: H96 trial evaluated a risk-adapted salvage treatment with single or tandem ASCT for 245 HL patients. Poor-risk patients (n=150) had primary refractory HL (n=77) or unfavorable relapse (≥2 of the following risk factors at first relapse: time to relapse <12 months, stage III or IV at relapse, and relapse within previously irradiated sites, n=73) and were eligible for tandem ASCT. Intermediate-risk patients (n=95) had one risk factor at first relapse and were eligible for single ASCT.
Results: Among poor-risk patients, 105/150 (70%) received tandem ASCT, whereas 92/95 intermediate-risk patients (97%) received single ASCT. According to intent-to-treat analysis, the 10-year freedom from second failure (FF2F) and overall survival (OS) estimates were 64% and 70%, respectively, for the intermediate-risk group, and 40% and 47%, respectively, for the poor-risk group. In the poor-risk group, outcomes were similar for primary refractory HL and unfavorable relapse (10-y FF2F 33% vs 48%, P=0.06; 10-y OS 43% vs 51%, P=0.26). Disease status at time of ASCT (Cheson 1999) was a major prognos-

tic factor driving outcome. In the poor-risk group, the 10-y OS was 68%, 58%, 16% and 22% for patients in complete remission (CR)/unconfirmed CR (CRu), partial remission (PR), stable disease (SD) and progressive disease (PD), respectively (P<0.0001), without significant difference between CR/CRu and PR patients (P=0.12). In the intermediate-risk group, 92/95 patients were in CR/CRu or PR at time of transplantation. The 10-y OS was 72% and 66% for patients in CR/CRu and PR, respectively, without significant difference (P=0.96). In the poor-risk group, 76 patients relapsed with 5-y and 10-y cumulative incidence of relapse of 47% and 51%, respectively. In the intermediate-risk group, 25 patients relapsed with 5-y and 10-y cumulative incidence of relapse of 24% and 27%, respectively. After relapsing, 23 patients underwent allogeneic stem-cell transplantation (alloSCT), mainly after a non myeloablative conditioning regimen (n=20); their 5-y OS was 60% (intermediate-risk group, n=5) and 33% (poor-risk group, n=18). For relapsed patients who did not undergo alloSCT (n=78), 5-y OS was 45% (intermediate-risk group, n=20) and 14% (poor-risk group, n=58). In all, 110 patients died (intermediate-risk group, n=30; poor-risk group, n=80). The main cause of death was HL (n=83, 75% of causes of death) whereas other causes included solid tumor (n=3), acute leukemia (n=5), non-Hodgkin lymphoma (n=2), infection (n=5), cardiac toxicity (n=2), post-alloSCT complication (n=5) or other (n=5).

Summary / Conclusion: With long-term follow-up, single ASCT remains appropriate for intermediate-risk patients with 10-y OS at 70% and a low toxicity. For poor-risk-patients, tandem ASCT remains a valuable option with 10-y OS at 47%, especially for patients in CR/CRu or PR at time of ASCT.

S527

IMPACT OF DOSE DENSITY AND HEMATOTOXICITY OF DHAP-REINDUCTION THERAPY ON THE OUTCOME IN RELAPSED HODGKIN LYMPHOMA (HL): AN ANALYSIS OF THE GERMAN HODGKIN-STUDY GROUP (GHSG)

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Background: Despite of an intensive reinduction therapy followed by high-dose-chemotherapy (HDCT) and autologous stem cell transplantation (ASCT), only 50% of patients with relapsed Hodgkin Lymphoma (HL) achieve long-term remission. Response to reinduction treatment is predictive for the outcome. In order to optimize the reinduction therapy in relapsed HL, we aimed at increasing dose density of DHAP (dexamethasone, high-dose cytarabine, cisplatinum) in the HDR2 trial by shortening the cycle interval from 21 to 14 days.

Aims: In this retrospective analysis of the HDR2 trial, the clinical impact of dose density of DHAP was evaluated. In addition, we assessed the impact of DHAP-associated hematotoxicity on dose density and treatment outcome.

Methods: In the HDR2 trial, patients with relapsed HL without disease progression after two courses of DHAP were randomized to receive additional sequential HDCT or to directly proceed to HDCT with BEAM followed by ASCT. DHAP cycle interval was defined as the time interval between day 1 of the first course and start of the second DHAP course. Kaplan Meier and Cox regression analyses were used to estimate the prognostic value of the DHAP cycle interval and of hematotoxicity on progression free survival (PFS) and overall survival (OS) after relapse. The impact of different variables on the length of DHAP cycle interval was examined with linear regression analysis. The level of significance was set to P<0.05.

Results: Data of 269 patients were evaluable for this analysis. The median age was 36 years; 36% of the patients analyzed were female, 53% had advanced-stage disease at diagnosis of relapse, and 13% had received >1 previous chemotherapy. Median time from initial diagnosis to relapse was 44.3 months. Only 15% of the analyzed patients received the second DHAP course within the recommended time interval of 14 days; in 39% the DHAP course interval was 21 days or longer. 232 patients were randomized after two courses of DHAP. A significant association between the length of the DHAP cycle interval and the outcome was shown in univariate (PFS: P=0.017; OS: P= 0.012) as well as in multivariate cox regression analysis including early or multiple relapses, stage IV disease and anemia at relapse as established prognostic factors (PFS: P=0.035; OS: P=0.003). Patients who received the second DHAP course on day 21 or later had a significantly poorer PFS and OS when compared to those patients who proceeded before day 21 (3-year PFS 58% vs. 73%, P= 0.027; 3-year OS 76% vs. 87%, P=0.016, respectively).

DHAP-associated grade 4 hematotoxicity had a significant impact on the length of DHAP course intervals (P=0.022 in multivariate linear regression analysis). Those patients who developed thrombocytopenia or leukocytopenia grade 4 had significantly longer course intervals. In addition to the prognostic factors of the Josting score and the length of the DHAP course interval (> 21/≤21 days), severe hematotoxicity was associated with a significantly shorter PFS (HR 1.36: P= 0.036) and OS (HR 1.79; P= 0.012) in multivariate analysis.

Summary / Conclusion: Dose density of DHAP reinduction therapy is an independent prognostic factor with regard to PFS and OS in relapsed HL patients. Additionally, hematotoxicity grade 4 is a significant factor contributing to prolonged DHAP course intervals and has a negative impact on PFS and OS. These results support the mandatory use of G-CSF in this setting as well as an investigation of thrombopoietin analogues during DHAP reinduction therapy in order to maintain dose density.

S528

OUTCOME AND RISK FACTORS OF HODGKIN LYMPHOMA PATIENTS WITH _RELAPSE OR PROGRESSION AFTER AUTOLOGOUS STEM CELL TRANSPLANT:_ A REPORT FROM THE GERMAN HODGKIN STUDY GROUP (GHSG)

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Background: Today, most patients with Hodgkin Lymphoma (HL) can be cured at first diagnosis. For HL patients suffering from relapse or progression after first line therapy, high dose chemotherapy followed by autologous stem cell transplant (ASCT) is the treatment of choice and cures about 50% of these patients. Those who relapse or progress after ASCT have a very poor prognosis and clearly represent an area of unmet medical need. However, data on this small group of patients is rather limited and no established risk factors are available. Here, we present a recent retrospective analysis from the GHSG database on HL patients with recurrence after ASCT.

Aims: Our aims were to better characterize HL patients with relapse or progression after ASCT in order to define possible risk factors for the prediction of overall survival.

Methods: All trials in the GHSG database were searched for HL patients who relapsed or progressed after ASCT.The information for this retrospective analysis was updated in May 2012. Overall survival after progression of HLfollowing ASCT (OSrr) was the end point of this study. Descriptive statistics and Kaplan Meier estimates of OSrrwere used and risk factors for OSrranalyzed. The significance level was set to 0.05.

Results: 152 patients who relapsed or progressed with HL after ASCT were identified. 149 patients fulfilled the predefined inclusion criteria. With a median observation time of 65 months after progression, 112 patients (75%) had died. OSrrwas 63% (95% confidence interval [CI]; 54% to 70%), 51% (95% CI 42% to 58%), and 20% (95% CI 13% to 28%) at 1, 2 and 5 years, respectively. From several risk factors tested, stage (P=0.044) and presence of B-symptoms (P=0.005), both at relapse before ASCT, significantly predicted OSrr. Combination of these two risk factors allowed the identification of three distinct risk categories for the four significantly different groups (P=0.013, Figure 1).

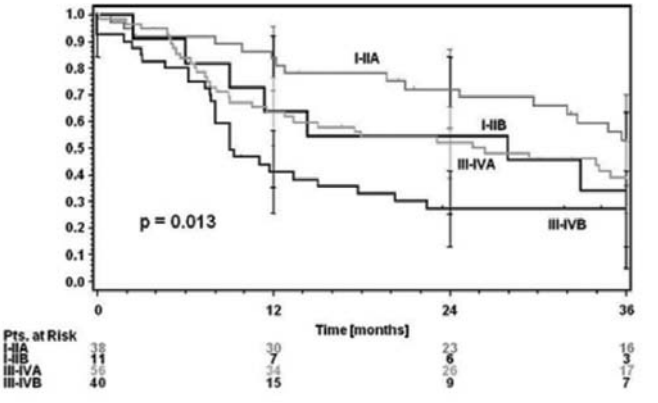

Figure 1.

Accordingly, patients in clinical stage I or II without B-symptoms had a better prognosis (OSrr84% [95% CI 67% to 92%], 72% [95% CI 54% to 84%] and 33% [95% CI 18% to 49%] at 1, 2 and 5 years, respectively) in comparison to patients with stage III or IV with B-symptoms (OSrr 41% [95% CI 26 to 57%], 27% [95% CI 13 to 42%] and 8% [95% CI 0 to 18%], respectively).Other potential risk factors such as stage at first diagnosis (P=0.80), early, late or multiple

relapses before ASCT (P=0.95), and time from ASCT to further progression of HL (P=0.81) were not significant.
Summary / Conclusion: 1. Stage and B symptoms at relapse before ASCT are significant predictors of OS in relapsed HL patients receiving ASCT.2. Current treatment approaches for patients with relapsed or progressive HL after ASCT showdisappointing results with 80% of this very high-risk group dying within 5 years. Emerging new drugs such asbrentuximabvedotinmight improve the outcome of these patients in the future.

S529

THE TH-INFILTRATE OF HODGKIN LYMPHOMA HAS A UNIQUE PHENOTYPE RICH IN ACTIVATION/PROLIFERATION MARKERS, CENTRAL MEMORY & TH1-POLARISED CELLS, WITHOUT EVIDENCE OF SENESCENCE, SUPPRESSION OR EXHAUSTION
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Background: CD4+ T cells (T-helper cells: TH) dominate the classical Hodgkin lymphoma (CHL) microenvironment but a detailed understanding of their function is lacking. We hypothesized that rather than being immunosuppressive, TH2-polarized cells, the TH-cells of CHL comprise activated, proinflammatory cells capable of sustaining malignant Hodgkin Reed Sternberg cells (HRS). Understanding the key intercellular mediators of survival signals may provide pharmacological targets in therapy-resistant patients.
Aims: Using TH-expressed markers of known or proposed functional importance in CHL the aim was to characterize pathognomonic features of HRS-helper T cells distinguishing them from the reactive TH cells of non-malignant inflammatory lymph nodes
Methods: Frozen single cell suspensions (SCSs) from diagnostic CHL-infiltrated lymph node biopsies (n=7), and control reactive cervical lymph nodes (n=5) were thawed and stained for CD3/CD4 and markers of functional importance in CHL, including tumour-necrosis-factor superfamily (TNFRSF) members, chemokine receptors (CCR), memory, activation, senescence, immunosuppression mediators and TH1/TH2 cytokines. Flow cytometry was performed, markers expressed as % total CD3+CD4+ population and medians calculated for each tissue (CHL vs reactive control). Mann-Whitney U tests determined significant differences between CHL and reactive node-derived cells
Results: CHL-infiltrating TH-cells comprised a similar proportion of total thawed cells in CHL and controls (40%), outnumbering CD8+ T cells. Central memory (CM) T cells (CD45RA-CCR7+, associated with superior activity) were a more dominant component of CHL TH-cells than controls (29% vs 11% P=0.03) while TEMRA cells (CD45RA+CCR7-, associated with exhaustion and impaired function) were more common in controls (1% vs 9% P=0.02). Markers of exhaustion and immunosuppression were expressed in non-significantly fewer TH-cells in CHL vs controls - CD57 (1% vs 22%) and PD1 and (6% vs 21%). Although FOXP3 was found at comparable levels in CHL and reactive node TH cells (10%), its effector molecule CTLA4 was expressed at higher levels in CHL (48% vs 19%, P=0.05). Ligands of HRS-expressed TNFRSF members CD30, OX40 and ICOS were expressed at substantially higher levels in CHL-derived TH cells (CD30L in 22.9% vs 6.5%, P=0.02; OX40+ICOS+ in 32.3% vs 10.5%, P=0.05). CCR expression patterns were profoundly discriminatory: despite similar levels of the receptor for the CHL-characteristic chemokine TARC/CCL17 (CCR4) in CHL and control TH cells (5.7% vs 10.5%), TH1-associated CCRs CXCR3 and CCR5 were dominant in CHL vs controls (53.5% vs 16.8%; P=0.02 and 18.9% vs 5.9%, P=0.02) while the T follicular helper cell marker CXCR5 was reduced in CHL (5.1% vs 24.1%). TH2 cytokines were entirely absent, while IFNg was expressed in similar numbers of cells (4%) and TNFa in more CHL-derived TH cells (20% vs 10%).
Summary / Conclusion: CHL-infiltrating TH cells are cytokine-secretory, activation marker-rich and lack exhaustion/suppression markers, hence are capable of mediating survival signals to HRS cells. The malignant node sequesters substantial numbers of central memory cells, which may contribute to the profound systemic immune defect encountered in advanced disease. An infiltrate of activated CD4+ cells rich in CM cells, lacking PD1, CD57 and CXCR5 and expressing CTLA4, CD30-L, OX40, ICOS, CXCR3 & CCR5 appears to be a pathognomonic signature even when compared to reactive control nodes, in contrast to the TH2-polarized suppressive cell-rich infiltrate currently proposed for CHL, and may be suitable for diagnostic purposes in difficult cases.

Myeloproliferative neoplasms - Biology

S530

MPN PATIENTS HARBOR RECURRENT TRUNCATING MUTATIONS IN TRANSCRIPTION FACTOR NF-E2
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Background: We have described overexpression of the transcription factor NF-E2 in MPN patients and shown that elevated NF-E2 levels cause a MPN phenotype in transgenic mice.
Aims: To further investigate the role of *NF-E2* in the pathophysiology of MPNs, we sequenced the *NF-E2* gene in a cohort of MPN patients and related myeloid disorders.
Methods: The *NF-E2* gene was sequenced in 456 MPN patients as well as in patients with MDS, CMML, post-MPN-AML and healthy controls. Functionality of the mutants was determined by DNA binding- and reporter gene assays. Hematopoietic colonies grown from MPN patient cells were assayed for NF-E2 and $JAK2^{V617F}$ mutations. The *in vivo* effect of NF-E2 mutations was assessed in a murine bone marrow transplant model.
Results: Acquired insertions and deletions leading to frameshift mutations were detected in the *NF-E2* coding sequence in patients with PV and MF. The frameshifts introduce premature stop codons, leading to truncations in the NF-E2 protein. Truncated NF-E2 proteins contain neither the DNA binding domain nor the leucine zipper required for dimerization to small Maf proteins.
Mutant NF-E2 proteins do not bind DNA in a gel-shift-assay. Furthermore, on their own, they do not transactivate reporter gene activity. However, since all *NF-E2* mutations were observed in a heterozygous state, we tested their ability to activate reporter gene expression in the context of wt NF-E2. Surprisingly, NF-E2 mutants, which on their own retain no transactivation activity, significantly enhance wt NF-E2 activity.
Analysis of individual hematopoietic colonies revealed that in three patients the *NF-E2* mutation was acquired subsequent to the $JAK2^{V617F}$ mutation. These assays revealed a proliferative advantage of NF-E2 mutant cells as cells that acquired both a *NF-E2* and a $JAK2^{V617F}$ mutation vastly outcompeted cells carrying the $JAK2^{V617F}$ mutation alone. This observation was recapitulated in a murine model, where mice expressing both the $JAK2^{V617F}$ mutation and mutant NF-E2 display a statistically significant increase in hemoglobin, MCV, MCH and WBC count above that affected by $JAK2^{V617F}$ alone. These data demonstrate that *in vivo*, mutant NF-E2 cooperates with $JAK2^{V617F}$ to augment the MPN phenotype. We determined the molecular mechanism mediating the selective advantage of cells carrying NF-E2 mutations. The presence of mutant NF-E2 conferred a proliferative advantage in the absence of cytokines beyond that conferred by $JAK2^{V617F}$ alone, witnessed by the accumulation of high cell numbers as well as by a significant increase in the proportion of cells in S-phase. Moreover, cells carrying both $JAK2^{V617F}$ and mutant NF-E2 expressed significantly higher levels of the cell cycle regulators cyclinD3, CDK4 and CDK6, which promote the G1-S transition.
Mice transplanted with NF-E2 mutants displayed a statistically significant elevation in hematocrit, RBCs, neutrophils and platelets. Both peripheral blood and bone marrow (BM) show a myeloid bias, with statistically significant increases in the proportion of myeloid cells in the peripheral blood and a corresponding elevation of the number of CMP, MEP and GMP in the BM. Moreover, following the myeloproliferative phase, NF-E2 mutation carrying mice transform to acute myeloid leukemia.
Summary / Conclusion: Our data identify *NF-E2* as a novel mutational target in MPN patients, one that enhances wtNF-E2 function *in vitro* and promotes erythrocytosis, thrombocytosis and a myeloid bias *in vivo*. Our data underscore the role of elevated NF-E2 activity in the pathophysiology of myeloproliferative neoplasms.

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A GENOMEWIDE ASSOCIATION STUDY IDENTIFIES NOVEL LOCI THAT PREDISPOSE TO MYELOPROLIFERATIVE NEOPLASMS
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Background: Epidemiological studies have suggested the presence of common genetic variation that predisposes to the development of myeloproliferative neoplasms (MPN) in the general population. Indeed, we have previously found that a specific *JAK2* haplotype, termed 46/1 or GGCC, strongly predisposes to *JAK2* V617F positive disease. This haplotype, however, only accounts for approximately 50% of the overall population attributable risk of developing an MPN and explains only a very minor component of V617F-negative disease. Thus, other predisposition factors remain to be identified.
Aims: To identify common, low penetrance genetic factors that predispose to the development of myeloproliferative neoplasms.
Methods: We undertook a three stage genomewide association study (GWAS), focusing on MPN that were negative for *JAK2* V617F. The study group (n=2506) included cases with either essential thrombocythaemia or primary myelofibrosis recruited from the UK, Italy, Germany, Austria and Greece. Established cohorts (WTCCC, KORA, INCHIANTI) were used as population controls as well as locally collected samples.
Results: In Stage 1 Affymetrix SNP 6.0 analysis was performed on 585 UK cases and, after extensive quality control, the genotypes of 642,633 SNPs were compared to WTCCC2 data. Stage 2 consisted of targeted analysis of the top 203 SNPs from Stage 1 (based on statistical and biological criteria) in 956 cases and 5707 controls from the UK, Greece, Germany, and Austria. This analysis identified 7 SNPs that remained significant after correction for false discovery rate ($P \leq 0.0016$) and had effects in the same direction as stage 1. At stage 3, these 7 SNPs were tested in a further 965 cases and 4,408 controls from 2 independent cohorts from the UK and Italy. The final effect size and significance of these 7 SNPs was determined by a fixed effects meta-analysis of the 6 cohorts. According to methodology described by the WTCCC2 and estimating that this GWAS has 80% power, SNPs with p-values $\leq 5 \times 10^{-7}$ were determined to be statistically significant. Following meta-analysis, 2 SNPs with genome wide levels of significance and without heterogeneity between cohorts were identified. The most significant of these SNPs lies 153 kb downstream of *EVI1* (chr 3q26; HR = 0.84, p-value=2.1×10^{-8}) and the other is located in an intron of *ITGA8* (chr 10p13; HR = 1.88, p-value=1.1×10^{-7}). Two further associations, one between *HB1SL* and *MYB* (chr 6q23; p-value = 2.5×10^{-6}) and the other between *THRB* and *RARB* (chr 3p24; P= 2.5×10^{-5}), approached genome wide significance but the heterogeneity between cohorts was also significant. Since reduced *MYB* expression has been associated with development of ET-like disease in mouse models, we analysed gene expression in myeloid progenitors cultured *in vitro* from a series of healthy controls by qRT-PCR. Reduced *MYB* expression, but not *HBS1L*, relative to the housekeeping gene *GUSB* was significantly associated with the chromosome 6 risk allele (n=18; P=0.0001).
Summary / Conclusion: We have identified SNPs in or close to two genes, *EVI1* and *ITGA8*, which alter the risk of developing *JAK2* V617F negative MPN. Two further loci approach statistical significance and one of these is associated with reduced *MYB* expression.

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IDENTIFICATION OF A NOVEL MODE OF KINASE INHIBITOR RESISTANCE IN JAK2: JAK2 INHIBITOR RESISTANCE IS MEDIATED BY THE GENERATION OF 45-KDA JAK2 VARIANT WHICH ALTERS THE KINASE DOMAIN STRUCTURE

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Background: JAK2 V617F can be identified in the majority of cases of polycythemia vera (PV), and in 50% of cases in essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF). JAK2 inhibitors including ruxolitinib (Jakavi, INCB018424), TG101348 and lestaurtinib (CEP-701) display clinical activity in clinical trials in PV, ET and IMF, and ruxolitinib has recently been approved for the treatment of primary and secondary myelofibrosis. In other malignancies it has been demonstrated that acquired resistance to kinase inhibitors is due to emergence of secondary resistance mutations in the target kinase.
Aims: Identification of JAK2 point mutations or resistant mechanism against the ruxolitinib is very imporatnt in order to develop second generation JAK2 inhibiotrs or alteration of treatment staregy to the patients.
Methods: Cell based Screening strategy against the inhibitor is the most comprehensive method in order to identify the drug resistant mutations. For this we exposed JAK2 V617F expressing Ba/F3 cells to ruxolitinib.
Results: Surprisingly, sublines resistant to ruxolitinib at 1000nM, 2000nM and even 4000nM did not harbor point mutations neither in the kinase or the pseudokinase domain of JAK2. However, western blot analysis of sublines resistant to ruxolitinib revealed a 45-kDa JAK2 variant together with full length JAK2V617F protein in 87% of the cases. Sequencing of the short form in drug resistant clones revealed a novel JAK2 variant missing amino acids 76 to 820 resulting in the N-terminal FERM domain directly fused to the kinase domain of JAK2 (FERM-JAK2). FERM-JAK2 was highly resistant to the ATP-competitive JAK2 inhibitors ruxolitinib and TG101348. JAK2 exists in an either active or inactive state, which is largely regulated by phosphorylation of tandem tyrosines located within the activation loop. Substitution of phenylalanine at tyr1007, tyr1008 or both leads to complete inactivation of V617FJAK2 and WT-JAK2.In contrast, Y to F mutation of tyr1007, tyr1008 or both in FERM-JAK2 does not prevent activation of STAT5 and transformation of Ba/F3 cells, suggesting that FERM-JAK2 preferentially exist in an inactive state. This would impede drug binding and explain the resistant phenotype of FERM-JAK2. Phospho-deficient mutant studies further provided evidence that tyr866, tyr966, and tyr972 are crucial for the activation of FERM-JAK2. Ectopic expression of FERM-JAK2 in HEK293T, NIH3T3 and Ba/F3 cells showed activation of STAT5 and transformation of Ba/F3 cells to factor independence. Interestingly, co-immunoprecipitation studies revealed that FERM-JAK2 does not bind or activate the IL-3R in contrast to V617FJAK2. Ectopic expression of FERM-JAK2, in contrast to V617FJAK2, was able to activate STAT5 in a cytokine receptor deficient cell line. *in vitro* binding studies revealed that FERM-JAK2 directly binds and activates STAT5. Using flag- and myc-tagged FERM-JAK2 we show that the FERM domain is sufficient for an efficient dimerization and activation of FERM-JAK2 in the absence of an cytokine receptor. Transplantation of retrovirally transduced murine bone marrow with FERM-JAK2 compared to JAK2 V617F showed an accelerated phenotype with marked increases in WBC, HCT, RBC, HB, reticulocytes, pronounced splenomegaly and loss of body weight in mice. In contrast to V617FJAK2 expressing mice, FERM-JAK2 expressing mice displayed a lethal phenotype.
Summary / Conclusion: Taken together, we could identify a novel JAK2 variant which alters the kinase domain structure, leads to direct, cytokine receptor independent STAT5 activation, and gives rise to an accelerated, PV-like disease in mice.

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THE MUTATIONAL LANDSCAPE AND CLONAL ARCHITECTURE OF MPN PATIENTS DETERMINED BY TARGETED NEXT GENERATION SEQUENCING

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Background: Myeloproliferative neoplasms (MPNs) are a group of diseases characterized by aberrant proliferation of the myeloid, erythroid and/or megakaryocytic lineages. In addition to JAK2-V617F mutations, a number of acquired alterations have been found to be involved in the initiation and progression of MPN. However, since the sequencing studies to date focus on examining mutations in only single or low number of genes, the interplay between different mutations is difficult to appreciate. Also, many of the mutations reported in MPN are rare (1-10% of patients carry them) and larger cohort studies are necessary to understand the impact of these less abundant mutations. Here we report the mutational spectra of a cohort of 214 MPN patients

analyzed using multiplexed next generation sequencing for mutations in 110 genes. In addition, we also demonstrate the clonal architecture for patients harboring multiple mutations.

Aims: We aim at defining the mutational spectra of a cohort consisting of 214 MPN patients and dissecting the clonal architecture of patients carrying multiple alterations. We developed a rapid, robust and cost efficient method that can be used for large cohort studies to analyze correlations between mutations and clinical presentation.

Methods: DNA from 214 MPN patients was fragmented, barcoded using 48 indexed adapters and enriched using Agilent SureSelect. The genes in the enrichment panel consisted of all genes reported to be mutated in MPN, as well as 100 additional genes found to be mutated in other myeloid malignancies or involved in hematopoietic signaling. The enriched DNA was sequenced using Illumina HiSeq2000 and each unique barcode assigned to its respective patient (48 patients/reaction). All patients were analyzed in duplicates and only mutations present in duplicate samples were considered candidate mutations. All candidate mutations were confirmed using an orthogonal NGS platform (Ion Torrent) and the somatic nature of the mutations was proven by comparing the mutations with paired non-hematopoietic DNA. Single colonies were picked and analyzed from patients with multiple mutations to assess the clonal architecture and temporal order of acquisition.

Results: We sequenced 110 genes (>1200 exons) in 214 MPN patients obtaining an average coverage of ~350-fold for the target regions. We used the JAK2-V617F mutations to estimate the sensitivity of the method. We were able to detect in JAK2-V617F 128/130 patients with an allelic burden >5%, which was the cutoff used for the Illumina mutation calling. Analysis of somatic mutations showed that 45/214 (21%) patients harbored 2 or more somatic mutations, and 7/214 (3%) patients carried 3 or more somatic mutations and no specific trend of mutation co-existance was observed (Figure 1, left circos plot shows JAK2-V617F positive patients and left circos plot shows JAK2-V617F negative patients). Sequencing of serial samples and single colonies from the same patient to assess the clonal architecture in patients with multiple mutations showed that no strict order of acquisition could be observed, and no genetic aberration was in all patients exclusively acquired before JAK2-V617F. Sequencing of serial samples for mutations described to be responsible for leukemic transformation (TP53 and KRAS/NRAS) assesses the kinetics between acquisition of the mutation and leukemic transformation. Furthermore, we detected novel somatic mutations in 9 genes not previously described to be involved in the MPN pathogenesis.

Summary / Conclusion: We were able to develop a tool allowing multiplexed cohort analysis using NGS. We found that ~21% of MPN patients harbored 2 or more somatic mutations. Analysis of the clonal architecture showed that none of the mutations exclusively preceded the acquisition of JAK2-V617F and no clear patter of co-existance between mutations was observed. Furthermore, we were also able to detect somatic mutations in 9 genes previously not described to be involved in the MPN pathogenesis.

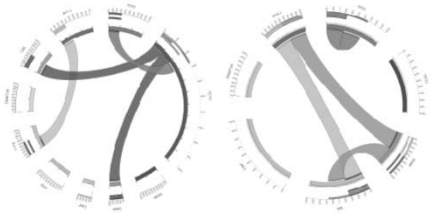

Figure 1.

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IPS CELLS FROM PV PATIENTS: A PHARMACOLOGICAL MODEL TO SCREEN INTERFERON ALPHA IMPACT IN A DEFINED MUTATIONAL CONTEXT

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Background: Myeloproliferative neoplasms (MPN) may present an oligoclonal pattern in which the hematopoietic stem/progenitor compartment displays molecular complexity, with coexisting acquired mutations. The most frequent mutation is $JAK2^{V617F}$ (JAK2VF), shown to deregulate myeloid progenitor cells proliferation. We and others have shown a differential response of PV subclones to treatment with peg-IFNα-2a according to the mutational pattern, but the precise cellular targets and mechanisms of action of this drug remain unknown.

Aims: To study the hematopoietic differentiation of induced pluripotent stem cells (iPSC) derived from somatic cells of MPN patients with complex mutational presentation and develop cellular models for IFNα pharmacological study.

Methods: The hematopoietic differentiation of a wild-type (WT) and a heterozygous JAK2VF iPSC line was assessed using two protocols : protocol A after derivation of embryoid bodies with an EPO containing cytokine cocktail, and protocol B using a Matrigel™ matrix in a medium with or without EPO. Hematopoietic differentiation was assessed by FACS analysis of myeloid surface markers and by methylcellulose assays for progenitor functionality. IFNα pharmacological studies were performed in both differentiation protocols in WT and JAK2VF iPSC lines.

Results: In the WT iPSC line, both protocols gave similar results for hematopoietic differentiation efficacy as assessed by the relative percentages of the various intermediate stage progenitors and mature cells. Nevertheless, culture on Matrigel™ gave higher yields in total cell number. In both WT or JAK2VF iPSC, CD34+ cells appear at the same timepoint (day 6) but the proportion of CD34+ cells was higher in JAK2VF cell cultures (15% vs 7,5%). As expected, the erythroid lineage was more abundant during the JAK2VF cell line differentiation with a peak of glycophorine A positive (GpA+) cells at day 20 of 70% in average compared to 45% for WT cells, along with spontaneous progenitor growth in the absence of erythropoietin (EPO). 2) IFNα was added 6 days after initiation of the differentiation protocol in both WT and JAK2VF cell lines. In JAK2VF cells, a delay and a 88% decrease in the emergence of CD43+ early mature hematopoietic cells was observed. In contrast, no delay was noted in CD43+ cell production in the WT cell line with only a 28% decrease compared to untreated cells. Likewise, GpA+ erythroid cell production was reduced by 62% after addition of IFNα in the JAK2VF cell line compared to a 30% decrease in the WT cell line. The decrease in erythroid progenitors, was also more pronounced in the treated JAK2VF iPSC-derived cells than in the WT-derived cells in clonogenic assays.

Summary / Conclusion: Using two different hematopoietic differentiation protocols, the JAK2VF iPSC line reproduced several features observed in PV patients such as an increase in erythroid cells production and EPO-independence. Treatment of the JAK2VF iPSC line with IFNα during hematopoietic differentiation reduces the emergence of early mature hematopoietic cells, erythroid progenitor growth and differentiation while sparing the erythroid growth and differentiation of a wild-type iPSC line. These results reproduce the in vivo efficacy of IFNα against JAK2VF clones. Thus, similar analysis performed on different iPS cell lines representing the different mutational combinations of a given PV patient should provide a useful model to study the precise mechanisms of action of IFNα on MPN hematopoiesis applicable in various complex molecular contexts.

Molecular Markers in acute lymphoblastic leukemia

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NOVEL AND RECURRENT GENES INVOLVED IN STRUCTURAL AND SEQUENCE VARIATIONS IN RELAPSED CHILDHOOD HIGH HYPERDIPLOID ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: High hyperdiploid acute lymphoblastic leukemia (HH-ALL) is characterized by 51-67 chromosomes and nonrandom gains of specific chromosomes (X, 4, 6, 10, 14, 17, 18, or 21). Occuring in 25-30% of cases, it is the most frequent numerical cytogenetic alteration in pediatric B-cell precursor ALL. Pre-leukemic clones are generated already *in utero*, but cooperating oncogenic lesions are necessary for overt leukemia. Children suffering from HH-ALL have a good prognosis, but recurrent disease will affect 15-20%. The underlying genetic mechanisms leading to overt leukemia and relapse remain to be determined.
Aims: The objective of this study was to comprehensively assess and validate genetic lesions associated with genesis and relapse of leukemia in a cohort of pediatric patients (n=6) with recurrent HH-ALL.
Methods: Whole genome and whole exome next-generation-sequencing was applied to analyze matched sample sets of six children with recurrent HH-ALL taken at initial diagnosis and/or relapse and remission. Paired-end genomic libraries were sequenced on a Genome Analyzer IIx or a HiSeq 2000 (Illumina). Reads were aligned against the human reference genome (GRCh37) using BWA. Unique reads were analyzed with GASV to detect translocations and inversions. Somatically acquired variations not present in the Database of Genomic Variants were reported. FREEC was employed to detect copy number alterations. Targeted enrichment of whole exomic regions was carried out employing SeqCap EZ libraries (Roche) and 100 bp single reads were sequenced on a HiSeq 2000. Mutations were called and LOH detected using an in-house bioinformatic pipeline. Putative somatically acquired mutations were validated by PCR, Sanger sequencing and FISH analysis.
Results: We detected and validated 11 interchromosomal translocations affecting genes coding for proteins (ART4, C12orf60, MACROD2, TBL1XR1, LRRN4, KIAA1467, ELMO1) and several miRNAs (e.g. miR1200), lincRNAs and nuclear RNAs involved in splicing (U6, U13). A MACROD2/KIAA1467-fusion potentially encoded a novel chimeric protein. All other rearrangements presented loss-of-function or -expression alterations. Most translocations were associated with copy number alterations. One case showed oscillating copy numbers at clustered breakpoints indicative of shattering/rejoining of chromosomal fragments, termed chromothripsis. Furthermore, deletions, inversions and loss-of-heterozygosity were detected. Exome sequencing revealed recurring mutations of CREBBP and members of the Ras family of small GTPases. One patient expressing wildtype NRAS and KRAS harbored mutated PAR4, a tumorsuppressor that is downregulated by oncogenic RAS for efficient transformation. Further mutations (non synonymous coding, splice site mutations or gained stop codons) were detected and validated in various transcription factors and signaling molecules involved in differentiation, cell cycle regulation and apoptosis. Relapse was associated with partial chromosomal gain (1q), loss (4q), a novel translocation (t(4;7)), deletions (IKZF1), increasing dominance of a chromothriptic clone and selection for RAS and CREBBP alterations.
Summary / Conclusion: Our data indicate a central role for RAS and CREBBP regulated pathways as assisting oncogenic lesions in pathogenesis and relapse of HH-ALL. Additional mutations indicate disturbance of B-cell differentiation, enhanced proliferation, suppression of cell death and a possible role of regulatory RNAs in HH-ALL.

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DIFFERENT MINIMAL RESIDUAL DISEASE (MRD) LEVELS PREDICT POST-TRANSPLANTATION OUTCOME IN MRD+ ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Background: MRD is the most powerful indicator of the risk of relapse, and is increasingly adopted in ALL for the risk-oriented application of stem cell transplantation (SCT) in MRD-positive (^{pos}) patients. Retrospective analyses indicated a lesser efficacy of SCT in MRD^{pos} cases, however without defining the quantitative MRD ranges associated with SCT failure. Such a definition could improve the ability to distinguish MRD^{pos} patients for whom SCT is an appropriate choice from those in whom other treatments should be considered instead or prior to SCT.
Aims: To define which post-induction/consolidation MRD levels can negatively affect post-transplantation outcome in adult ALL.
Methods: The long-term results of a prospective Northern Italy Leukemia Group trial conducted between 2000 and 2006 and enrolling a total of 304 consecutive unselected patients with Ph- ALL were reviewed. In this study, SCT was prescribed only to MRD^{pos} patients, regardless of clinical risk class. MRD was assessed by RQ-PCR methodology using one-two case-specific sensitive molecular probes, examining the bone marrow at weeks 10, 16 and 22, i.e. after chemotherapy cycles no. 3, 5 and 7. MRD results from all three time-points were pooled, and the highest value registered in individual patients was used to identify the MRD-negative group (always MRD^{neg}) and three other subsets characterized by increasing levels of residual ALL, from low-positive (<10^{-4} [MRD^{pos1}]) to positive (10^{-4} to <10^{-3} [MRD^{pos2}]) to strongly positive (≥10^{-3} [MRD^{pos3}]). MRD^{neg} patients were not offered SCT unless t(4;11)+. For study purposes, disease-free survival (DFS) and relapse incidence (RI) were assessed and compared among different MRD^{pos} groups, specifically in patients selected for SCT at end of consolidation phase after completion of the MRD study as per study design (at least MRD^{pos2} at week 16 and/or MRD^{pos1} at week 22).
Results: Of 304 patients treated (median age 35 years, range 16-68; male gender 57%) 258 (85%) entered complete remission (CR). Sensitive probe(s) were available for 200 (77.5%) CR patients, of whom 141 completed consolidation and 59 did not (early SCT 13, relapse 41, toxicity 5). Of 141 evaluable patients, 136 completed the MRD study, 64 being MRD^{neg} (47%), 21 MRD^{pos1} (15.5%), 17 MRD^{pos2} (12.5%) and 34 MRD^{pos3} (25%). With a minimum observation time of 4 years and a maximum close to 12 years, estimated 6-year DFS and RI were 57% and 32% in MRD^{pos1}, 46% and 50% in MRD^{pos2} and 15% and 76% in MRD^{pos3} cohorts, respectively (all P's <0.0001). Of all 72 MRD^{pos} patients, 44 (61%) underwent SCT as per protocol design (allogeneic SCT 26, "hypercycles" with autologous blood stem cell rescue 18). Although 6-year DFS rate was improved after allogeneic SCT (42% vs 20% with autologous SCT, P=0.09), MRD level was highly influential for post-transplantation outcome (DFS 48% in MRD^{pos1-2} group [n=25] vs 16% in MRD^{pos3} group [n19], P=0.025; RI 42% vs 69%, P=0.13), and the best overall result was obtained with allogeneic SCT in MRD^{pos1-2} group (DFS 60% [n=15] vs 18% in MRD^{pos3} group [n=11], P=0.08; RI 23% vs 64%, P=0.09).
Summary / Conclusion: In this prospective study, about one half of MRD^{pos1-2} patients were salvaged by SCT, mainly by allogeneic rather than autologous SCT (DFS 60%). Because of the poorer SCT results in MRD^{pos3} group, patients with post-induction/consolidation MRD ≥10^{-3} should receive further/experimental therapy and not proceed to SCT until the MRD signal is brought below the 10^{-3} cutoff.

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COMPARISON OF NEXT-GENERATION SEQUENCING AND ASO-PCR METHODS FOR MRD DETECTION IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The clinical management of patients with acute lymphoblastic leukemia (ALL) relies on accurate prediction of relapse risk to inform treatment decisions (Pui *et al*, JCO 2011). The measurement of minimal residual disease (MRD) has emerged as the most important predictor of outcome in ALL (Campana, Hematology Am Soc Hematol Educ Program 2010). Allele-specific oligonucleotide (ASO)-PCR can be used to assess MRD; however, this technique requires preparation of clonotype-specific primers for each individual which is laborious and time-consuming. We developed the LymphoSIGHT™ platform, a high-throughput sequencing method, which universally amplifies antigen-receptor gene segments and can identify all leukemia-specific sequences at diagnosis, allowing monitoring of disease progression and clonal evolution during therapy (Faham *et al*, Blood 2012).
Aims: In this study, we compared the sequencing and ASO-PCR methods for measuring MRD in follow-up samples and analyzed the extent of clonal evolution present in diagnostic and follow-up samples from 37 ALL patients.
Methods: Using the sequencing assay, we analyzed diagnostic blood and bone marrow samples from 37 ALL patients for clonal rearrangements of immunoglobulin (IGH-VDJ, IGH-DJ, IGK) and T cell receptor (TRB, TRD, TRG)

genes. Clonal rearrangements had been previously detected in all 37 patients using ASO-PCR methods. We assessed MRD at the IGH and/or TRG locus in 99 follow-up samples, and analysis of the concordance between MRD results obtained by the sequencing method and ASO-PCR is ongoing. Finally, we looked for cases with clone evolution and assessed the frequency of evolved clones over time in serial samples.

Results: Sequencing detected the presence of a high-frequency clonal rearrangement of at least one receptor ("calibrating receptor") in 100% of the 37 ALL patients; 36 patients had at least 2 calibrating receptors at diagnosis, 29 patients had at least 3 calibrating receptors, and 12 patients had more than 3. The TRG assay was the most frequent gene rearrangement: at least one TRG clonal rearrangement was detected in 29 of the diagnostic ALL samples. IGH-VDJ was the second most informative receptor, with clonal rearrangements being detected in 23 patients. We analyzed follow-up samples for the presence of MRD. Sequencing detected MRD levels of >10% in 4 samples, 1-10% in 4 samples, 0.1-1% in 8 samples, 0.01-0.1% in 12 samples, 0.001-0.01% in 8 samples, and <0.001% in 11 samples. MRD was undetectable by sequencing in 45 samples. Being able to track more than one clonal sequence allows the follow up of the dynamics of these clones. In at least one patient, the relative frequencies of the 3 clonal sequences changed dramatically over the disease course (Figure 1).

Summary / Conclusion: This high-throughput sequencing method enables MRD detection without the need for development of patient-specific reagents. The sequencing method allows monitoring of clonal dynamics over time, which may provide valuable insight into tumor evolution and resistance to therapy at the individual clone level. Concordance data between sequencing and ASO-PCR will be presented.

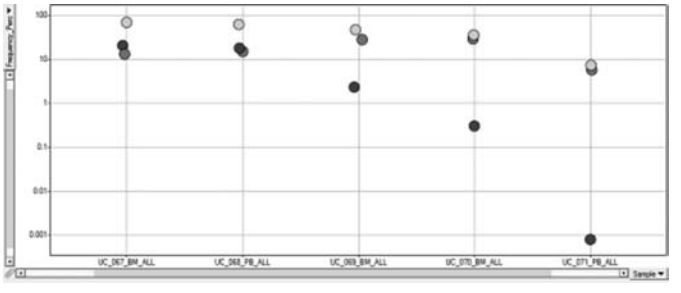

Figure 1. Dynamics of clone frequency over time in one ALL patient across 5 samples. Colors indicate three different clonal sequences. The 5 samples are shown on the X axis. The clone frequency is shown on the Y axis.

S538

ERG DELETION EXERTS A CD2-DEPENDENT POSITIVE PROGNOSTIC IMPACT ON IKZF1-DELETED CHILDHOOD ALL

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Background: B-cell precursor (BCP) acute lymphoblastic leukemia (ALL) is a clinically and biologically heterogeneous disease. Based on the presence of routinely screened recurrent genetic aberrations, most cases can be classified into genetically defined subgroups. Despite this success, a still significant proportion of patients falls into the so-called "B-other" category with largely unknown genetic background and heterogeneous outcome. Importantly, the majority of relapses occur within patients who lack any known strong prognostic feature. To improve the prognosis of this group, identification of new and more accurate risk predictors is of ongoing interest.

Aims: Using SNP microarray analysis, intragenic deletions of the *ERG* gene on chromosome 21q22.3 (*ERG*del) were recently described as a recurrent abnormality in childhood ALL. However, detailed characterization of this aberration in the context of large modern clinical trials has not been performed so far. In our study we aimed to assess the incidence of *ERG*del in childhood ALL and to evaluate its clinical value as a prognostic marker.

Methods: We developed a multiplex PCR assay to screen *ERG*del on the genomic level and screened 1323 patients treated on the multicentre ALL-BFM 2000 protocol. A commercially available multiplex ligation-dependent probe amplification (MLPA) kit was used to analyze *IKZF1* deletions. Other clinical and laboratory parameters were acquired by routine diagnostic procedures.

Results: We identified 60 cases with *ERG*del, all exclusively within BCP-ALL. The majority of positive patients were observed in the "B-other" subgroup (44/403). Interestingly, in at least 1/3 of cases the deletion was bi-/oligoclonal and it was significantly associated with higher age, CD2-positivity and IKZF1 deletion. We found no or only weak associations of *ERG*del alone with prognosis. However, when combined with CD2 status, the CD2-positive/*ERG*del patients demonstrated superior outcome. This effect was significant even within cases harbouring *IKZF1* deletions which have previously been shown to have a negative prognostic impact in the ALL-BFM 2000 trial population. In addition, we analyzed stability of *ERG*del between diagnosis and relapse in all six CD2-negative/*ERG*del relapses. In 3/6 cases the *ERG* deletions were lost and in the remaining 3 cases the relapse deletions differed from those found at diagnosis.

Summary and Conclusions: To conclude, we describe a high incidence of *ERG*del in B-other ALL. Our data suggest that the *ERG* locus is specifically prone to deletion in this subgroup. Nevertheless, the deletion does not seem to play a driving role in the pathogenesis of the disease. We show that combined with CD2-positivity the *ERG*del confers a superior prognosis which even overcomes the negative impact of concurrent *IKZF1* deletions.

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S539

HIGH HYPERDIPLOIDY (HEH) AMONG ADOLESCENTS AND ADULTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL): CYTOGENETIC FEATURES, CLINICAL CHARACTERISTICS AND OUTCOME

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Background: HeH is the massive clonal gain of chromosomes resulting in a modal number of 51-65 chromosomes. The pattern of chromosome gain is non-random and distinctive with 8 chromosomes accounting for >75% gains. HeH is the most prevalent genetic subtype in paediatric ALL occurring in 33% patients and is associated with a good prognosis. We have previously reported the incidence and outcome of HeH among adolescents and adults treated on UKALLXII/ECOG2993 both with and without Philadelphia positive (Ph+) disease (Blood, 2007;109:3189 & 2009;113:4489). Among paediatric patients, several cytogenetic features, including the presence of specific trisomies, have been associated with outcome. In particular, +4, +10, +17, +18 and high modal number have been associated with improved survival.
Aims: There are no previous studies of HeH in adults describing the pattern of chromosome gain or the relationship with outcome. As adolescent and adult ALL patients generally have an inferior outcome compared to children the prognostic relevance of specific trisomies may be more relevant in this group.
Results: Among 1232 BCP-ALL patients treated on UKALLXII/ECOG2993 (1993-2006) tested for HeH, 159 (13%) were positive. The incidence of HeH was similar among Ph+ (48/340, 14%) and Ph- (111/892, 12%) patients. Ph+ HeH patients were older than Ph- HeH patients (38 v 26 years, P=0.001). Although ~60% of Ph- patients were <25 years, ~25% were 40-59 years. Ph+ HeH karyotypes had significantly lower modal numbers whereas the distribution for Ph- cases was similar to paediatric ALL with a peak at 55 chromosomes. There were significant differences in the pattern of chromosome gain: Ph+ karyotypes were more likely to have +2, +15 and +der(22)t(9;22)(q34;q11), and less likely to have +10, +17 and +18. Gains of chromosomes 4, 6, 14, 21 and X were equally prevalent in both groups. The median follow-up time for the 111 Ph- HeH patients was 8 years. The majority (106, 96%) achieved a complete remission (CR) with 31 (29%) cases subsequently relapsing and 17 (16%) dying in first remission. The 5 year survival was 58% (95% CI 48-66%). Patients who had gained a chromosome 4, 10, 14 or X had a significantly improved outcome: HR (95% CI) 0.41 (0.22-0.72), 0.55 (0.31-0.98), 0.56 (0.32-0.99) and 0.49 (0.27-0.90) respectively. However, only +4 and +10 remained significant (P=0.003 & 0.027 respectively) in a multivariate model with age and white cell count. There was no evidence to suggest a relationship between modal chromosome number and outcome. Survival data was only available for 26 Ph+ patients treated prior to the introduction of imatinib (median follow-up 11 years). All patients, bar one, achieved CR (96%) but 9 (35%) subsequently relapsed and 10 (40%) died in first remission. Thus the 5 year survival was 38% (20-56). There was little evidence to suggest outcome heterogeneity according to specific trisomies or modal chromosome number; except for a borderline result for chromosome 4 [0.45 (0.18-1.16)].
Summary / Conclusion: In conclusion, the cytogenetic landscape of Ph- HeH ALL is almost identical to that observed in paediatric ALL whereas it is distinctive from Ph+ HeH ALL. There is significant evidence to suggest outcome heterogeneity according to the presence of specific trisomies in Ph- HeH ALL. Further studies based on more contemporary cohorts will be needed to evaluate these results among young adults treated on paediatric or paediatric-like protocols.

Multiple Myeloma - Biology

S540

IN VIVO TARGETING OF STROMAL-DERIVED FACTOR-1 AS A STRATEGY TO PREVENT MYELOMA CELL DISSEMINATION TO DISTANT BONE MARROW NICHES

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Background: Active multiple myeloma (MM) is characterized by the presence of continuous dissemination of MM cells from the primary site of tumor development to multiple distant bone marrow (BM) niches. We hypothesized that stromal-derived factor-1 (SDF-1) may represent a target for preventing transition from MGUS to active-MM; thus resulting in inhibition of MM progression; and tested NOX-A12, a high affinity l-oligonucleotide-based inhibitor of SDF-1 in MM, looking at its ability to mold the BM milieu, thus modulating MM cell growth and homing to the BM *in vivo* and *in vitro*.
Aims: 1) To demonstrate that SDF-1-neutralization in the BM niches inhibits cell dissemination *in vivo* in a MM model. 2) To demonstrate the delivery of NOX-A12 *in vivo*; and its ability to modulate MM tumor growth and MM cell dissemination.
Methods: SDF-1 levels were evaluated by immunohistochemistry on BM specimens obtained from patients with MGUS, active-MM, or healthy individuals; and confirmed by ELISA, using conditioned-medium of BM-mesenchymal stromal cells (BM-MSCs) obtained from MGUS, active-MM and healthy individuals. Co-localization of MM tumor cells (MM.1S-GFP+) and NOX-A12 with SDF-1-positive areas was tested by *in vivo* confocal microscopy, using both AlexaFluor633-conjugated-anti-SDF-1 monoclonal antibody and AlexaFluor647-conjugated-NOX-A12. Effect of NOX-A12 in modulating MM cell dissemination was tested *in vivo*, by using *in vivo* confocal microscopy. *In vivo* homing and *in vivo* tumor growth of MM cells (MM.1S-GFP+/luc+) were assessed by using *in vivo* confocal microscopy and *in vivo* bioluminescence, in SCID mice treated with a) vehicle; b) NOX-A12; c) bortezomib; d) NOX-A12+bortezomib. Detection of mobilized MM-GFP+ cells *ex vivo* was performed by flow cytometry. Effects of drug combination on dissemination of MM cells to distant BM niches were evaluated *ex vivo* by using immunofluorescence on mice femurs. DNA synthesis and adhesion of MM cells in the context of NOX-A12 (50-100nM) treated primary MM BM-MSCs in presence or absence of bortezomib (2.5-5nM) were tested by thymidine uptake and adhesion *in vitro* assay, respectively. Synergism was calculated by using CalcuSyn software. NOX-A12-dependent-modulation of signaling was evaluated by western blot on MM cells exposed or not to NOX-A12-treated primary BM-MSCs.
Results: Patients with active-MM present with higher BM SDF-1 expression than MGUS patients and healthy individuals. *In vivo* confocal imaging revealed higher SDF-1 expression in MM cell-enriched BM areas compared to non-MM colonized BM niches. We next demonstrated that NOX-A12 was delivered to BM areas in MM-tumor bearing mice. Mice pre-treated with NOX-A12 showed a significant reduction of MM cell growth and dissemination compared to untreated mice, indicating indeed that modulation of the pre-metastatic niche with NOX-A12 reduced MM cell dissemination. We examined NOX-A12-dependent the *in vivo* transcriptional changes on the murine BM milieu and found that the BM cells of NOX-A12-pretreated mice showed transcriptional downregulation of SDF-1 as well as downregulation of angiogenesis-, cell growth-, and cell adhesion-related genes. We next dissected the ability of NOX-A12-dependent neutralization of SDF-1 to disrupt the interaction of MM cells with the surrounding BM milieu and found that NOX-A12 induced MM cell mobilization from the BM to the peripheral blood *ex vivo*. We next showed that NOX-A12-dependent de-adhesion of MM cells from BM-MSCs enhanced MM cell sensitivity to bortezomib, as shown both *in vitro* and *in vivo*. Specifically, significant reduction of tumor burden in those mice treated with sequential administration of NOX-A12 and bortezomib was observed, compared to bortezomib alone-treated mice (P <0.05). Similarly, NOX-A12+bortezomib combination induced significant inhibition of MM cell homing, as shown by *in vivo* confocal microscopy.
Summary / Conclusion: NOX-A12-dependent neutralization of SDF-1 modulates the pre-metastatic BM niche *in vivo*, thus resulting in inhibition of tumor cell dissemination in an MM model. Our data suggest that NOX-A12 may represent a first in class niche-targeting agent that can prevent or disrupt bone marrow metastasis in MM and possibly other tumors that metastasize to the bone marrow.

S541

PLASMA CELL PHENOTYPE AND B-CELL STATUS ARE INDEPENDENT PREDICTORS OF PROGRESSION-FREE AND OVERALL SURVIVAL IN MGUS.

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Background: Monoclonal Gammopathy of Undetermined Significance (MGUS) shows progression to myeloma or other B-lymphoproliferative disorders in approximately 1% of cases per year. MGUS is associated with immune suppression and shows approximately 2% per year excess infectious mortality. Depletion of normal plasma cells and abnormal sFLC ratio predict an increased risk of progression. Changes in normal B-cell subsets are also reported although it is not clear how these relate to outcome.
Aims: To identify B-cell related factors which affect progression and survival in MGUS.
Methods: Prospective analysis of plasma cell and B-cell immunophenotype was performed since 2004 on the bone marrow in 1206 MGUS cases with records of the standard diagnostic and clinical features. B-cell clonality was included in the analysis to assess for the presence of an underlying B-cell disorder.
Results: During follow-up (median 3.5, range 0.5-8yrs), 48 developed myeloma and 9 a B-lymphoproliferative disorder. There were 281 deaths of which 151 were potentially MGUS-related (i.e. myeloma, infectious or renal condition). Normal plasma cells were depleted (i.e. represented <5% of total plasma cells) in 18% of cases and this was strongly associated with disease progression (Log-rank P<0.001). There was 63% concordance between PC phenotype and sFLC ratio; either factor alone was equally predictive of disease progression (Log-rank P=0.026). B-cell abnormalities (i.e. depleted normal subsets, perturbed K:L ratio, or MBL) were detected in 26% of cases with no discernable clonal relationship to the neoplastic plasma cells. B-cell abnormalities were strongly associated with progression and disease-related mortality (univariate P=0.001, multivariate P=0.021).
Summary / Conclusion: In addition to the neoplastic plasma cell expansion, MGUS is characterised by abnormal B-cell homeostasis and humoral immunity. All of these factors predict for an increased risk of disease progression. B-cell and plasma cell abnormalities are also strongly associated with excess infectious mortality. Current treatment for myeloma can reverse B-cell/humoral abnormalities with low toxicity and it may be appropriate to consider a clinical trial to evaluate early intervention in the subset of MGUS patients identified in this study who are at an increased risk of infectious mortality.

S542

HETEROGENEITY OF MUTATIONAL PROCESSES IN MULTIPLE MYELOMA

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Background: The catalog of somatic mutations in a cancer is the aggregate outcome of strength and duration of exposure to one or more mutational processes. Signatures underlying mutational processes have never been investigated in multiple myeloma (MM), a plasma cell malignancy with incompletely understood molecular pathogenesis. While chromosomal abnormalities are involved in MM initiation, they are insufficient for its progression, which is characterized by secondary events, particularly gene mutations. Despite novel treatment options, relapses are frequent and ultimately chemoresistant, thus MM remains an incurable disease. Understanding the molecular processes shaping the MM genome at diagnosis and those contributing to emergence of chemoresistant clones can therefore provide new insights into disease biology and treatment.
Aims: We have previously showed how the mutational landscape, clonal architecture and evolutionary progression were heterogeneous across 84 samples from 67 patients with MM (Bolli et al, Haematologica, 2012, Vol 97-S1, a0571). In this study, we set out to extract the mutation signatures characterizing the mutational processes underlying the landscape of mutational changes in MM.
Methods: We validated 4421 variants in 67 patients. We showed variability in the numbers and relative contributions of each base substitution (C>A, C>G, C>T, T>A, T>C, and T>G). To provide insight into the processes underlying such a heterogeneous catalogue of mutations, we incorporated the sequence context in which mutations occurred by considering the bases 5' and 3' to each mutation to generate 96 possible mutation types. Werepresented the fraction of mutations of each type as a heatmap for each case. We employed nonnegative matrix factorization (NMF) and model selection to extract the quantitative contribution of each mutational signature.
Results: Most cases showed a predominance of Signature A, C>T changes at CpG trinucleotides. This is likely to represent spontaneous deamination of methylated cytosine to thymine, and is the dominant process in myeloid malignancies, a major contributor to early mutations in breast cancer, and seen at high rates in the germline. Signature B, consisting of C >T, C>G and C>A mutations in a TpCpX context, contributed 5 to 45% of the variants in most cases, but 100% in the 2 samples characterized by the highest number of variants, suggesting that this signature is associated with a hypermutator phenotype. In another 2 patients, we found clusters of 5-10 mutations from Signature B, all within a region <200bp showing strand specificity, a process known as 'kataegis'. This signature was first documented in breast cancer, and others and we have speculated to arise from aberrant activity of APOBEC proteins. In serial samples, relative contribution of each signature changed up to 2-fold. Importantly, progression towards aggressive disease was associated with an increased contribution of signature B and the appearance of kataegis, suggesting that this signature may be linked to phenotypic evolution. Another process leading to regional clustering of mutations was somatic hypermutation driven by the AID protein, resulting in clusters of mutations of *CCND1* in two cases, both characterized by t(11;14), juxtaposing *CCND1* with the *IGH* locus. Finally, copy number analysis revealed the presence of chromothripsis, a mechanism of genomic instability defined by tens to hundreds of chromosomal rearrangements involving localized genomic regions, associated with poor outcome.
Summary / Conclusion: We have described for the first time a catalogue of several heterogeneous mutational processes active in MM, including spontaneous deamination of methylated cytosine, kataegis, somatic hypermutation, and chromothripsis. We have shown that mutational processes are active across different cancer types and retain the same biological features, such as the hypermutator phenotype of Signature B. The extent of such mutational processes can vary from genome-wide to localized clusters, and their relative contributions change over time. This analysis represents advancement in our understanding of MM biology, and of the processes underlying its genomic heterogeneity.

S543

CC-292, A NOVEL BRUTON'S TYROSINE KINASE INHIBITOR ALONE AND IN COMBINATION WITH CARFILZOMIB IMPACTS BONE RESORPTION IN MULTIPLE MYELOMA BY BLOCKING OSTEOCLAST SEALING ZONE FORMATION

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Background: The activation of Bruton's tyrosine kinase (Btk), a member of the Tec family of tyrosine kinases, modulates B-cell development and activation and plays an important role in antibody production. Interestingly, Btk and Tec (the other Tec kinase family) regulate the differentiation of osteoclasts (OC), which are an integral part of the bone microenvironment. Osteolytic bone lesions occur in 70-80% of patients with multiple myeloma (MM), and are associated with increased morbidity and mortality. The imbalance between OC and osteoblasts leads to increased bone resorption and induces osteolytic lesions. Inhibition of OC is important for the improvement of osteolytic bone disease and for the treatment of MM. Btk and Tec regulates OC function via receptor activator of nuclear factor κB (RANK) signaling. RANK signaling activates c-Src which controls OC bone resorption by regulating actin organization via cortactin. c-Src also phosphorylates proline-rich tyrosine kinase (Pyk) 2, which plays an important role in OC activation and localizes to the sealing zone in OC.
Aims: We sought to assess the effects and molecular mechanism of a potent and specific BTK inhibitor, CC-292 in the context of OC. We also evaluated the effect of CC-292 used in combination with the proteasome inhibitor carfilzomib (CFZ) in our *in vitro* and *in vivo* MM model.
Methods: OC were derived from MM patient monocytes. OC number and function was evaluated by TRAP staining and pit formation assay, respectively. The effect of CC-292 on the molecules involved in sealing zone formation, such as c-Src and Pyk2 signaling was investigated by Western blotting. OC sealing zone formation was detected by immunofluorescence. The *in vivo* study was performed in the disseminated xenograft MM model. Human Luc-GFP$^+$-MM.1S cells were injected IV in NOD-SCID mice (n=40). Treatment with CC-292 (30mg/kg per os× 5 for 6 weeks), CFZ (3 mg/kg i.v.× 2 for 4 weeks and 2 mg/kg i.v.× 2 for 2 weeks) and combinations of both agents were administered. Tumor burden was evaluated by bioluminescence imaging (BLI) once-weekly.
Results: OC function was significantly inhibited in the presence of CC-292. However, OC number, size and differentiation marker expression were increased in the presence of CC-292. CC-292 inhibited c-Src and Pyk2 phosphorylation. Furthermore, CC-292 inhibited cortactin protein and mRNA expression and upregulated c-Cbl protein (E3 ubiquitin ligase for c-Src) expression in OC. Importantly, CC-292 disrupted the OC sealing zone formation. In contrast, the proteasome inhibitor CFZ had no impact on OC sealing zone formation but instead inhibited OC differentiation. CC-292 in combination with CFZ inhibited both sealing zone formation

as well as OC differentiation, resulting in a more pronounced suppression of OC function than either agent alone.
In our *in vivo* study, the combination of CC-292 and CFZ inhibited the tumor burden by a statistically significant margin (control vs combination, P=0.0159).
Summary / Conclusion: These data demonstrate that the novel Btk inhibitor CC-292 inhibits OC function through inhibition of OC sealing zone formation.
CC-292 in combination with CFZ induced potent suppression of OC function. The *in vivo* study demonstrated a significant decrease in tumor burden in the combination group vs control group. Ongoing static and dynamic histomorphometric analyses will help delineate the effect of this combination on bone remodeling.

S544

INTRACELLULAR NAMPT INHIBITION ENHANCES BORTEZOMIB ACTIVITY OVERCOMING DRUG RESISTANCE IN MULTIPLE MYELOMA
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Background: A prominent feature of malignant cells is the acquisition of characteristics that enable uncontrolled proliferation, including the capability to modify or reprogram cellular metabolism. In such a scenario, tumor cells exhibit highly increased rates of energy consuming reactions due to elevated intracellular NAD+. This realization has provided the basis for molecular studies of NAD+ metabolism to identify novel targeted therapeutic strategies. Recently we demonstrated that Nicotinamide phosphoribosyl transferase (Nampt), a key enzyme involved in NAD+ metabolism, is essential for maintenance of multiple myeloma (MM) cell viability; and conversely, that Nampt inhibition/depletion potently kills MM cells. In vivo studies in murine xenograft models of human MM showed that the chemical inhibitor of Nampt, FK866, is well tolerated, prolongs survival, and reduces tumor growth. Proteasome inhibitor bortezomib has transformed therapy of relapsed MM, as well as prolonged event free and overall survival when used as initial therapy for newly diagnosis disease. However, prolonged bortezomib exposure may result in cumulative toxicity and acquisition of drug resistance. Combination approaches aimed to prevent or overcome mechanism(s) of bortezomib-resistance offer great potential to improve outcome.
Aims: In this study we aim to demonstrate that the combination of FK866, with Bortezomib induces synergistic anti-MM cell death both *in vitro* using MM cell lines or patient CD138+MM cells and *in vivo* in a human plasmacytoma xenograft model.
Methods: We utilized MM.1S, MM.1R, RPMI-8226, U266 and ANBL6 BR human MM cell lines, as well as purified tumor cells from patients relapsing after prior bortezomib-based therapies. Cell viability and apoptosis assays were performed using Annexin V/PI staining. Intracellular NAD+ level and proteasome activity were quantified after exposure to single/combination drugs by specific assays. *in vitro* angiogenesis was assessed by Matrigel capillary-like tube structure formation assay. Immunoblot analysis was performed using antibodies to caspase-8, caspase-9, caspase-3, PARP, and tubulin. The expression level of Nampt transcript (probe ID 217739_s_at) as well as its prognostic role was evaluated in publically available database (GSE9782) of tumor cells from Bortezomib-treated MM patients. CB-17 SCID male mice (n = 28; 7 mice/EA group) were subcutaneously inoculated with 5.0 x 10^6 MM.1S cells in 100 microliters of serum free RPMI-1640 medium. When tumors were measurable (3 weeks after MM cell injection), mice were treated for three weeks with vehicle alone, FK866 (30 mg/kg 4 days weekly), Bortezomib (0.5 mg/kg twice weekly), or FK866 (30 mg/kg) plus Bortezomib (0.5 mg/kg). Statistical significance of differences observed in FK866, Bortezomib or combination-treated mice was determined using a Student t test. Isobologram analysis was performed using "CalcuSyn" software program. A combination index <1.0 indicates synergism.
Results: We observed higher Nampt mRNA levels in bortezomib-resistant patient MM cells, which correlated with decreased overall survival. We therefore demonstrated that combining the NAD+ depleting agent FK866 with bortezomib induces synergistic anti-MM cell death and overcomes bortezomib-resistance in MM dell lines as well as in patient CD138-positive MM cells (P< 0.004). This effect is associated with: 1) activation of caspase-8, caspase-9, caspase-3, and PARP; 2) enhanced intracellular NAD+ depletion; 3) inhibition of chymotrypsin-like, caspase-like and trypsin-like proteasome activities; 4) inhibition of NF-kappa B signaling; and 5) inhibition of angiogenesis. Furthermore, Nampt knockdown significantly enhances the anti-MM effect of bortezomib, which can be rescued by ectopically overexpressing Nampt. In murine xenograft MM model, low-dose combination FK866 and Bortezomib is well tolerated, significantly inhibits tumor growth, and prolongs host survival. (2–2.5 months P=0.001).
Summary / Conclusion: Our *in vitro* and *in vivo* findings demonstrate that intracellular NAD+ levels represent a major determinant in the ability of bortezomib to induce apoptosis of MM cells, providing the rationale for clinical protocols evaluating FK866 together with Bortezomib to improve patient outcome in MM.

Red blood cells - Biology

S545

INHIBITION OF SMAD2/3 SIGNALING BY ACE-536 MITIGATES INEFFECTIVE ERYTHROPOIESIS AND CORRECTS ANEMIA IN BETA-THALASSEMIA
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Background: Members of the TGF-β superfamily are known to be involved in erythropoiesis and iron homeostasis. Elevated levels of Smad 2/3 signaling ligands have been reported in diseases with ineffective erythropoiesis such as β-thalassemia and myelodysplastic syndromes (MDS). β-Thalassemia is caused by a deficiency in β-globin production in erythroid cells, resulting in decreased hemoglobin levels and anemia. Excess α-globin chains accumulate in the cell, causing proteotoxicity and apoptosis. Ineffective erythropoiesis in β-thalassemia leads to arrest of terminal erythroid differentiation, erythroid precursor expansion in bone marrow and spleen, apoptosis, and hemolysis of RBCs.
Aims: We hypothesized that inhibition of Smad 2/3 signaling could mitigate ineffective erythropoiesis and correct anemia in β-thalassemia.
Methods: ACE-536 is a modified activin type IIB (ActRIIB) receptor fusion protein which acts as a ligand trap for certain members of the TGF-β superfamily, including GDF8 and GDF11. Unlike wild type ActRIIB, ACE-536 does not bind activin A or bone morphogenetic proteins (BMPs). Circulating levels of GDF11 were measured in β-thalassemia patients (N=19) and found to be ~3-fold higher compared with healthy controls (N=5) (286±38 vs 87.8±6 pg/mL, P<0.05). In wild-type mice, ACE-536 treatment promotes maturation of terminal differentiating erythroblasts, unlike EPO which increases precursor proliferation. We used the murine model of β-thalassemia intermedia (*Hbb*$^{th1/th1}$) to study the effects of RAP-536 treatment (murine ortholog of ACE-536). β-Thalassemic mice were treated SC twice weekly with RAP-536 (1 mg/kg) or TBS vehicle (VEH) control for 2 months (N=12/group). Wild-type littermates were dosed similarly (N=17/ group).
Results: Following 2 months of treatment, RAP-536 significantly increased RBC (+28.0%, P<0.001), hemoglobin (+15.0%, p<0.001), and hematocrit (+18.5%, p<0.001), and decreased reticulocytes (-33.4%, P<0.05) in β -thalassemic mice compared to VEH treatment. Analyses of bone marrow and spleen from β -thalassemic mice treated with RAP-536 revealed significantly decreased basophilic erythroblasts and increased late stage orthochromatic erythroblasts. RAPssemic mice had significantly reduced serum EPO levels (639.7±111 vs. 1769.7± 517 pg/mL, P<0.05), and spleen weights (418.3±28 vs. 677.1± 65 mgs, P<0.01) compared to VEH treatment, indicative of decreased extramedullary erythropoiesis. βreated with RAP-536 had increased tibial bone mineral density by dual-energy X-ray absorptiometry (DXA) and increased trabecular bone volume by micro-computed tomography (μCT) compared to vehicle treatment, possibly due to decreased erythropoietic activity in the marrow space.Quantitative tissue iron measurement and Perl's Prussian blue staining revealed decreased spleen, liver and kidney iron levels in RAP-536 treated βehydrogenase levels (334.6±33 vs. 424.6±76 IU/mL) were lower in β-thalassemic mice treated with RAP-536 compared to VEH-treated mice, demonstrating decreased hemolysis. Morphological assessment of peripheral blood smears showed decreased hemolysis, α-globin inclusion bodies and poikilocytosis.
Summary / Conclusion: In summary, these data demonstrate that RAP-536 attenuates ineffective erythropoiesis, corrects anemia, and improves associated co-morbidities in a murine model of βently being tested in Phase II clinical trials in MDS and β-thalassemia patients.

S546

CHARACTERIZATION OF SEC23B HYPOMORPHIC ALLELES IN CDA II PATIENTS
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Background: Congenital dyserythropoietic anemia type II (CDA II) is a genetic hyporegenerative anemia characterized by ineffective erythropoiesis. This condition belongs to COPII-related human genetic disorders. Indeed, it is due to mutations in SEC23B, a component of COPII complex, the core trafficking machinery of the endoplasmic reticulum-Golgi. To date, 60 different causative mutations have been described. The most frequent mutations are nucleotide substitutions (75% missense/nonsense), whereas frameshift and splicing mutations were observed in 15% and 10% respectively. The vast majority of patients have two mutations (in the homozygous or compound heterozygous state),

according to the pattern of autosomal recessive inheritance. In no case homozygosity or compound heterozygosity for two nonsense mutations was found, a situation likely to be lethal. However, few cases with two hypomorphic mutations have been described so far.
Aims: To characterize molecular mechanisms underlying SEC23B alleles and to correlate them with clinical phenotype.
Methods: Diagnosis of CDA II was based on history, clinical findings, laboratory data, morphological analysis of aspirated bone marrow and, whenever possible, on evidence of hypoglycosylated band 3 by SDS-PAGE. Prediction analyses for splice sites mutations were performed by web server tools, Splice Site Prediction by Neural Network and Human Splicing Finder. Total RNA was isolated from peripheral blood mononuclear cells (PBMCs). Relative gene expression was calculated by using the $2^{-\Delta Ct}$ method. P value has been calculated by Student *t* test. Proteins were extracted from PBMCs and western blotting (WB) was performed using a specific rabbit anti-SEC23B antibody (1:500). WB analysis on gradient 4-15% SDS PAGE was performed by precast gel.
Results: We found five novel nucleotide replacements in *SEC23B*: three intronic mutations (c.834+3A>C; c.221+163A>G; c.1404+5G>A), one nucleotide insertion (c.1419_1423insC, p.I473Ifs*47) and one G>A transition (c.221G>A, p.C74Y). None of these mutations is present in the 1000 Genome project. Accordingly to recessive inheritance pattern, the patients were compound heterozygotes for two mutations. Patients A-II.1 and B-II.1, compound heterozygotes for two hypomorphic mutations, showed a marked reduction of SEC23B expression at both mRNA and protein level, while the patient with a missense/nonsense genotype (C-II.1) showed only a slight reduction. Interestingly, patients A-II.1 and B-II.1 exhibited a milder phenotype than patient C-II.1. When we analyzed *SEC23A* expression in all three patients, we found an upregulation of approximately 4 and 3 fold in respect with the paralog SEC23B in compound heterozygotes patients for two hypomorphic mutations. Conversely, no compensatory effect of *SEC23A* expression has been found neither in the patient compound heterozygotes for one missense and one nonsense mutation nor in control subjects.
Summary / Conclusion: Our analyses suggested that the association of two hypomorphic alleles led to a strong reduction of SEC23B expression, without generating severe clinical presentation. We proposed a compensatory mechanism SEC23A-mediated which could balance the reduced expression of its paralog SEC23B. This is in agreement with studies on zebrafish morphants which showed that both Sec23 genes carry specific but partially redundant roles, at least in craniofacial cartilage maturation. The comprehension of the role of SEC23A-B paralogs in humans may provide a means of therapeutic intervention by modulating their expression.

S547

NF-E2 REGULATES PUTATIVE RATE-LIMITING ENZYMES IN THE HEME BIOSYNTHESIS PATHWAY

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Background: During erythropoiesis, an increase in heme biosynthesis is crucial for correct maturation. Although it has been shown that this augmented synthesis is achieved by a sequential activation of the eight heme biosynthetic enzymes, the exact regulatory mechanisms remain unclear.
The transcription factor Nuclear Factor Erythroid 2 (NFor erythroid differentiation. It is known to regulate expression of the β-globin gene as well as the genes for two enzymes involved in heme biosynthesis, porphobilinogen deaminase and ferrochelatase.
Aims: We investigated the role of NF-E2 in the regulation of five of the remaining heme biosynthetic enzymes.
Methods: Possible NF-E2 binding sites in the heme biosynthesis enzymes were identified by *in silico* analysis of ChIP-sequencing data from the Encyclopedia of DNA Elements (ENCODE) Consortium. In addition, the promoter regions of aminolevulinic acid dehydratase (ALAD), uroporphyrinogen synthase (UROS), uroporphyrinogen decarboxylase (UROD), coproporphyrinogen oxidase (CPOX) and protoporphyrinogen oxidase (PPOX) were examined for NF-E2 consensus sequences by computational analysis. Binding of NF-E2 to the predicted DNA sequences *in vivo* was verified by chromatin immunoprecipitation (ChIP). Subsequently, functionality of the identified NF-E2 binding sites was determined by luciferase reporter gene assays. Finally, HEL cells were treated with lentiviral constructs containing a shRNA against NF-E2 or a control shRNA and heme content was determined.
Results: Putative binding sites for NF-E2 were detected in the promoter regions of ALAD, UROS, UROD, CPOX and PPOX. In UROS, UROD and CPOX, *in vivo* binding of NF-E2 to the respective locus was verified by chromatin immunoprecipitation. In luciferase reporter gene assays, expression ofNF-E2 induced the erythroid promoter of uroporphyrinogen synthase. Site-directed mutagenesis of the NF-E2 binding site in the erythroid promoter of uroporphyrinogen synthase abolished the NF-E2 mediated activation, establishing this enzyme as a novel NF-E2 target gene. During the increased demand for heme synthesis in erythropoiesis, CPOX has been proposed to be rate-limiting. NF-E2's role in regulating CPOX expression underlines its function as a regulator of heme biosynthesis. Consistent with this regulatory function, knockdown of NF-E2 in HEL cells resulted in a reduction in heme content by 20%.
Summary / Conclusion: Our results demonstrate that NF-E2 modulates the heme biosynthesis pathway at multiple, putatively rate-limiting, steps and thereby establishes a positive feedback loop that secures a constant heme supply for the assembly of hemoglobin during erythroid differentiation.

S548

PRETREATMENT WITH A RHO KINASE INHIBITOR PREVENTS ACUTE LUNG INFLAMMATION IN THE SICKLE CELL MICE MODEL

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Background: Rho kinases are involved in NF-kB activation, suggesting a potential role of Rho kinases in LPS-induced inflammation. The Rho kinase inhibitor, Y-27632, reduced leukocyte infiltration in several models of inflammation such as endotoxic liver and lung injury. Lungs are particularly vulnerable to vaso-occlusive events because of their anatomic features in SCD. Studies *in vitro*, and *in vivo* using animal models show that leukocytes play a key role in vasoocclusion. Previous studies have shown that LPS challenge causes exaggerated inflammation in sickle cell transgenic mice, associated with increased serum and broncoalveolar lavage (BAL) of TNF-α, IL-1, chemokines (KC and MIP-2) and sVCAM-1.
Aims: The aim of this study was to examine the contribution of the Rho kinase pathway to acute airway LPS inflammation in SCD using the sickle mice model.
Methods: Berkeley SCD mice bone marrow was transplanted into irradiated C57BL/6 to generate age- and gender-matched genetically-identical cohorts of SCD mice. Acute lung inflammation and injury were induced in female wild- type (WT) and SCD mice by intranasal administration of lipopolysaccharide (LPS, 50 µl of 250 µg/ml). The specific inhibitor of ROCK, Y-27632 (5mg/kg), dissolved in sterile saline was applied by intraperitoneal injection 1h before LPS instillation. The vehicle mice group received a similar volume of sterile saline. BAL was performed 5h (early response) and 24h (late response), after LPS challenge. qRT-PCR analysis was used to examine gene expression and ELISA was used to determine protein production.
Results: After 5h of LPS challenge in mice, a huge influx of leukocytes (neutrophils, NS) was triggered in BAL of WT and SCD mice, compared with the respective vehicle groups; however, this influx was greater in SCD mice, when compared with WT (9.3±2.9 vs 3.5±0.4 WBC (x10^6cell/mL); 6.5±2.7 vs 1.8±0.4 NS (x10^6cell/mlL; P<0.05, respectively; n=4). Pretreatment of SCD mice with Y-27632 significantly reduced the WBC and NS influx to the lung (1.9±0.2; 0.24±0.08 (x10^6cell/mL); P<0.05, respectively; n=4). Corresponding with influx of NS, lung lavage levels of KC, MIP-2 and TNF-α were significantly higher in SCD mice BAL compared to WT (1446±66 vs 469±0.9; 1893±390 vs 796±78; 396.9±68 vs 149.8±16.3;pg/ml, respectively; n=4). Y-27632 given 1h before LPS effectively reversed the LPS-induced increase in KC, MIP-2 and TNF-α BAL levels in SCD mice (68.01±21.2; 394.3±208; 128.8±85.7, pg/ml, respectively; n=4). Pre-treatment with Y-27632 was also able to significantly decrease TNF-α levels at 24h after LPS instillation in SCD mice (278.4±86.04 vs 61.9±10.76, pg/ml; n=4). Moreover, Rho-kinase inhibition significantly reduced lung gene expression of KC and TNF-α in SCD mice at 5h after LPS challenge (1.75±0.15 vs 0.28 ±0.14, 0.94± 0.22 vs 0.10±0.05, respectively; n=4).
Summary / Conclusion: In conclusion, these data indicate that the Rho-kinase signaling pathway may play a role in pulmonary infiltration of neutrophils via regulation of TNF-α and chemokine production in the acute lung injury induced by LPS in sickle cell mice. The findings from this study suggest that ROCK inhibition may have important implications for the control of the inflammatory response in the SCD.

S549

THE EFFECTS OF RPS19 KNOCKDOWN ON GATA1 EXPRESSION IN A CELL LINE MODEL OF DIAMOND BLACKFAN ANEMIA

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Background: Diamond Blackfan Anemia (DBA) is a rare bone marrow failure disorder characterised predominantly by severe erythroid failure. The causative mutation remains unknown in ~30% of patients, however a mutation or deletion of one of a number of ribosomal proteins (RPS or RPL) has been identified in the remaining ~70% of cases, leading to DBA being classified as a 'ribosomopathy'(Dianzani & Loreni Haematologica. 2008). While haploinsufficiency of RPs appears to be the major cause of DBA, a recent study (Sankaran VG, *et al*. J. Clin. Invest. 2012) reported mutations in the transcription factor GATA1, in the absence of any known RP mutation. While GATA1 mutations have only

been detected in two DBA pedigrees to date, this exception to the 'ribosomopathy' rule may help identify the link between ribosomal protein disruption and the tissue-specificity of the erythroid defect.
Aims: To investigate whether GATA1 (and selected downstream targets) are affected by RPS19 knockdown.
Methods: We have developed a Dox-inducible shRNA RPS19 knockdown model of DBA in the human erythroleukaemic cell line TF1.8. Two independent shRNA triggers targeting RPS19 and a vector control were introduced into cells andcultured in Tet-free media. Supplementation with 5IU/mL EPO was used to induce differentiation along the erythroid lineage.Q-RT PCR was used to measure expression of selected genes on days 0, 3, 5 and 7 of culture. All results are the combined average of 3 independent biological replicates and were normalised to β-actin. Flow cytometry analysis of GlyA and CD13 was also performed to monitor changes in erythroid and myeloid lineages.
Results: Q-RT PCR resultsconfirm RPS19 knockdown (Fig. 1A)and revealed decreased expression ofGATA1 followingRPS19 knockdown (Fig. 1B).The most striking affect on GATA1 can be seen at Day 3, where GATA1 levels are increasing in control cells, but decreasing in cells with the RPS19 shRNA constructs. Furthermore, we found that the expression of the GATA1 target gene, KLF1 (EKLF) is decreased in these cells compared to controls.
Flow cytometry analysis confirmed that the TF1.8 cells respond to EPO with a progressive increase in the percentage of GlyA positive cells and reduction of CD13. Interestingly, cells transduced with RPS19 shRNA do not differentiate to the same extent as control cells, with the proportion of GlyA+ cells decreased in cells with RPS19 knocked down.
Summary / Conclusion: We haveidentified that GATA1 mRNA is down-regulated in response toRPS19 knockdown in an inducible shRNA cell line model of DBA. Furthermore we demonstrate downstream effects on the key GATA1 target gene KLF1, consistent with a recent finding that EKLF (KLF1) mRNA levels were significantly decreased in the blood and bone marrow of DBA and 5q-syndrome MDS patients (Neuwirtova *et al.* Ann Hematol. 2013). Together these results suggest thatchanges to GATA1 (and downstream target gene) levels may be important not only in the rare cases of DBA where GATA1 is mutated, but also in classical DBA associated with ribosomal protein haploinsufficiency. Further studies are being undertaken to validate the effects of RPS changes on GATA1 and downstream genes. This will involve assays for GATA1 transcription factor activity, and assays in a DBA model based on CD34+ cord blood cells infected with our validated RPS19 knockdown constructs.

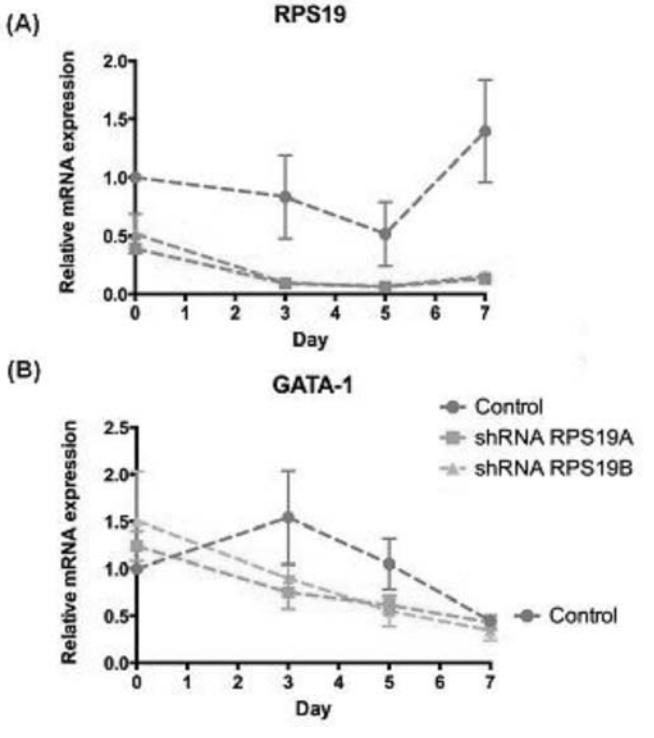

Figure 1 (A) Doxycycline-induced RPS19 mRNA knockdown with two independent RPS19 shRNA constructs compared to vector control in culture for 7 days with EPO supplementation. (B) The effect of RPS19 knockdown on GATA1 mRNA levels in the same cells.

Stem cell transplantation - Clinical 1

S550

INFLUENCE OF TWO DIFFERENT DOSES OF ANTITHYMOCYTE GLOBULIN ON CLINICAL OUTCOMES OF LEUKEMIA PATIENTS FOLLOWING HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION: RESULTS OF A RANDOMIZED TRIAL

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Background: Several previous studies have suggested the suitable dose of ATG in matched unrelated transplantation (MUD). But until now, we haven't known the optimal dose of ATG with respect to prevention of graft failure and severe GVHD in the haploidentical transplantation.
Aims: To evaluate the effect of the two different doses of antithymocyte globulin (ATG)in conditioning regimen on graft failure, incidence of acute graft-versus-host-disease (GVHD), relapse and survival among patients receiving hematopoietic stem cell transplantation (HSCT) without *ex vivo* T-cell-depletion (TCD) from haploidentical donors
Methods: Two hundred and twenty-four patients with leukemia in remission and with HLA haploidentical donors were randomized in this study. One hundred and twelve received 6mg/kg ATG while the others received 10 mg/kg ATG. In addition to major HSCT end-points being established, a comparative evaluation of incidence of infection after transplantation was performed. Informed consent was obtained. This study is registered with www.chictr.org, number ChiCTR-TRC-11001761.
Results: Groups were balanced for patient, donor, and transplant characteristics. Incidence of acute GVHD grades III-IV was higher in the ATG-6 group (12.5%) than that in the ATG-10 group (2.7%, P =0.006, 95 percent confidence interval for the difference, -16.6 to -3.0 percentage points). The cumulative rate of platelet engraftment was comparable between the two groups (day 100, 91.9% vs. 88.7%, P=0.19).
Higher dose of ATG required more corticosteroid for resolution of toxicity (P=0.001). EBV reactivation occurred more frequently in the ATG-10 group (25.3%) than in the ATG-6 group (9.6%), (P=0.001). The incidence of relapse was 7.6% in ATG-6 group and 4.6% in ATG-10 group, respectively (P=0.377) and the one-year DFS was 87.5% and 88.1% for ATG-6 group and ATG-10 group, respectively (P=0.877).
Summary / Conclusion: Although 6mg/kg ATG applied in conditioning regimen for haploidentical transplant recipients with non *in vitro* TCD grafts decreased risk of EBV reactivation comparing to 10 mg/kg ATG, it exposes patients to a higher risk for severe acute GVHD. Therefore, the results suggested that replacement of 10 mg/kg ATG with 6 mg/kg ATG should be cautious.

S551

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

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Background: Chronic graft versus host disease (cGvHD) remains the major cause of morbidity and mortality after allogeneic hemopoietic stem cell transplants. Identifying objective predictors on day +100, would allow the identification of patients at high risk of developing cGvHD and subsequent mortality.
Aims: We have tested whether chronic graft versus host disease (cGvHD) , transplant related mortality (TRM) and survival , can be predicted on day +100 by standard laboratory values.
Methods: The patient population consisted of 715 consecutive patients with hematologic disorders allografted between 2001 and 2011, alive on day +100 and free of cGvHD. All patients had laboratory values available on day +100 (±20 days). The donor was an HLA identical sibling (n=335) or an alternative donor (n=380). The median follow-up was 1741 days (range 116-4346). In multivariate Cox analysis, the predictive variables were platelet count (Plt), serum cholinesterase (CHE), gamma-glutamyltransferase (_GT), serum albumin (ALB) and serum immunoglobulin A. Median levels at day +100 were used as cut off and a score of 1 was given for Plt <110x10^9/L, CHE <3772 IU/mL, _GT >56 IU/L, ALB <3,93 mgr/dL and IgA <49 mg/dl.
Results: Results Two prognostic groups were identified: low risk (0-2 negative predictors)(n=497), and high risk (3-5 negative predictors) (n=218). The two risk groups identified patients at different risk of cGvHD (19% vs 37%,P=<0.0001) and of TRM (8% vs 31%, P<0.0001. The actuarial survival of the 2 groups at 10 years was 53% vs 38% (P<0.0001) and disease free survival 56% vs 36% (P<0.0001). In multivariate analysis, including donor/recipient age, female/male grafts, phase of disease, donor type, and year of transplant, the RR for patients at high risk on day+100 was 2.1 for overall mortality, 3.0 for cGvHD and 4.5 for TRM. Other significant predictors were disease phase (on TRM and survival) and alternative donors (on cGvHD). Causes of death in the low and high risk group, were as follows: leukemia (24% vs 26%), GvHD (4% vs 15%) and infetions (3% vs 15%).
Summary / Conclusion: Conclusions: The use of 5 simple laboratory tests (Plt, CHE, _GT, Albumin and IgA) on day +100 after an allograft, predicts cGvHD and mortality, irrespective of donor/patient and disease variables. Prospective trials to prevent cGvHD and mortality in high risk patients, may include immunemodulation with or without intensified infection prophylaxis.

S552

MESENCHYMAL STEM CELL AS A SALVAGE TREATMENT FOR SEVERE REFRACTORY ACUTE GRAFT-VERSUS-HOST DISEASE: A PILOT PROSPECTIVE STUDY

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Background: Refractory acute graft versus host disease (aGVHD) is one of the major causes of death after allogenetic haematopoietic stem cell transplantation (allo-HSCT).
Aims: This study evaluated the efficacy and safety of mesenchymal stem cells (MSCs) from the bone marrow of a third-party donor to refractory aGVHD.
Methods: Twenty-seven patients with refractory aGVHD were enrolled in this prospective study. These patients all failed to first-line treatment with corticosteroids and at least one second-line therapy. MSCs were given at a median dose of 1×10^6 cells/kg once weeklyuntil aGVHD was complete responseor for a total of 8 weeks. The response, infection and adverse events were recorded during treatment and follow-up.
Results: After a total of 65 doses of MSC were administered, with a median of 3 (range:1-9) doses per patient, the overall response rate for aGVHD was 74.1%_including complete response in 59.3% and partial response in 14.8%. The efficacy of MSCs to aGVHD was significantly related with timing of onset and type or severity of aGVHD at the initiation of MSC treatment. It had demonstrated that the main target organs (skin,gutand liver) of aGVHD were responsive to MSCs. Skin and liver were superiorto gut in the response to MSCs. In addition, we observed that 6 lung aGVHD patients were all responsive to MSCs. The incidenceof CMV and EBV infections, including viremia and diseases, were not different between aGVHD with MSCs and aGVHD without MSCs during MSCs treatment and follow-up (P=0.68). Tumor relapse were also not different between aGVHD with MSCs and aGVHD without MSCs (P=0.75). No short-term toxic side effects were observed and other secondary tumor occured after MSC treatment except for one EBV-associated PTLD. Compared with that before MSCs treatment, the proportion of $CD3^+CD4^+$_$CD3^+CD8^+$ was significantly higher at 4 weeks after treatment (P=0.06).The incidenceof chronic GVHD in aGVHD with MSCs is lower than that in aGVHD without MSCs (P=0.37).
Summary / Conclusion: The results concluded that MSCs derived from bone marrow of third-party donors are effective to treat refractory aGVHD. MSCs do not increase the risks of tumor relapse and infections.

S553

THE COMPARISON BETWEEN HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION AND UMBILICAL CORD BLOOD TRANSPLANTATION FOR TREATMENT OF HEMATOLOGICAL MALIGNANCES IN CHILDREN

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Background: Little information is available regarding the priority in selecting the alternative donors for childhood hematological malignances.
Aims: To compare the therapeutic effects of haploidentical hematopoietic stem cell transplantation (haplo-HSCT) and umbilical cord blood transplantation (UCBT) in childhood hematological malignances.
Methods: A total of 281 children received either single UCBT (n=37) or haplo-HSCT from a family donor (n=244) between January 2000 and December 2010 were enrolled. Long-term outcomes were reported and a risk-factor analysis was provided.
Results: Hematopoietic recovery was significantly faster in haplo-HSCT recipients compared with UCBT recipients (100-day myeloid engraftment: haplo-HSCT group, 99.59±0.01%, UCBT group, 86.49±0.35%, P < 0.001; 100-day platelet engraftment: haplo-HSCT group, 94.26±0.02%, UCBT group, 72.97±0.58%, P<0.001), whereas the incidence of graft-versus-host disease was higher in the haplo-HSCT group (100-day total acute GVHD: haplo-HSCT group, 71.72±0.08%, UCBT group, 51.35±0.70%, P = 0.015; 2-year total chronic GVHD: Haplo-HSCT group, 51.68±0.10%, UCBT group, 18.92±0.43%, P = 0.001). The haplo-HSCT recipients had better overall survival (OS: 65.44±0.09% versus 51.21±0.71%, P=0.044) and lower non-relapse mortality (NRM: 19.36±0.06% versus 37.98±0.66%, P=0.007) compared with UCBT recipients, and relapse and disease-free survival (DFS) rates did not differ significantly between the groups. Among high-risk patients, haplo-HSCT recipients had better OS and DFS rates compared with UCBT recipients (OS: 46.43±0.46% versus 16.67±4.07%, P=0.015; DFS: 42.29±0.46% versus 16.67±4.07%, P=0.032). Multivariate analysis showed that UCBT was associated with poorer OS (HR=2.63, 95% CI 1.52-4.55, P=0.001) and increased NRM (HR=2.50, 95% CI 1.37-4.55, P=0.003).
Summary / Conclusion: In summary, we firstly observed that haplo-HSCT can improve the outcomes of children with hematological malignances compared with single UCBT.

S554

IMPROVED SURVIVAL OF PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES ADMITTED TO AN INTENSIVE CARE UNIT: A PROSPECTIVE DUTCH NATIONAL INTENSIVE CARE EVALUATION

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Background: Improved survival rates for patients in the general intensive care unit (ICU) led to less reluctance in admitting those with haematological malignancies to the ICU.
Aims: The aim of this study was to explore trends in the characteristics, severity of illness, indications and outcomes over time of these patients.
Methods: Data of patients admitted from haematology to an ICU from 2004 till 2012 and registered in the Dutch National Intensive Care Evaluation (NICE) database were analyzed. Four strata were created: patients with leucocytes (L) $<1.0x10^9$, $\geq1.0x10^9$ and $\leq4.0x10^9$, $>4.0x10^9$ and $\leq12.0x10^9$ and $>12.0x10^9$ cells/L. The effect of the time periods was calculated in the different linear regression models using time, stratum classification and the interaction between stratum classification and time as covariates. When analyzing changes over time for length of stay (LoS) and severity of illness score, time in fractional years was used. The standardized mortality ratio (SMR, ratio between the observed number of deaths and the expected number of deaths) was employed to measure the outcome for mortality.
Results: Data of 2,414 first ICU admissions of patients with a haematological malignancy were available for analysis. The proportion of these admissions compared to all medical ICU admissions increased over time from 374 (1.75%) in 2004/5 to 460 (1.94%) in 2006/7 to 590 (2.10%) in 2008/9 and 781 patients (2.43%) in 2010/11 (P<0.01). This increase was observed in all strata except for patients with leucocytes $<1.0x10^9$. The median ICU-LoS for hospital survivors was 2.7 days [interquartile range (IQR) 1.0-7.5] and hospital-LoS was 23.0 days [IQR 11.0-40.7]. Both were different across the strata (Table 1, P<0.01) but did not change over time (P=0.56 for ICU-LoS and P=0.37 for hospital-LoS). The mean APACHE-II score on ICU admission was 24.2±9.3 and differed between the strata (table 1, P<0.01), but did not change over time (P=0.14).The calculated ICU SMRs decreased over time from 1.10 to 0.89 (L<1.0, P=0.03) and from 1.56 to 0.77 (L 1.0 to $4.0x10^9$, P=0.03).
Summary / Conclusion: Significantly more patients with haematological diseases were admitted to Dutch ICUs from 2004 to 2012. While their disease severity at ICU admission, as determined by APACHE-II, remained similar, ICU survival improved particularly for patients with leucocytes below 4. Based on these results, neutropenia is no longer an argument for not admitting patients with a haematological malignancy to the ICU.

Table 1. Length of stay, APCHEII severity of illness and standardized mortality ratios per stratum.

	Leucocytes $<1.0x10^9$	$1.0x10^9\leq$ Leucocytes $\leq4x10^9$	$4.0x10^9<$ Leucocytes $\leq12.0x10^9$	Leucocytes $>12.0x10^9$	*p*-value
LoS ICU[1]	3.7 [1.3-9.1]	2.6 [1.2-7.8]	2.7 [1.1-6.9]	3.4 [1.3-8.2]	<0.01
LoS hospital[1]	36.0 [21.0-52.4]	24.9 [11.0-42.3]	17.3 [9.0-36.0]	21.6 [11.0-39.5]	<0.01
APACHE II[2]	28.3 (±8.4)	25.7 (±9.0)	22.1 (±8.1)	25.5 (±8.5)	<0.01
SMR[3]	0.92 (0.80-1.05)	0.95 (0.81-1.11)	1.12 (0.99-1.26)	0.90 (0.80-1.00)	n.a.

[1] expressed as median number of days with interquartile range
[2] expressed as mean with standard deviation
[3] expressed as mean with 95% confidence interval
n.a. = not applicable

Therapeutic targets in acute leukemia

S555

IMMUNOPHARMACODYNAMIC RESPONSES FOLLOWING TREATMENT WITH THE BISPECIFIC T-CELL ENGAGER ANTIBODY BLINATUMOMAB AMONG PATIENTS WITH RELAPSED/REFRACTORY B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Blinatumomab (AMG 103) is an investigational, anti-CD19/anti-CD3, bispecific T-cell engager (BiTE®) antibody that induces target-cell dependent, polyclonal T-cell activation and proliferation resulting in targeted serial lysis of $CD19^+$ B-cells.
Aims: We sought to characterize the effects of blinatumomab on lymphocyte subpopulations and markers of T-cell activation in adult patients with relapsed/refractory B-precursor acute lymphoblastic leukemia (ALL) enrolled in a phase 2 study.
Methods: Adult patients (N=36) with relapsed/refractory B-precursor ALL received blinatumomab via continuous intravenous infusion for 28 days followed by a 14-day treatment-free interval. Patients could receive up to 5 treatment cycles. Whole blood and serum samples were collected at multiple time points during treatment. Changes in lymphocyte subpopulations, cytokines, granzyme B, and blinatumomab concentrations in the serum were evaluated. All patients provided written informed consent.
Results: The lymphocyte response to blinatumomab was similar in all patients. Peripheral B-cell counts dropped to ≤1 B cell/µL within the first week of infusion and remained undetectable for the duration of treatment. Peripheral T-cells disappeared within the first 2–6 hours of infusion but recovered to baseline within several days. Apart from this initial redistribution, T-cell counts remained at least stable in most patients throughout treatment. In some patients, even an expansion of both, $CD4^+$ and $CD8^+$, T-cell compartments occurred, likely due to proliferation of blinatumomab-activated T-cells; however, this expansion did not appear to be required for achieving a complete remission (CR). In the period immediately following initiation of blinatumomab infusion, markers indicative of T-cell activation were present: CD69 was transiently expressed on a significant proportion of T-cells within 48 hours, and peak granzyme B levels in the serum were detected approximately after 24 hours. In most patients, there was a transient elevation of cytokine levels in the serum dominated by interleukin-10, interleukin-6, and interferon-γ during the first 48 hours of the first cycle, but this response was diminished or absent in subsequent cycles. At doses of 5 and 15 µg/m²/d, respectively, mean (±SD) blinatumomab serum steady-state concentrations were 198 (±61) pg/mL and 694 (±236) pg/mL. These values were consistent with those from previous studies.
Summary / Conclusion: Key elements of the immunopharmacodynamic response to blinatumomab included B-cell depletion, T-cell activation and redistribution, and transient cytokine and granzyme B release. These responses were consistent with T-cell engagement via the established BiTE® mode of action. The pharmacodynamic markers evaluated did not allow for predictive differentiation between patients who achieved a hematologic response and those who did not.

S556

GLUCOCORTICOID INDUCED LEUCINE ZIPPER PROTEIN (GILZ) REGULATES HEMATOPOIETIC STEM CELL FUNCTION AND MYELOID DIFFERENTIATION IN A MOUSE MODEL OF CEBPA MUTANT ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) arises through the stepwise acquisition of genetic and epigenetic changes, recently evidenced in mouse model of biallelic *CEBPA* mutations. Hematopoietic stem cell (HSC) expansion precedes the formation of leukemia initiating cells with committed myeloid phenotype in mice bearing two types of patient-derived *CEBPA* alleles. C-terminal *CEBPA* mutations lead to a loss of HSC quiescence, expansion of pre-malignant pool of cells and accelerated AML, whereas N-terminal mutations provide necessary residual myeloid commitment capacity. Understanding the mechanisms underlying mutant HSCs proliferation and leading to leukemia progression is critical to combat leukemia and prevent tumor relapse. We have found that proliferating mutant HSCs downregulate Glucocorticoid Induced Leucine

Zipper protein (GILZ), suggesting that GILZ plays a role in normal and leukemic HSCs and regulates leukemogenesis.
Aims: We would like to address the role of GILZ in normal and aberrant hematopoiesis and its potential role in biology of AML using GILZ knock-out (KO) mice and *CEBPA* mutant mouse model of AML.
Methods: We have analyzed HSC and myeloid progenitors compartments in recently generated GILZ KO mice by multi-color flow cytometry. In addition, mice bearing GILZ KO alleles were crossed to *CEBPA* mutant allele bearing mice (N- and C- terminal mutations). Littermates heterozygous for both GILZ and *CEBPA* mutations (of *GILZ* +/- *CEBPA* N/+ and *GILZ* +/- *CEBPA* C/+ genotypes) were intercrossed and fetal livers with compound *CEBPA* N/C *GILZ* Y/- genotypes were collected and transplanted along with the wild type competitor bone marrow cells into lethally irradiated hosts. Effect of GILZ deficiency on *CEBPA* mutant HSC function, myeloid differentiation and long term leukemogenesis was followed overtime.
Results: Here we demonstrate that young GILZ KO mice have normal HSC and myeloid progenitors frequency and number. However, when combined with leukemogenic *CEBPA* mutations, GILZ deficiency dramatically affects the number of engrafting *CEBPA* mutant HSCs. Moreover, GILZ deficiency rescued the block of myeloid differentiation caused by biallelic *CEBPA* mutations, as normal frequency of Mac-1+ cells were produced by *CEBPA* N/C GILZ Y/- cells. This suggests that GILZ regulates the function of C/EBPa and/or C/EBP family members in normal and malignant myelopoiesis. Importantly, none of the mice transplanted with *CEBPA* N/C GILZ Y/- cells succumbed to leukemia over the 8-months period of the follow-up, despite their sustained presence in bone marrows and spleens.
Summary / Conclusion: These data suggest that GILZ deficiency rescues normal myelopoiesis in *CEBPA* mutant cells and therefore abrogates the leukemogenic function of *CEBPA* mutant proteins. Overall these data unravel a novel player in the regulation of normal and malignant myelopoiesis with a potential for therapeutic exploration.

S557

GENOME-SCALE EXPRESSION AND TRANSCRIPTION FACTOR BINDING PROFILES REVEAL THERAPEUTIC TARGETS IN TRANSGENIC ERG MYELOID LEUKEMIA

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Background: Aberrant expression of hematopoietic transcription factors perturbs normal hematopoiesis and may lead to the development of leukemia. The ETS transcription factor, ERG, plays a central role in definitive hematopoiesis and its overexpression in patients with acute myeloid leukemia (AML) is associated with a stem cell signature and bad prognosis. Yet how ERG causes leukemia is unclear.
Aims: To decipher the molecular pathogenesis of ERG driven acute myeloid leukemia and to identify therapeutic targets
Methods: Transgenic mice expressing the human ERG3 isoform under the pan-haematopoietic Vav promoter were created. Leukemias were characterized by flow cytometry, gene expression profiling and Chromatin Immunoprecipitation Sequencing (Chip-Seq) of ERG. Gene Set Enrichment Analysis (GSEA) was used to compare with human AML and Stem cell gene expression database. Enhancer analysis was done by transgenic reporter mice. Therapeutic experiments performed in-vitro and in-vivo in mice transplanted with transgenic leukemias.
Results: Levels of ERG expression in transgenic mice were similar to those observed in human AML and hematopoietic stem cells. ERG induced an early progenitor myeloid leukemia in transgenic mice, equivalent to the M0 phenotype observed in human AML with high ERG expression. Integrated genome-scale analysis of gene expression and ERG binding profiles, determined by ChIP-Seq, revealed that ERG activates a transcriptional program similar to human AML stem/progenitor cells and human AML with high ERG expression. We further show that ERG induces expression of the Pim1 kinase oncogene through a novel hematopoietic enhancer element that we further validated in transgenic reporter mice. Pharmacologic and molecular inhibition of Pim1 disrupted growth and induced apoptosis of ERG-driven leukemic cells. In addition, ERG indirectly induces the RAS pathway and direct RAS inhibition by a RAS inhibitor blocked growth of leukemia cells *in vitro* and *in vivo*.
Summary / Conclusion: ERG causes myeloid progenitor leukemia characterized by a direct induction of leukemia stem and progenitor cell transcriptional program. Pim-1 and the RAS pathway are potential therapeutic targets of these high risk leukemias.

S558

EFFICACY AND SAFETY OF QUIZARTINIB (AC220) IN PATIENTS AGE ≥60 YEARS WITH FLT3-ITD–POSITIVE RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA (AML)

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Background: Advanced age and FMS-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD) in acute myeloid leukemia (AML) are each associated with early relapse after standard chemotherapy and poor survival. Quizartinib (AC220), an oral FLT3 inhibitor active against ITD mutant and wild type FLT3, has shown promising activity in phase 1 and 2 studies.
Aims: To determine safety and confirmed rates of composite complete remission (CRc) and complete remission (CR) in an elderly population of FLT3-ITD–positive patients with relapsed/refractory AML treated with quizartinib monotherapy.
Methods: We conducted a phase 2 study to assess the efficacy and safety of quizartinib monotherapy in FLT3-ITD–positive and FLT3-ITD–negative patients in 2 cohorts (N=333). Subjects were tested by a central laboratory for the FLT3-ITD mutation with a >10% ratio of ITD to total FLT3 defined as positive. We present here data on a subset of 110 patients aged ≥60 years, which included 54 patients aged ≥70 years, with the FLT3-ITD mutation and AML relapsed in <1 year or refractory to first-line chemotherapy who were treated with quizartinib. Quizartinib was administered once daily as an oral solution during 28-day treatment cycles. The initial starting dose in the first 17 patients was 200 mg/day, but because of the occurrence of QT interval prolongation, the dose was reduced to 135 mg/day for men and 90 mg/day for women. The CRc rate included CR, CR with incomplete platelet recovery (CRp), and CR with incomplete hematologic recovery (CRi).
Results: Efficacy results are summarized in the Table 1. Median duration of treatment for patients ≥60 years of age was 14.1 weeks (range 0.1–70.6 weeks), and 63 (57%) had a CRc (3 CR, 4 CRp, 56 CRi). For patients aged ≥70 years, median duration of treatment was 14.9 weeks, and 31 (57%) had a CRc (1 CR, 3 CRp, 27 CRi). Median overall survival (OS) in patients ≥60 years of age was 25.3 wks and 16 (15%) survived >52 weeks. The median age of the patients surviving >52 weeks was 69.5 years (range 66–80 years) and median time on quizartinib was 52.1 weeks (range 6.6–70.6 weeks). All of these patients responded to quizartinib (2 CR, 2 CRp, 8 CRi, 4 partial remission). Two patients were still alive at last follow up with survival of more than 1.5 years (OS 93.0 and 96.0 weeks). The median survival of subjects that survived for more than 28 days and achieved a CRc or PR was 31.1 weeks compared to 11.8 weeks for non-responders. The most common (≥10%) Grade 3 or 4 treatment-related adverse events (TRAEs) were anemia (25%), febrile neutropenia (22%), thrombocytopenia (12%), transient QT interval prolongation (10%; no Grade 4 events), and neutropenia (10%). 20 patients (18%) had TRAEs resulting in discontinuation.
Summary / Conclusion: The data presented in this abstract show that elderly FLT3-ITD–positive patients treated with quizartinib, even those aged ≥70 years, with chemotherapy-resistant AML, have preserved high response rates, and promising survival.

Table 1. Efficacy in relapsed/refractory FLT3-ITD-positive AML patients aged ≥60 years and ≥70 years.

	FLT3-ITD–positive AML patients	
	≥60 years (N=110)	≥70 years (N=54)
Cumulative CRc, n (%)	63 (57)	31 (57)
CRc+PR, n (%)	86 (78)	39 (72)
Median CRc duration, weeks (95% CI)	12.1 (6.3, 15.7)	13.9 (8.0, 16.5)
Median OS, weeks (95% CI)	25.3 (21.3, 30.0)	22.7 (17.0, 26.8)

CRc=composite complete remission; OS=overall survival; PR=partial remission.

S559

MCLA-117: A CLEC12AXCD3 BISPECIFIC IGG TARGETING A NOVEL STEM CELL ASSOCIATED ANTIGEN IN AML

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Background: Although the current intensive chemotherapies to treat acute myeloid leukemia (AML) induce complete remission in most AML patients, the long term prognosis of these patients is very poor. Therefore, novel targeted therapies with potent mechanisms of action that attack the tumor cells and their progenitor cellsare much needed. CLEC12A is a myeloid differentiation antigen that is expressed on 90-95% of denovo and relapsed AML and is selectively expressed on leukemic stem cells. Bispecific antibodies can be used to redirect T cells to tumor cells for efficient tumor killing. A T cell-redirecting bispecific antibody binds and activates the T cells with one arm while the second arm binding to CLEC12A directs the cytotoxic T cells to the tumor cells.
Aims: We aimed to develop a human bispecific IgG antibody that targets CD3 expressed on T cells and CLEC12A expressed on AML blasts and residual leukemic stem cells.
Methods: γ
We have developed MCLA-117 which is a highly potent human CLEC12AxCD3 bispecific IgG antibody binding to CD3 on T cells and CLEC12A expressed on AML blasts and residual leukemic stem cells. MCLA-117 was selected from panels of common light chain human monoclonal antibodies derived from the MeMo® transgenic mouse. To produce the bispecific MCLA-117 antibody in the absence of contaminating monospecific antibodies, minor amino acid changes in the CH3 domain of the heavy chain constant region were introduced to drive efficient heterodimerization resulting in production of >95% pure bispecific antibodies by transfected cells.
Results: Co-incubation of resting patient T cells and AML tumor cells with MCLA-117 full length IgG bispecific antibody resulted in efficient tumor cell lysis. An initial version of the CLEC12AxCD3 bispecific IgG induced the release of non-specific pro-inflammatory cytokines IL-2, IFNγ and TNFα via binding of its Fc-tail to activating Fcγ receptors. Therefore, we introduced mutations in the heavy chain constant region CH2 domain to abrogate Fc receptor binding to prevent unwanted cytokine release while retaining binding to FcRn for long half life. Indeed, MCLA-117 antibody with silenced CH2 domains was shown in PBMC assays to prevent the release of non-specific pro-inflammatory cytokines, while retaining its full capacity to induce T cell mediated lysis of AML tumor cells.
Summary / Conclusion: MLCA-117 is a highly potent bispecific antibody that induces T cell mediated lysis of AML tumor cells by targeting the CLEC12A antigen on AML blasts and residual leukemic stem cells. Clinical application of the MCLA-117 product candidate potentially provides a therapy in AML that more efficiently eradicates the cancer cells and prevents relapse.

Granulocytes

S560

HEMATOPOIETIC-SPECIFIC LYN-SUBSTRATE 1 (HCLS) IS DEACETYLATED BY NAMPT/NAD+/SIRT1 PATHWAY

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Background: Recently we identified HCLS1 protein to be an essential player in the G-CSFR signalling pathway in myeloid cells (Skokowa et al., 2012). We demonstrated that upon G-CSF stimulation, phosphorylated and activated HCLS1 together with its binding partner HAX1 binds to LEF-1 transcription factor leading to LEF-1 activation and subsequently myeloid differentiation. In patients with severe congenital neutropenia (CN) harbouring HAX1 mutations, HCLS1 expression and functions are severely downregulated, leading to "maturation arrest" of myelopoiesis. We also described a new G-CSFR signalling through activation of Nicotinamide phosphoribosyltransferase (NAMPT)/NAD^+/Sirtuins in healthy individuals and in CN patients. We found that G-CSF-triggered deacetylation of myeloid specific factors is essential for myeloid differentiation (Skokowa *et al.*, 2009).
Aims: In the present study we aimed to investigate whether HCLS1 is regulated by NAMPT/NAD^+/Sirtuin pathway via deacetylation.
Methods: We identified four acetyl-lysine sites in HCLS1 protein and generated rabbit polyclonal antibodies specifically recognized each of acetylated lysines for western blot. We also generated lentiviral constructs contained HCLS1 cDNA in which one of acetylated lysines of HCLS1 protein is replaced by a single amino acid.
Results: Using immunoprecipitation with anti-acetyl-lysine antibody we found that HCLS1 is acetylated in HL60 myeloid cells upon pre-treatment of cells with histone deacetylase inhibitors. We also showed that treatment of HL60 and NB4 cells with high doses of Nicotinamide but not Trichostatin A enhanced HCLS1 acetylation on lysine 123 and 241, suggesting that class III histone deacetylases (Sirtuin family) could deacetylate HCLS1 protein.
We further found that NAD^+ and NAMPT treatment of the acute myeloid leukemia cell line HL60 decreased HCLS1 acetylation on both lysines 123 and 241. Similar effects were observed in HL60 cells transduced with lentiviral construct expressing NAMPT cDNA. Inhibition of NAMPT using specific inhibitor FK866 increased HCLS1 acetylation on acetyl-lysines 123 and 241 in NB4 and on acetyl-lysine 241 in HL60 cells. To evaluate the mechanism of NAMPT-dependent deacetylation of HCLS1, we performed immunoprecipitation experiments in cell lysates of HL60 cells using anti-SIRT1 antibody and found interaction between endogenous HCLS1 and SIRT1 proteins. Moreover, interaction between HCLS1 and SIRT1 proteins were detected by immunoprecipitation in 293T cells over-expressing HCLS1 and SIRT1.
Summary / Conclusion: Taken together, we concluded that HCLS1 is deacetylated through NAMPT/NAD^+/SIRT1 pathway and that in patients with severe congenital neutropenia not only diminished expression and phosphorylation but also elevated NAMPT-triggered deacetylation may contribute to the neutropenic phenotype.

S561

PRESENCE OF PERIPHERAL BLOOD CELLS WITH PNH PHENOTYPE IN PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA

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Background: Chronic idiopathic neutropenia (CIN) is a disorder of granulopoiesis characterized by increased apoptosis of the granulocytic progenitor cells and presence of activated, oligoclonal T-lymphocytes with myelosuppressive properties in the peripheral blood (PB) and bone marrow (BM). Based on the existing evidence, we have suggested that CIN represents the mild form of the spectrum of immune-mediated BM failure syndromes.
Aims: To probe the hypothesis that similar to other BM failure syndromes, i.e. aplastic anaemia and myelodysplatic syndromes, CIN patients display PB cells with PNH phenotype.
Methods: We have studied 102 patients fulfilling the diagnostic criteria for CIN and 43 age- and sex-matched healthy individuals. All patients had absolute neutrophi counts lower than 1800/μl (White) or 1500/μl /ml (Black) for more than three months, had no evidence of any underlying disease that might be associated with neutropenia, no history of exposure to irradiation, use of chemical compounds or intake of drugs to which neutropenia might be ascribed, normal BM karyotype and negative anti-neutrophil antibody activity. Flow cytometry was performed for the identification of peripheral blood erythrocytes, granulocytes and monocytes displaying PNH phenotype. Specifically, the CD59dim (type II) and CD59negative (type III) PNH red cells were evaluated within the Glycophorin A (CD235) positive cell fraction. The FLAER negative PNH gran-

ulocytes and monocytes were assessed within the CD24 and CD14 cell fraction, respectively.
Results: The proportion of type II and type III PNH red cells were significantly increased in CIN patients (0.0587±0.337 and 0.0282±0.094, respectively) compared to healthy individuals (0.0064± 0.0067 and 0.0030±0.0021, respectively) (P<0.0001 and P=0.0088, respectively). Similarly, the proportion of FLAER negative (PNH phenotype) monocytes were significantly increased in CIN patients (1.47±1.795) compared to healthy controls (0.1651±0.173) (P<0.0001). No statistically significant difference was identified between patients and controls in the proportion of PNH granulocytes .
Summary / Conclusion: Patients with CIN display increased proportion of PNH red cells and monocytes in the PB. These data support further the concept that CIN shares common pathogenetic features with immune-mediated BM failure syndromes.

S562

RISK OF TRANSFORMATION TO MYELODYSPLASIA/LEUKAEMIA AND SEPSIS/DEATH IN PATIENTS AFFECTED WITH SEVERE CONGENITAL NEUTROPENIA:DATA FROM THE ITALIAN NEUTROPENIA REGISTRY (INR)
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Background: Severe Congenital neutropenia (SCN) is a disease characterized by persistent absolute neutrophil count below 500/mmc, early onset of bacterial infections and block of maturation of neutrophils in the bone marrow. SCN is a premalignant disorder. Granulokine-colony-stimulating factor (G-CSF) has radically changed the prognosis of these patients conferring defence against infections, but it probably favours the process of transformation to myelodysplasia (MDS)/leukaemia (AL). The cumulative risk of sepsis/death and transformation to MDS/AL is different according to the cohort studied (Severe Congenital Neutropenia International Registry-SCNIR, French registry and the Swedish Cohort)
Aims: To describe the cumulative incidence of MDS/AL and sepsis causing death in the cohort of SCN patients enrolled in the INR.
Methods: Anagraphical and historical data (date of birth, gender, date of emergence of neutropenia, date of diagnosis, date of last follow up) as well as data on bone marrow morphology, type of molecular mutation, presence of G-CSF mutation, G-CSF dose, number and type of infections, cause of death if any, were extracted from the INR.
Results: From June 2003 to December 2012, among 306 registered in INR, 22 patients were diagnosed with SCN and considered eligible for the study. Nine out of 22 (41%) were females and 13/22 (59%) were males; median aged, at last follow up, 6,3 years (range 0,5-40,2). The patients were diagnosed with SCN at a median age of 9 months (range 0-418). All subjects had the maturative arrest at promyelocyte or myelocyte stage. In the cohort, 16/22 (72%) carried the ELANE mutation, 2/22 (9%) were HAX1 mutated and the remaining 4/22 (18%) had no known mutation. All subjects were treated with G-CSF started at a median age of 9 months (range 0,5-418). Median G-CSF dose exposure was ≤5 mcg/kg/day in 10/21(48%), 6-10 mcg/kg/day in 5/21 (24 %) , 11-19 mcg/kg/day in 3/21 (14%) and ≥20 mcg/kg/day in the remaining 3/21 (14 %). In the cohort none developed AL, while one patient/22 (4%) progressed to myelodysplasia after 4 years of G-CSF treatment at dose of 5 mcg/kg/day. Four out of 22 pts (18%) died , 2 patients for sepsis during follow-up and the other 2 for transplant related causes (infection and acute GVHD). The two sepsis/death events before transplantation were related to absent compliance to G-CSF. The cumulative incidence of MDS/AL trasformation was of 6% at 4 years after the beginning of G-CSF and the probability to develop MDS was 8% at 8 years of life. The cumulative risk of sepsis causing death was 10% after 3 years after the beginning of G-CSF ; the risk to die for sepsis was calculated as 10% at 3,5 years of life.
Summary / Conclusion: The cumulative incidence of MDS in our cohort is lower than the one calculated in the Swedish, French and in the SCNIR cohort mainly after 10-15 years of follow up. This might be due to younger and smaller Italian cohort if compared at least with the french and the SCNIR ones. The incidence of sepsis/death is almost comparable with the French and the SCNIR cohort. The explanation of the deaths might be caused mainly to low/absent compliance to G-CSF therapy. Early diagnosis, amelioration of compliance to therapy and very strict monitoring of clinical status and bone marrow features might reduce these complications in SCN population.

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ACETYLATION OF LEF-1 TRANSCRIPTION FACTOR REGULATING HEMATOPOIETIC DIFFERENTIATION
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Results: Previously we identified that NAMPT is essential for the G-CSF –triggered granulocytic differentiation via a NAD^+- sirtuin-1-dependent pathway. Sirtuins are NAD+-dependent protein deacetylases and we found that myeloid-specific transcription factors C/EBPaand C/EBPß are deacetylated and activated by SIRT1. We and others demonstrated, that lymphoid enhancer-binding factor 1 (LEF-1) transcription factor is crucial for neutrophil granulocytopoiesis and lymphopoiesis. LEF-1 mediates the proliferation, survival and differentiation of granulocyte and lymphocyte progenitor cells by binding and activation of cell type-specific transcription factors. LEF-1 expression was severely diminished in myeloid progenitor cells of severe congenital neutropenia patients, who show "maturation arrest" of granulopoiesis. NAMPT, NAD+ and SIRT1 levels were elevated in myeloid cells and in plasma of severe congenital neutropenia patients, demonstrating hyperactivation of this pathway. To evaluate the mechanism of NAMPT-triggered granulocytic differentiation, we analysed whether LEF-1 could be de-/acetylated. Immunoprecipitation with acetylated-Lysine Ab in Jurkat cells showed acetylated form of LEF-1 protein. Moreover, by means of immunoprecipitation with SIRT1 antibody we found interaction between LEF-1 and SIRT1. We further identified lysine residues in LEF-1 protein, which could be acetylated and generated specific rabbit polyclonal antibody specifically recognized acetyl-Lys of the LEF-1 protein. Western blot experiments using these antibody confirmed acetylation of LEF-1 protein on the particular lysine residue. To study the involvement of NAMPT in the deacetylation of LEF-1 protein we treated Jurkat cells with human recombinant NAMPT for 24 hr. Indeed, we found significantly decreased levels of acetylated LEF-1 protein and elevated mRNA expression of LEF-1 after treatment with NAMPT. At the same time, inhibition of NAMPT using specificinhibitor FK866 resulted in increased levels of acetylated LEF-1 protein after 24 hr of FK866 treatment. This was in line with elevated mRNA expression levels of LEF1 (2 folds) and its target genes Survivin (3 folds), c-myc (2 folds), HCLS1 (1,5 folds) and Cyclin D1 (1,5 folds) after 48 hours of FK866 treatment. Further we analysed whether NAMPT effects on LEF-1 trans-activating functions are through direct acetylation of LEF1 protein itself. We made LEF-1 mutant with a single amino acid substitution, which removes lysine acetylation site in the LEF1 protein and performed reporter gene assay using TOP promoter construct. TOP promoter construct contains six LEF-1/TCFs binding sites and luciferase promoter. We found that if acetylation is removed, LEF-1 protein activated TOP promoter 17- 26 fold stronger, as compared to WT LEF-1. This data shows that LEF-1 binding and activation at LEF-1/TCFs binding sites of regulatory DNA is due to direct changes of acetylation of the LEF1 protein itself. Hence, deacetylation of LEF-1 might be crucial process in the mechanism of NAMPT-triggered granulocytic differentiation and might be an important part of the pathomechanism of neutropenia.
In conclusion, in patients with congenital neutropenia, LEF-1 is severely downregulated and the remaining LEF-1 protein is deacetylated. LEF-1 is therefore physically and functionally missing.

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THYROID DISEASE-ASSOCIATED NEUTROPENIA: FREQUENCY, CLINICAL, LABORATORY AND IMMUNOPHENOTYPICAL FINDINGS
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Background: Granulopoiesis abnormalities have been described in association with thyroid disease. However data regarding systematic evaluation of adult neutropenia and concurrent or prior thyroid disease are scarce.
Aims: To investigate the frequency of thyroid disorders among patients presenting with neutropenia, to evaluate possible differences in peripheral lymphocyte subsets in neutropenic patients with thyroid diseases, as well as their immunological profile.
Methods: We retrospectively analyzed the clinical and laboratory findings of 218 consecutive patients who presented with neutropenia to the outpatient

Hematology Clinic of our Department between 2005 and 2008. Laboratory evaluation included CBC, blood smear, chemistries, folate, vitamin B12 and iron status, serology for HBV, HCV, HIV, EBV and toxoplasmosis, ANA, anti-DNA Abs, RF, complement factors C3 and C4, thyroid function tests (FT3, FT4, TSH, anti-TPO Abs, anti-TG Abs, TRAbs), and antineutrophil antibodies (anti-PMN Abs) by GIFT, GAT and LIFT methodology. Furthermore immunophenotype of blood lymphocytes was performed by three-color flow cytometry.
Results: Among 218 patients with neutropenia, 95 had thyroid disease (43.5%), 65 chronic immunologic neutropenia, 33 T-large granular lymphocytosis, 11 autoimmune disorders and 14 other diagnoses. Patients with thyroid disorders had an increased frequency of recurrent infections compared to other patients. (P=0.04). The following significant correlations were found: a. negative correlation between FT3 levels and absolute neutrophil counts (ANC) (r=-0.274, P=0.007), b. negative correlation between T4 levels and absolute CD4 counts (r=-0.274, P=0.02), c. positive correlation between TSH levels and absolute CD4 counts (r=-0.16, P=0.05), d: negative correlations between TPO-Abs and TG-Abs levels and C4 levels (r=0.16, P=0.05; r=0.266, P=0.001). In addition CD2+ and CD4+ lymphocyte subsets were significantly higher in patients with thyroid disease compared to patients with normal thyroid function (P=0.05, P=0.002). Among the 95 neutropenic patients with thyroid disease, 51 had Hashimoto thyroiditis (HT, 23.4%), 9 Graves' disease (GD, 4.1%), 6 total thyreoidectomy associated with nodules (TTM, 2.8%), 18 non-toxic multinodular goiter (NTMG, 8.2%) and 11 hypothyroidism (HP, 5%). Patients with autoimmune thyroid disease had an increased frequency of concomitant autoimmune cutaneous or systemic manifestations (P=0.03). Among the various thyroid disorders there was a statistically significant difference in the distribution of CD20+ lymphocytes (P=0.038): HT patients had an increase in the percentage of B-lymphocytes, while the opposite was evident for the TTM-subgroup. In patients with GD an increase of the proportion of NK cells and a decrease in the percentage of TCRγδ+ lymphocytes were noted (P=0.05, P=0.005). Patients with NTMG had significantly higher ANC (P=0.004) compared to other thyroidopathies. Anti-PMN Abs were found in 37.2% of thyroid disorders. A positive correlation between anti-PMN Abs titers and anti-TPO levels was evident (P<0.05).
Summary / Conclusion: The frequency of thyroid disease among neutropenic patients may be higher than previously reported. The existence of anti-PMN antibodies, as well as the different distribution of lymphocyte subsets among patients with different thyroid diseases indicates both humoral and cellular mechanisms in the pathophysiology of thyroid disease-associated neutropenia.

Presidential Symposium

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COMBINED HAPLOINSUFFICIENCY OF SYNCRIP/HNRNP-Q AND SNHG5/SNORNAS IN DELETION 6Q PROMOTES T-CELL ACUTE LEUKEMOGENESIS

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy characterized by leukemic transformation of T-cell progenitors. Large deletion of chromosome 6q (del6q) is a frequent abnormality in T-ALL, the oncogenic target of which remains elusive.
Aims: To identify the oncogenic molecular target(s) of the deletion 6q in T-ALL.
Methods: We used integrated genomics tools including array-CGH, next-generation sequencing (NGS) and gene expression profiling on French and Dutch T-ALL cohorts to search for candidate genes. For in vivo experiments, genes were silenced using lentiviraly transduced short hairpin RNA in transgenic Tal1Lmo1 mouse cells and in human primary T-ALL cells, then cells were injected to *Rag*$^{-/-}$*γc*$^{-/-}$ mice and NSG mice, respectively, and the mice were monitored for the onset of an overt T-ALL.
Results: Array-CGH analysis in a first cohort of primary T-ALL cases showed that del(6q) was highly associated to TAL1-expressing T-ALL. The deletion was monoallelic in all cases, interstitial, often subclonal, and mapped to 6q14. Using a custom tiling array designed for 6q14 region in 86 TAL1-related cases from three T-ALL cohorts, we focused on a common deleted region of 4.9 Mb. This region was sequenced using NGS technologies in 6q deleted or non-deleted cases. Two contiguous genes, namely *SYNCRIP* and *SNHG5*, were found to be altered in two otherwise "non-deleted" cases. *SYNCRIP* (encoding hnRNP-Q) and *SHNG5* (a non-protein-coding gene that hosts snoRNAs) are both involved in the regulation of RNA maturation and protein translation. In parallel, gene expression profiling study identified a del(6q) signature in which *SYNCRIP* appeared as the most relevant gene considering its localization in the common deleted region and expression levels throughout normal thymic differentiation. Altogether, *SYNCRIP* and *SHNG5* were two strong candidates to be considered as haploinsufficient tumor suppressor genes involved in T-ALL oncogenesis. We then functionally tested the involvement of these two genes in T-ALL acceleration models. In a first model, transgenic *Tal1Lmo1* cells were injected to *Rag*$^{-/-}$*γc*$^{-/-}$ recipient mice after lentiviral silencing of either *Syncrip*, *Snhg5*, or both genes, together with the retroviral activation of Notch1. Strikingly, the combined silencing of both *Syncrip* and *Snhg5* genes, but not from each gene isolated, accelerated the onset of an overt T-ALL. In a second model, the combined silencing of *SYNCRIP* and *SNHG5* in primary T-ALL cells from a *SIL-TAL* patient significantly increased the leukemia initiating activity when the cells were injected into immunodeficient NSG mice. Gene expression profiling of the resulting T-ALLs showed a Gene Ontology signature of splicing, ribosomal and mitochondrial pathways.
Summary / Conclusion: Using integrated genomics and functional models, we have identified two genes at 6q14 which are recurrently co-deleted in the TAL1-related T-ALL subtype, resulting in leukemia acceleration.

S566

GENETIC BASIS OF MYELOID LEUKEMOGENESIS IN DOWN SYNDROME

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Background: Transient abnormal myelopoiesis (TAM) is known as a clonal myeloid proliferation affecting ~10% of neonatal infants with Down syndrome (DS). Although spontaneous regression is as a rule in most cases, about 20-30% of the survived infants develop acute megakaryoblastic leukemia (AMKL) years after the remission. As for the molecular pathogenesis of TAM and DS-AMKL, it has been well established that *GATA1* mutations are detected in virtually all TAM cases as well as DS-AMKL. However, it is still open to question whether a *GATA1* mutation and trisomy 21 are sufficient for the development of TAM, what is the cellular origin of the relapsed AMKL and whether additional gene mutations are required for the progression to AMKL.
Aims: The purpose of this study is to identify the spectrum of gene mutations in TAM/AMKL cases and intra-tumor heterogeneity and clonal evolution patterns in TAM/DS-AMKL using high-throughput sequencing.
Methods: We performed a comprehensive analysis of somatic mutations in TAM/AMKL cases using whole genome sequencing of four trio samples obtained at the initial presentation of TAM, during remission and at the relapse phase of AMKL. Subsequently, deep resequencing was performed to accurately determine the allele frequencies of each detected single nucleotide variant. Whole exome sequencing was also performed for TAM (N=15) and DS-AMKL (N=14) samples (discovery cohort). The recurrent mutations in the discovery cohort, in addition to known mutational targets in myeloid malignancies, were further screened in an extended cohort of TAM (N = 41) as well as DS-AMKL (N = 49) and non-DS-AMKL cases (N=19).
Results: Whole genome sequencing and subsequent deep sequencing of the individual mutations unmasked detailed clonological pictures of clonal evolution/expansion leading to AMKL. Intratumoral heterogeneity was evident at the time of initial diagnosis of TAM and also present in AMKL in all cases. In three patients, relapsed AMKL evolved from one of the major subclones in TAM phase with a shared *GATA1* mutation, as previously reported in relapsed AML in adults. On the other hand, one patient showed a unique pattern, in which the relapsed AMKL originated from a minor subclone in TAM phase that was totally unrelated to the predominant clone, carrying an independent *GATA1* mutation. DS-AMKL samples had significantly higher numbers of somatic mutations compared to TAM samples with the mean numbers of nonsynonymous mutations of 5.8 and 1.7 per sample in AMKL and TAM cases, respectively (P=0.0002). While *GATA1* was the only recurrent mutational target in the TAM phase, 8 genes were recurrently mutated in AMKL samples in whole exome sequencing, including *NRAS*, *TP53* and other novel gene targets. Targeted sequencing revealed secondary mutations other than *GATA1* mutations in 42 out of 49 (85.8%) DS-AMKL, 5 out of 41 (12.2%) TAM and 11 out of 19 (57.9%) non-DS-AMKL cases. RAS pathway and Tyrosine kinases/cytokine receptors mutations were confirmed in 8 and 17 out of 49 DS-AMKL cases, respectively. Lower allelic burden than corresponding *GATA1* mutations indicated that they were more likely to be subclonal mutations.
Summary / Conclusion: TAM is characterized by a paucity of somatic mutations and thought to be virtually caused by a *GATA1* mutation in combination with constitutive trisomy 21. We found two major clonal evolution patterns during DS-AMKL relapse. Secondary mutations involving genes such as RAS pathway genes, tyrosine kinase and epigenetic regulators play a major role in clonal evolution into DS-AMKL.

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OBINUTUZUMAB (GA101) OR RITUXIMAB (R) + CHLORAMBUCIL (CLB) VERSUS CLB ALONE IN PATIENTS WITH CLL AND PRE-EXISTING MEDICAL CONDITIONS (COMORBIDITIES): FINAL STAGE 1 RESULTS OF THE CLL11 PHASE 3 TRIAL

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Background: Chemoimmunotherapy (CIT) is standard of care in young and physically fit patients (pts) with CLL. Development of CIT for older and less fit CLL pts is ongoing, but data from phase 3 trials are sparse.
Aims: CLL11 is the largest trial to evaluate 3 treatments in previously untreated CLL pts with comorbidities: Clb alone, GA101 + Clb (GClb), R + Clb (RClb). The final analysis of CLL11 stage 1 efficacy and safety results is presented here.
Methods: Treatment-naïve CLL pts with a Cumulative Illness Rating Scale (CIRS) total score >6 and/or an estimated creatinine clearance (CrCl) <70 mL/min were eligible. Pts received Clb alone (0.5 mg/kg po d1, d15 q28 days, 6 cycles), GClb (100 mg iv d1, 900 mg d2, 1000 mg d8, d15 of cycle 1, 1000 mg d1 cycles 2-6), or RClb (375 mg/m^2 iv d1 cycle 1, 500 mg/m^2 d1 cycles 2-6). Primary endpoint was investigator-assessed progression-free survival (PFS).
Results: Median age, CIRS score, and CrCl at baseline were 73 years, 8, and 61.1 mL/min for stage 1a (Clb vs GClb, 356 pts) and 73 years, 8, and 62.1 mL/min for stage 1b (Clb vs RClb, 351 pts, triggered by a different event rate). Key efficacy and safety results are below. Grade 3-4 infusion-related reactions with GClb occurred at first infusion only. Management required splitting the first dose over 2 days.
Summary / Conclusion: CIT with GClb or RClb significantly prolongs PFS vs Clb alone. The results demonstrate that GClb and RClb are very active in CLL and superior treament options in this population. GClb vs RClb will be compared in stage 2 analysis with more follow-up available.

Table 1.

Total Stage 1 N=589	Stage 1a		Stage 1b	
	Clb N=118	GClb N=238	Clb N=118	RClb N=233
Median observation time, months	13.6	14.5	14.2	15.3
Overall response rate, %	30.2	75.5	30.0	65.9
Complete responses, %	0	22.2	0	8.3
Median PFS, months	10.9	23.0*	10.8	15.7
HR, CI, *p*	0.14, 0.09-0.21, <.0001		0.32, 0.24-0.44, <.0001	
Grade 3-5 adverse events during treatment, %	41	67	41	46
Infusion-related reaction	-	21	-	4
Neutropenia	15	34	15	25
Infections	11	6	11	8

(* still immature, < 20% at risk at time of median)

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JAK-STAT PATHWAY IN MALIGNANT AND NON-MALIGNANT CELLS CONTRIBUTES TO MPN PATHOGENESIS AND THERAPEUTIC RESPONSE

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Background: Myeloproliferative neoplasms (MPN) are clonal blood disorders characterized by excessive production of mature blood cells. Patients present with high levels of circulating pro-inflammatory cytokines that contribute to MPN-associated symptoms and sequelae. The identification of somatic mutations in *JAK2*, *MPL* and *LNK* in the majority of MPN patients has underscored the importance of JAK-STAT signaling to MPN pathogenesis and led to the development of JAK kinase inhibitors. JAK1/2 inhibitors improve splenomegaly and systemic symptoms, but thus far, have not been shown to reduce or eliminate the malignant clone. It is unclear if the rapid reduction in spleen size and improvement in constitutional symptoms upon JAK kinase inhibition are due to JAK2 inhibition in MPN cells, in non-malignant cells, or targeting of the JAK-STAT pathway in both populations.
Aims: To assess the contribution of JAK-STAT signaling to inflammatory signaling in MPN cells and in non-clonal hematopoietic cells and the effects of JAK1/2 inhibition on cytokine production in clonal and non-clonal populations.
Results: To identify cytokines altered in PMF, we measured serum cytokine levels in MPLW515L engrafted mice and in control mice. We found that a set of cytokines, including Il6 and Cxcl9, were markedly elevated in the serum of diseased mice compared to control mice and importantly, many of these same cytokines are elevated in PMF patients. Further, the JAK1/2 inhibitor ruxolitinib normalized aberrant cytokine levels in PMF mice, consistent with the anti-inflammatory effects of ruxolitinib seen in the clinical setting.
Analysisof cytokine expression levels in sorted mutant and non-mutant cells showed that MPLW515L-mutant cells highly express Il6 and Cxcl9, consistent with tumor derived cytokine production. By contrast, other elevated cytokines (Ccl5, Cxcl9) are largely derived from non-clonal hematopoietic cells. Ruxolitinib treatment normalized cytokine production from both cell populations, in line with an effect of JAK inhibition in malignant and non-malignant cells *in vivo*.
STAT3 is known to contribute to cytokine production in different malignant and inflammatory contexts, but the role of STAT3 signaling in MPN initiation, inflammatory signaling and progression remains unclear. Hematopoietic-specific Stat3 deletion improved survival, reduced disease severity, and reduced cytokine-mediated inflammation in the MPLW515L bone marrow transplant model. These mice had lower blood counts, reduced circulating cytokine levels, lower spleen weights, and reduced fibrosis. In contrast to the significant effects seen with complete hematopoietic-specific Stat3 deletion, deleting Stat3 exclusively in the mutant compartment did not reduce cytokine production, disease severity, or improve survival. These data are consistent with a requirement for STAT3 signaling in both tumor and in non-malignant hematopoietic cells in PMF.
Summary / Conclusion: Our data demonstrate STAT3 as an important effector of cytokine signaling in MPN, and that STAT3 signaling contributes to MPN pathogenesis. Notably, our results suggest JAK-STAT activation in non-clonal cells contributes to MPN pathogenesis. We demonstrate that signaling cross-talk between mutant and non-mutant populations is an important feature of PMF. Finally, our studies support the notion that JAK kinase inhibition in malignant and non-malignant cells is required to improve symptoms, reduce disease severity, and to prevent malignant progression in MPN patients.

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IRON DEFICIENT ANEMIA INVOLVES RECONFIGURATION OF ERYTHROPOIETIC PROGRAM ORCHESTRATED BY HEME RECEPTOR BACH1

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Background: Developing erythroid cells must synthesize balanced amounts of globin proteins and heme because of their intrinsic toxicities. Free globin proteins without heme precipitate easily, causing proteotoxicity, whereas free heme produces reactive oxygen species. Since iron deficient anemia (IDA) is one of the most prevalent diseases, coordination of globin and heme syntheses according to available iron level in erythroid cells must be critical. It has been considered that living bodies have exquisite systems, which sense the amount of iron or heme to regulate globin synthesis and erythropoiesis. While heme-regulated eIF2a kinase HRI is known to regulate the translation of globin by sensing heme, little is known about the transcriptional mechanism for balancing heme and globin levels. *In vitro* studies have shown that BTB and CNC homology1 (Bach1) is a heme-binding transcriptional repressor and binds to the β-globin locus control region and the enhancer regions of Hmox1 encoding heme oxygenase-1 (HO-1). Heme binds to Bach1 to inhibit its DNA binding and to induce its degradation, achieving derepression of Bach1 target genes. Based on these lines of evidence, we hypothesized that Bach1 would adapt synthesis of globin according to heme and iron levels.
Aims: To clarify the function of Bach1 during erythropoiesis under IDA.
Methods: *Bach1*-deficient mice were used for the analysis. To induce iron deficient anemia, the mice were fed with low iron diet. Erythroid progenitors were separated from flow cytometry. Quantitative chromatin immunoprecipitation (ChIP) analysis was performed with TER119-positive erythroblasts using anti-MafK (Santa Cruz) and anti-Bach1 serum A1-6 (Sun et al., 2004). Serum hepcidin level was determined with LC-MS/MS method.
Results: *Bach1*-deficient mice did not exhibit defects in erythropoiesis. In contrast, we found aggravation of IDA in *Bach1*-deficient mice. Based on electron microscopic analysis, massive aggregates were observed in peripheral red blood cells under IDA in *Bach1*-deficient mice, presumably reflecting denatured globin. While expression of both HO-1 and globin mRNA expressions was repressed in wild-type erythroblasts upon iron depletion, their expression was higher in *Bach1*-deficient erythroblasts. ChIP analysis confirmed endogenous BACH1 occupancy at the regulatory regions of these genes. Changes in the systemic iron distribution are also predisposing factors for IDA. Thus, we next assessed serum hepcidin, known as a main regulator of systemic iron level. We found that the serum hepcidin level was significantly higher in *Bach1*-deficient mice.
Summary / Conclusion: We propose a model, referred to as “hemoglobin regulon”, in which the balance between heme and globin in iron deficient erythroid cells is coordinated through Bach1. In iron deficient erythroid cells, heme concentration is decreased, leading to the activation of Bach1 and thereby an inhibition of globin gene transcription. The expression of HO-1 is also kept low in iron deficient erythroid cells, precluding degradation of the newly synthesized heme using the scarce iron. Therefore, both heme and globin are maintained to similar levels by Bach1 and an accumulation of harmful excessive globin is avoided. IDA is not a mere result of iron shortage but is based on an adaptive gene response within erythroid cells. In addition, Bach1 represses hepcidin expression and thereby regulates systemic iron homeostasis. Heme-initiated transcriptional regulations by Bach1 are essential for erythropoiesis and iron homeostasis.

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THE ASSOCIATION OF MUTATIONS IN RUNX1 AND CSF3R WITH THE DEVELOPMENT OF LEUKEMIA IN SEVERE CONGENITAL NEUTROPENIA: A NEW PATHWAY IN LEUKEMOGENESIS

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Background: Congenital neutropenia (CN) is a rare inherited disorder of hematopoiesis with a 20% risk to evolve to acute myeloid leukemia or myelodysplastic syndrome (AML/MDS).
Aims: We aimed to investigate the patterns of acquisition of leukemia-associated-mutations in 31 CN patients developing leukemia.
Methods: We used next-generation DNA deep sequencing.
Results: Twenty of the 31 patients (64.5%) demonstrated mutations within *RUNX1*. Of 20 the patients with *RUNX1* mutations, 19 had mutations known to be associated with severe congenital neutropenia: *ELANE* (11), *ELANE+GFI1* (1), *WAS* (3), *HAX1* (3) and *G6PT* (*SLC37A4*) (1).None of the patients acquired an AML associated mutations in *CEBPA, DNMT3A, IDH1, IDH2, NPM1* or *TET2*. Intriguingly, the majority of patients with *RUNX1* mutations (75%) had acquired *CSF3R* mutations. Other leukemia-associated mutatons in the patients with *RUNX1* mutations were found infrequently: *FLT3*-ITD (two patients), *EP300* mutations (two patients*), CBL* (one patient) and *SUZ12* (one patient). Cytogenetics of the leukemic cells revealed that 10 patients with *RUNX1* mutations developed monosomy 7, and 6 patients had trisomy 21. Sequential analysis of the time prior to overt leukemia revealed that *RUNX1* mutation is a late event in leukemogenic transformation. We also evaluated if *RUNX1* and *CSF3R* mutations were presented in the same malignant clone or whether there were two different clones carrying either *RUNX1* or *CSF3R* mutations. We performed CFU assay and sequenced DNA isolated from colonies of leukemia blasts of one CN/AML patient. Both *RUNX1* and *CSF3R* mutations were detected in the same leukemic colonies, suggesting that the RUNX1 mutations occured within the clones harbouring CSF3R mutations in the process of malignant transformation.
Summary / Conclusion: The high frequency of *RUNX*1 mutations in CN patients who developed leukemia and the high incidence of *CSF3R* mutations preceeding these mutations suggests a unique molecular pathway of leukemia development. The detection of *RUNX1* mutations in combination with *CSF3R* mutations could be a useful marker for identifying CN patients progressing to leukemia or MDS.

SIMULTANEOUS SESSION II

Novel chronic lymphocytic leukemia genetics - Clinical implications

S571

GENETIC DETERMINANTS OF FLUDARABINE-RESISTANCE IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: The current first line standard of care for fit CLL patients is fludarabine, cyclophosphamide and rituximab, which offers overall response and complete remission rates of 95% and 44%, respectively. Fludarabine-refractoriness (FR), defined as failure to respond or relapse within 6 months of therapy, accounts for 5-10% of treated patients, and continues to represent a challenging clinical problem. The only established player in determining FR is *TP53*, disrupted in 30-40% of FR-CLL. More recently, next generation sequencing studies led to the identification of new genes (*NOTCH1*, *SF3B1*, *BIRC3*) that are frequently mutated in FR-CLL. However, a comprehensive evaluation of the FR-CLL genome has not been reported.
Aims: We explored the genetic landscape of FR-CLL to provide a comprehensive description of the load and spectrum of alterations that are associated with this condition and to identify novel molecular determinants of FR.
Methods: We integrated Whole-Exome Sequencing (WES, Agilent SureSelect Human All Exon 50Mb, Illumina HiSeq 2000) and Copy Number (CN) analysis (SNP 6.0 arrays, Affymetrix) to study 10 FR-CLL cases sampled prior to the treatment that resulted in refractoriness and their paired germline DNA (discovery panel). Candidate somatic mutations were verified by Sanger sequencing and the entire coding region of recurrently mutated genes (>1 case) was then sequenced in an independent set of 48 FR-CLLs, of which 29 were also subjected to CN analysis. Mutation recurrence was compared versus 174 unselected CLL at diagnosis, to determine preferential association with FR-CLL.
Results: In the 10 discovery cases, we identified 163 non-silent sequence variants (average 16.3/sample, range 9-23), distributed in 151 genes and predominantly represented by missense mutations; additionally, 40 CNAs were found (average 4/sample, range 0-13), accounting for an overall load of genomic alterations of ~20 lesions/sample (range 11-26). The extended analysis of 58 FR-CLLs revealed several recurrently mutated genes - including the previously reported *TP53* (27.5%), *SF3B1* (24.1%), *NOTCH1* (17%) and *BIRC3* (15.5%) - but also novel candidates, with the tumor suppressor related gene *FAT1*, encoding for a cadherin like protein, being the most common (10.3%). *FAT1* mutations introduce amino acid changes in the cadherin and intracytoplasmic domains, and are similar to those recently reported in multiple solid tumors, where inactivation of FAT1 promotes Wnt signaling and tumor growth, consistent with a tumor-suppressor role. Importantly, the frequency of *FAT1*-mutated cases in FR-CLL was significantly higher than that recorded in unselected CLLs at diagnosis (N=2/174, 1.1%, P=0.004), suggesting a specific role in FR. SNP array analysis of 39 patients showed a broader load of CNAs in FR-CLL (4.8/case), than in untreated CLLs described in the literature (~2/case, Edelmann *et al*, Blood 2012). Furthermore, 10% of cases were characterized by a particularly high number of CNAs within one single chromosome, consistent with the chromotripsis phenomenon.
Summary / Conclusion: *FAT1* is a novel commonly mutated gene in FR-CLL (10.3%), which is rarely detected in unselected CLL cases at diagnosis, suggesting a potential functional role in the disease. Patients with FR-CLL present a higher burden of CNAs and chromotripsis compared to untreated CLL described in the literature. The contribution of *FAT1* mutation, CNAs and chromotripsis to FR in CLL is under investigation.

S572

GENOME-WIDE GENETIC ANALYSIS REVEALS GLOBAL DEREGULATION OF METHYLTRANSFERASE GENES IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Of the biological processes that contribute to clinical behaviour in chronic lymphocytic leukemia (CLL), few are more fundamentally important to the process of pathogenesis than the presence of somatically acquired chromosomal aberrations and gene mutations. However, our understanding of the pathways targeted by these lesions, which contribute to CLL biology, remains incomplete.
Aims: To identify and characterise genomic aberrations and somatic mutations that target methyltransferase (MT) genes in patients with CLL.
Methods: Our initial cohort comprised 261 CLL patients sampled at disease progression (mean time from diagnosis to sampling = 45.8 months), including 95 enrolled on the UK CLL4 trial. Affymetrix SNP 6.0 profiling was performed to identify somatically acquired copy number alterations (CNA) and copy number neutral LOH (cnn-LOH). Aberrations detected in at least 2% of patients (n=6) were considered recurrent. Selective confirmatory array-CGH (Agilent Technologies) was also employed. The RNA expression levels of candidate genes within recurrent CNA were investigated using qRT-PCR and high resolution melt analysis (HRM) and Sanger sequencing were used to screen for, and validate mutations. We then designed a next generation sequencing (NGS) platform (Agilent Haloplex Target Enrichment system) to identify somatic mutations in 82 selected genes, including 55 with MT activity.
Results: Affymetrix SNP 6.0 profiling facilitated the characterisation of established CNA and identified 22 other recurrent CNA including novel deletions of 18p12 (6%), 8p21.2-23.3 (4.6%), 4p16.1 (3%) and 20q12.1-13 (2.7%). Of specific interest was a previously un-described recurrent deletion of chromosome 3p21.31 (46.93-47.37mb), observed in eight cases (3%), with cnn-LOH observed in an additional case. The minimally deleted region encompasses five genes; *CCDC12*, *NBEAL2*, *KIF9*, *KLHL19* and the histone MT gene, *SETD2*. Quantitative RT-PCR demonstrated that *SETD2* was the likely target gene as it was the most significantly under-expressed gene in the region (P=0.001). In total 30 MT genes were targeted by CNA as follows: 1) established CNA resulted in the deletion or duplication of six MT genes; *EED* (13.8%) and *MLL* (8%)(del11q), *SETDB2* (43%)(del13q) and *MLL2*, *PRMT8* and *SETD8* (6%)(Tri12); and 2) a further 24 MT genes were targeted by other large (non-focal) CNA including recurrent deletions of *ASH2*L (3.4%), *WHSC1L1* (3.4%), *WHSC1* (2.7%) and *PRMD1* (2.7%). Using HRM to screen *SETD2* (exons 3-21) we could not identify mutations in the 3p deleted cases, but in 100 progressive patients without deletions of the 3p region we identified two mutated individuals, with a non-synonymous mutation and a frameshift deletion. Based on this preliminary data and recent publications we hypothesized that whilst *SETD2* mutations were rare, CLL might be characterized by deregulation of epigenetic control mechanisms with somatic DNA mutations targeting multiple genes with MT activity. An initial analysis of six cases using our customised NGS platform confirmed the presence of *SETD2* mutations (and deletions), and identified mutations in two additional MT genes (*MLL2* and *NSD1*) in individual cases.
Summary / Conclusion: In total, genes with MT activity were targeted by CNA and/or mutations in the majority of CLL patients in our study. This implicates the disruption of epigenetic modifying genes in the pathogenesis of CLL. Whilst therapeutics that target these processes have limited activity in CLL in general, our work may identify patient sub-groups that are more sensitive to these agents, thereby providing improved clinical management of patients with CLL.

S573

ASSOCIATION BETWEEN MOLECULAR LESIONS AND SPECIFIC B-CELL RECEPTOR SUBSETS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Genetic lesions and B-cell receptor (BCR) signaling are both oncogenic drivers in chronic lymphocytic leukemia (CLL). However, little is known regarding the association between specific genetic aberrations and distinct CLL subsets with stereotyped BCR.
Aims: The study aimed at verifying whether stereotyped BCR patterns may selectively promote the occurrence of specific genetic lesions in CLL.
Methods: Mutations (*TP53*, *NOTCH1*, *SF3B1*, *BIRC3*, *MYD88*), chromosomal abnormalities (del13q, +12, del11q, del17p, *BIRC3* deletion), and BCR stereotypy were investigated in 1419 newly diagnosed CLL. Associations between genetic lesions and BCR features were assessed by non-parametric binomial test corrected for multiple comparisons.
Results: BCR stereotypy as a whole did not impact on CLL genetics. However, two BCR subsets, namely subset 2 (*IGHV3-21*) and 8 (*IGHV4-39*), recurrently showed a distinctive pattern of molecular alterations. CLL subset 2 was significantly enriched in *SF3B1* mutations, which occurred in 52% of cases (P<.001). Among *IGHV3-21* CLL, *SF3B1* mutation enrichment was selective for cases showing a subset 2 BCR pattern, while they occurred at lower prevalence in *IGHV3-21* CLL with heterogeneous BCR (13%; P=.002). Subset 2 CLL lacked *TP53* abnormalities, that were otherwise evenly represented in all the other major subsets, thus pointing to *SF3B1*, rather than *TP53*, as the main driver of progressiveness in subset 2 CLL. Consistently, subset 2 CLL harboring *SF3B1* mutations showed a higher probability of being treated at 5 years (67%) compared to subset 2 CLL with wild type *SF3B1* (38%; P=.064) and to *IGHV3-21* CLL with heterogeneous BCR (46%; P=.076). Subset 8 CLL were significantly enriched in +12 (87% of cases; P<.001) and *NOTCH1* mutations (62% of cases). Among *IGHV4-39* CLL, +12 and *NOTCH1* mutations enrichment was selective for cases harboring a subset 8 BCR configuration, while their prevalence was significantly lower in *IGHV4-39* CLL with non-stereotyped BCR (27%; P=.003 and 8%; P=.006, respectively). From a clinical standpoint, the majority (62%) of subset 8 patients had transformed to Richter syndrome. All transformed cases carried both *NOTCH1* mutations and +12, while this genetic association was never observed among subset 8 patients that had not transformed to Richter syndrome (P=.017).
Summary / Conclusion: These data suggest that: i) synergy of *SF3B1* mutations and subset 2 BCR configuration promotes disease progression in *IGHV3-21* CLL; and ii) cooperation between *NOTCH1* mutations, +12, and subset 8 BCR configuration primes RS transformation in *IGHV4-39* CLL. Taken together, our observations provide a proof of concept that specific BCR configurations may contribute to clonal selection of specific genetic lesions influencing CLL outcome.

S574

DISTINCT FREQUENCY AND PROFILES OF TP53 GENE MUTATIONS IN CLL SUBGROUPS WITH DISTINCT ANTIGEN RECEPTORS: EVIDENCE FOR ANTIGEN-DRIVEN SELECTION OF GENOMIC ABERRATIONS

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Background: In CLL, mutations of the *TP53* gene negatively impact on patient outcome and are strongly associated with deletion of chromosome 17p and unmutated IGHV genes (U-CLL).
Aims: Here we sought to explore the distribution and spectrum of *TP53* mutations with regards to immunogenetic features in a series of 1160 patients intentionally selected for belonging to prognostically adverse immunogenetic CLL subgroups.
Methods: We investigated (i) U-CLL and/or (ii) cases expressing the IGHV3-21 gene, especially in stereotyped B cell receptors (BcRs) assigned to subset #2 with variable IGHV mutational status. For this reason, only 49/1160 (4.2%) cases of the cohort carried mutated IGHV genes (M-CLL; germline identity, GI, <98%). The remaining cases (1111/1160, 95.8%) concerned U-CLL; of these, 271 (24.4%) and 840 (75.6%) cases expressed IGHV genes with 98-99.9% and 100% GI, respectively. For the purposes of the present study, we particularly focused on missense *TP53* mutations in DNA binding motifs (DBMs), described to be associated with a markedly adverse prognosis.
Results: Overall, 206 cases (17.8%) were found to carry *TP53* mutations; 99/206 (48%; or 8.5% of the entire cohort) carried mutations in DBMs. The high frequency of mutations can be explained by the composition of the present cohort which essentially consisted of prognostically adverse immunogenetic subgroups. As expected, *TP53* mutations (all types as well as mutations in DBMs) were significantly more frequent in cases with del(17p) vs. all other aberrations in the hierarchical model (P<0.0001). Association analyses regarding immunogenetic features concerned the following: (1) somatic hypermutation (SHM) status. The frequency of all types of *TP53* mutations was significantly higher in cases expressing IGHV genes with GI=100% vs. GI=98-99.9% (19.6%

vs 13.6%; P=0.02). (2) IGHV gene repertoire. Among U-CLL cases, significant differences in the incidence of *TP53* mutations in general and mutations in DBMs in particular (P=0.03 for both) were identified in cases utilizing different IGHV genes: in particular, there were more than 20% *TP53*-mutated cases in the IGHV1-69, IGHV1-3, IGHV3-30 and IGHV4-39 groups, thus contrasting the IGHV5-51 (7%) and, especially, the IGHV3-21 group (4%). (3) BcR stereotypy. In addition to subset #2 (n=66 cases, variable SHM status), several other well-characterized subsets were represented in the present cohort (all concerning U-CLL), including #1 (n=61), #8 (n=15) (both already documented as poor-prognostic) as well as five subsets utilizing the IGHV1-69 gene albeit with distinct VH CDR3 motifs, namely #3 (n=16), #5 (n=14), #6 (n=25) and #7 (n=44). *TP53* mutation frequencies varied significantly (P=0.01) between these subsets, ranging from 0% in subset #5 to 25% in subset #3; of note, these subsets have previously been reported to exhibit distinct prognosis (shorter time-to-first-treatment in #3). The two largest subsets, namely #1 and #2 had *TP53* mutation frequencies of 14.8% and 6% respectively. Differences were more pronounced (P=0.008) concerning *TP53* mutation frequencies in DBMs.
Summary / Conclusion: We demonstrate markedly different incidence and spectra of *TP53* mutations, especially missense mutations in DBMs, in different stereotyped CLL subsets. These findings confirm and significantly extend recent reports by us and others that certain BcR stereotypes are linked to distinct profiles of genomic aberrations. On these grounds, antigen-driven, BcR-mediated selection can be proposed as a possible mechanism for the acquisition of genomic aberrations in CLL.
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S575

COMPREHENSIVE GENOME-WIDE ANALYSIS OF CLL SAMPLES FROM UK 1ST LINE AND RELAPSED/REFRACTORY CLINICAL TRIALS
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Background: CLL is a biologically and clinically heterogeneous disease. Whilst important progress has been made in the treatment of CLL, 25% and 50% patients relapse within two years of 1st or 2nd line chemo-immunotherapy, respectively. Multiple recurrent chromosomal abnormalities have been identified and associated with prognosis. To date, the only clinically useful predictive markers remain deletions/mutations of TP53. However, these identify only up to a third of patients with chemo-immunotherapy refractory disease.
Aims: To design a comprehensive genome-wide next generation diagnostic tool of recurrent acquired genetic abnormalities in CLL that predicts treatment response using well-annotated clinical trials samples.
Methods: The cohort studied consisted of 261 CLL patients recruited into four national UK CLL trials, divided in two groups: 192first line treatment (ARCTIC, AdMiRe) and 69 relapsed/ refractory patients (NCRNCLL201, NCRNCLL202). Samples were tested using genome-wide high-resolution arrays (Illumina Inc.) and analyzed with the software Nexus v6.1 (BioDiscovery, Inc.). CNA and copy neutral Loss of Heterozygosity (cnLOH) events were verified visually and the frequency of CNAs and cnLOH noted. Overall genomic complexity was determined per sample using the number and total length of CNAs reported. Previously described (Knight et al 2012) and novel minimally overlapping regions (MORs) were interrogated for candidate genes. Custom-designed targeted sequencing panels consisting of 387 amplicons each (TruSeq Custom Amplicon, Illumina) were run on the Miseq (Illumina Inc) to identify 20 pathogenic mutations in genes known to be recurrently mutated in CLL and in new candidate genes from MOR analysis. Bio-informatics analysis was carried out using BaseSpace (Illumina Inc.) and our in-house pipeline.
Results: *Genome-wide array.* The total number of CNAs in CLL-associated genes and their frequency in the pre-treatment and relapsed/refractory cohorts are presented in the table 1. The array data revealed that genomic complexity and chromotrypsis were significantly higher in the relapsed/refractory group with 30% of the cases with an average cumulative length of abnormalities greater than 60 Mb, whereas only 18% of the first line treatment cohort fulfilled the same criteria. *Next-generation sequencing.* Of the 63 MORs published previously by Knight *et al.* (2012), we were able to refine 24, in some cases to only one or two genes. We identified also an additional 50 MORs. Over 90% of these involved copy number losses occurring at <3% frequency. Re-sequencing of candidate genes in MORs identified 14 novel genes with pathogenic mutations (1 frameshift insertion, 2 frameshift deletions, 2 stop gains and 18 non synonymous mutations) of which 6 were recurrently mutated. Mutation frequencies of known CLL associated genes were within the ranges published to date. An increased mutation burden in relapsed trial samples compared to pre-treatment samples was seen.
Summary / Conclusion: Our results represent the most comprehensive genome-wide array and mutation analysis of CLL patients recruited into clinical trial samples so far. They demonstrate the value of combining genome-wide array and targeted NGS approaches for high sensitivity, high throughput molecular studies of CLL. Once clinical outcome data becomes available a comprehensive hierarchical model of response prediction will be validated.

Clinical studies in Multiple Myeloma 1

S576

DARATUMUMAB, A CD38 MONOCLONAL ANTIBODY STUDY IN ADVANCED MULTIPLE MYELOMA – AN OPEN-LABEL, DOSE ESCALATION FOLLOWED BY OPEN-LABEL EXTENSION IN A SINGLE-ARM PHASE I/II STUDY
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Background: Daratumumab (DARA) is a human CD38 monoclonal antibody with broad-spectrum killing activity in multiple myeloma (MM). Preliminary safety and efficacy data from this first-in-human dose-escalation study of DARA in patients with relapsed or refractory (RR) MM have previously been reported.[1] Here, we present efficacy data from the dose escalation part and preliminary safety and efficacy data from the ongoing extension part of the study.
Aims: The primary aim was to evaluate the safety profile of DARA in this setting. The secondary aims were to find the maximum tolerated dose (MTD), assessment of efficacy, pharmacokinetics (PK) and immunogenicity through examining Anti-Drug-Antibodies (ADA).
Methods: Patients ≥18 years, diagnosed with MM, requiring systemic therapy, considered RR to at least 2 prior lines of therapy and ineligible for ASCT were enrolled. Dose escalation of the study was based on a classic 3+3 design. DARA was given over a 9-week period consisting of 2 pre- and 7 full doses. Bone marrow biopsy was performed before treatment, after end of treatment and at end of study. In the extension part, patients receive 8mg/kg DARA weekly for 8 weeks followed by dosing every 2nd week for 16 weeks and every 4th week until disease progression, toxicity or for maximum 24 months. DARA plasma concentrations were measured by ELISA. Evaluation of efficacy data was according to modified IMWG criteria.[2] An Electro-Chemi-Luminescence method was used to detect ADA. The results presented are based on data analyzed before database lock.
Results: Data from 32 patients were included in part 1, and data from 13 patients from part 2 have been collected to date. Median age was 61 years in both part 1 and part 2. The median number of prior treatment lines was 6 in part 1 and 4 in part 2. PK analysis showed plasma peak levels as expected, but relatively rapid clearance at low dose levels, with PK data for part 2 currently in process. Preliminary efficacy evaluation from part 1 was based on best response to paraprotein according to IMWG criteria. In the 4 mg/kg group and upwards (n=12), 5 PRs and 3 MRs were observed and 7 of these patients had a 50-100% concomitant reduction in the bone marrow plasma cells. Median PFS (Figure 1) in the ≥4mg/kg dose groups was not reached (median follow-up at data cut-off was 3.8mths (range: 0-9.6mths).
Safety data continue to be evaluated with no ADA responses detected so far. In part 1, the most common adverse events reported were infusion related (IREs) which occurred predominantly during the first full infusion. 44% of subjects across all dose groups had IREs grade 1-3, of which 2 were grade 3. Six related SAEs (1 anemia, 1 thrombocytopenia, 2 bronchospasm, 1 cytokine release, 1 AST increase) were reported. In part 2, preliminary safety data mainly show infusion related reactions grade 1–2.
Summary / Conclusion: DARA induced a marked reduction in paraprotein and bone marrow plasma cells at doses ≥4mg/kg in heavily pretreated patients with relapsed or relapsed and refractory MM. This is currently unprecedented for single-agent monoclonal antibody treatment of MM. No ADA responses were detected and toxicity proved manageable. All data available from the study will be presented at the meeting.

References
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2. Rajkumar et al. Blood 2011;117:4691-5

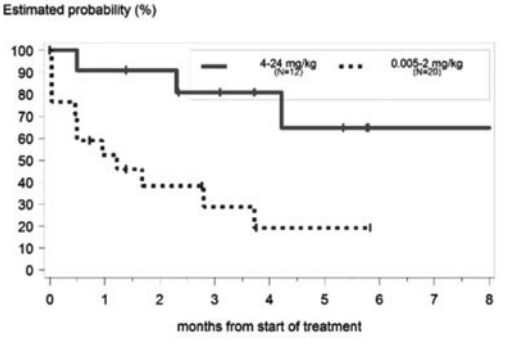

Figure 1.

S577

FINAL RESULTS FROM THE PHASE 1B/2 STUDY (PX-171-006) OF CARFILZOMIB (CFZ) IN COMBINATION WITH LENALIDOMIDE AND LOW-DOSE DEXAMETHASONE (CRD) FOR PATIENTS WITH RELAPSED OR PROGRESSIVE MULTIPLE MYELOMA

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Background: Carfilzomib (CFZ) is a selective inhibitor of the chymotrypsin-like activity of both the constitutive proteasome and the immunoproteasome, and was approved in the United States in 2012 as a single-agent treatment for patients with multiple myeloma (MM) who have received at least 2 prior therapies including bortezomib and an immunomodulatory agent and have demonstrated disease progression on or within 60 days of completion of the last therapy. We previously reported interim data from PX-171-006 (NCT00603447), a phase 1b/2 study of CRd in patients with relapsed or progressive MM (Wang M et al., ASCO 2011; Niesvizky R et al., *Clin Cancer Res*. 2013).
Aims: Herein we report final results from the PX-171-006 study.
Methods: Patients (1–3 prior treatments) received CRd in 28-day (D) cycles—CFZ IV on D1, 2, 8, 9, 15, and 16; lenalidomide PO D1–21; and dexamethasone weekly. During the phase 1 portion of the study, CFZ (15–27 mg/m^2) and lenalidomide (10–25 mg) doses were escalated to determine the maximum tolerated dose (MTD). The maximum planned dose (MPD) of CFZ was 20 mg/m^2 on D1 and 2 of Cycle 1 and 27 mg/m^2 thereafter, which was used in combination with lenalidomide (25 mg/d) and dexamethasone (40 mg/wk). During the phase 2 dose expansion portion of the study, CFZ was used at the MTD/MPD. Endpoints included overall response rate (ORR) (assessed by IMWG criteria), duration of response (DOR), progression-free survival (PFS), and safety.
Results: A total of 84 patients were enrolled since June 2008. Overall, prior treatments included bortezomib (77%; 18% refractory) and lenalidomide (70%; 35% refractory); 20% had high-risk cytogenetics/FISH. The MTD was not reached in phase 1, supporting expansion at the MPD (n=52; 23% bortezomib refractory and 42% lenalidomide refractory). As of November 2012 (median follow-up 24.4 mo), ORR was 69% overall and 76.9% at MPD. In addition, very good partial response was reported in 36.9% (overall) and 38.5% (MPD) of patients. Stringent complete response was reported in 3.6% (overall) and 3.8% (MPD) of patients. Responses were durable with a median DOR of 18.8 mo (95% CI 9.7–41.5) overall and 22.1 mo (95% CI 9.5–NE) at the MPD. Median PFS was 11.8 mo (95% CI 7.6–20.7) overall and 15.4 mo (95% CI 7.9–NE) at the MPD. In the 35% of patients refractory overall to lenalidomide, ORR was 58.6%, median DOR 13.8 mo (95% CI 6.47–NE), and median PFS 9.9 mo (95% CI 5.55-NE). In total, 10 and 12 patients at the MPD pursued other therapies and were censored for DOR and PFS, respectively. Seven patients at the MPD were censored for DOR and PFS after undergoing stem cell transplant/maintenance. Overall, a median of 8.5 (range 1−46) cycles of CFZ were started. Fifteen percent of patients discontinued CFZ due to adverse events (AEs); dose reductions were required by 4% of patients. The most common grade 3/4 hematologic AEs included neutropenia (35.7%), lymphopenia (29.8%), and thrombocytopenia (25.0%). The most common grade 3/4 non-hematologic AEs included hypophosphatemia (20.2%) and hyperglycemia (13.1%).Grade 3/4 peripheral neuropathy was infrequent (1%).
Summary / Conclusion: CRd was well tolerated and highly active and provided robust and durable responses in patients with relapsed or progressive MM where 35% were lenalidomide refractory. In patients who received the MPD, ORR was 76.9% with a DOR of 22.1 mo. Grade 3/4 hematologic AEs were generally consistent with earlier studies in advanced MM that used similar doses of single-agent CFZ. CRd is being further evaluated in several ongoing phase 2/3 trials.

S578

CARFILZOMIB, CYCLOPHOSPHAMIDE AND DEXAMETHASONE (CCD) FOR NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS: INITIAL RESULTS OF A MULTICENTER, OPEN LABEL PHASE II STUDY

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Background: The standard treatment for newly diagnosed elderly MM patients, VMP and MPT, induced 30% near-complete response/complete response (nCR/CR), with a discontinuation rate due to adverse events (EAs) of 35%. Carfilzomib is a novel, irreversible proteasome-inhibitor with a significant activity and favourable toxicity profile, including very low rates of peripheral neuropathy and neutropenia. Initial results on carfilzomib plus cyclophosphamide and dexamethasone (CCd) were presented at ASH 2012. Herein we report updated data after 8 months of follow up.
Aims: We evaluated efficacy and safety (primary end points) of the combination CCd in elderly newly diagnosed MM patients.
Methods: The Bryant and Day two-stage design was used to evaluate both efficacy and safety. Patients received oral cyclophosphamide (300 mg/m^2 on days 1,8,15), oral dexamethasone (40 mg on days 1, 8, 15, 22) and iv carfilzomib (20 mg/m^2 on days 1, 2, and 36 mg/m^2 on days 8, 9, 15, 16, cycle 1; 36 mg/m^2 on days 1, 2, 8, 9, 15, 16, cycles 2-9) every 28 days for 9 cycles, followed by maintenance with iv carfilzomib (36 mg/m^2 on days 1, 2, 15, 16) every 28 days until progression or intolerance.
Results: As of 9 October 2012, enrollment has been completed (58 pts): median age was 71 years, 28% of patients were older than 75 years, 40% had ISS stage III, and 35% had unfavorable FISH profile [t(4;14) or t (14;16) or del17p]. Forty one patients were evaluated for response after at least 4 cycles and 56 patients were evaluated for safety after at least 1 cycle. After a median follow-up of 8 months, 93% of patients achieved at least partial response (PR), 68% at least very good partial response (VGPR), 46% at least CR/near-CR, including 12% stringent-CR (Figure 1). Responses improved with the duration of treatment reaching after 9 cycles: 100% PR, 77% VGPR, 53% CR/nCR, including 23% stringent-CR. The 1-year PFS was 85% and the 1-year OS was 86%. Grade (G) 4 hematologic AE included neutropenia (2 pts, 4%). G3-4 non-hematologic AEs were infections (4 pts, 7%), cardiac (2 pts, 4%), constitutional (2 pts, 4%), renal (2 pts, 4%) and gastrointestinal complications (1 pt, 2%). At least one G3-4 event was reported in 11 patients (20%). Six pts (11%) discontinued treatment due to AEs and 9 pts (16%) required carfilzomib dose reductions due to AEs. No differences in response rate and safety between patients younger and older than 75 years were observed.
Summary / Conclusion: Carfilzomib-cyclophosphamide-dexamethasone showed encouraging activity in elderly patients with newly diagnosed MM. The combination was well tolerated with a discontinuation rate due to AEs of 11%. Results will be updated at the meeting.

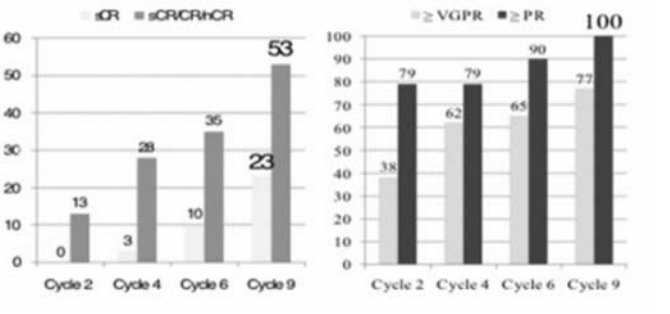

Figure 1.

S579

PHASE 1 STUDY OF THE NOVEL KINESIN SPINDLE PROTEIN INHIBITOR ARRY-520 + CARFILZOMIB(CAR) IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background: Background: ARRY-520, a novel kinesin spindle protein (KSP) inhibitor, with a unique mechansim of action has demonstrated promising clinical activity, both as a single agent and combined with dexamethasone in patients (pts) with bortezomib- and lenalidomide-refractory MM. The maximum tolerated dose (MTD) of ARRY-520 as a single agent was 1.5 mg/m^2 administered days 1, 2 every 2 weeks. Car, a novel irreversible proteasome inhibitor (PI), received Federal Drug Administration (FDA) regulatory approval in relapsed and refractory MM in 2012. Preclinical data demonstrate synergy with the combination of a PI and ARRY-520.
Aims: Aim: We aimed to combine these two agents for the first time, and report the findings from the phase I dose-escalation study in RRMM.
Methods: Methods: The primary objective was to determine the MTD and the safety/tolerability of the Car-ARRY combination. Secondary objectives were to determine efficacy as measured by the overall response rate, time to progression, progression free survival and time to next therapy. Pts had to have myeloma that was relapsed and/or refractory, be ineligible for autologous stem cell transplant, bortezomib refractory/intolerant, and have had prior lenalidomide exposure. ARRY-520 was administered intravenously (iv) over 1 hour on days 1, 2, 15 and 16, while car was administered intravenously over 30 minutes on days 1, 2, 8, 9, 15 and 16 on a 28 day cycle. All pts received growth factor support with filgrastim.
Dose-escalation used a standard 3+3 schema proceeded based on dose-limiting toxicities (DLTs) during cycle 1, with planned escalation of the dose of ARRY-520. Car dose was fixed and not escalated. Adverse events (AEs) were graded by NCI-CTCAE v4, while responses were assessed by the modified International Uniform Response Criteria.
Results: Results: To date, 17 pts have been enrolled in the ongoing dose escalation. In cohort 1, ARRY-520 was dosed at 0.75 mg/m^2. 3 pts were enrolled and no DLT was observed. In cohort 2, ARRY-520 was escalated to 1 mg/m^2 with Car at 20/27 mg/m^2, and among the first 3 pts, 1 DLT with non neutropenic fever associated with influenza pneumonia was observed. No DLT was encountered in the 3 additional pts in cohort 2. In cohort 3 ARRY-520 was escalated to 1.25 mg/m^2 with Car at 20/27 mg/m^2 and 5 pts were enrolled. The first 2 pts were not toxicity evaluable. Among the 3 evaluable pts, no DLT were encountered. Accrual is ongoing in the final cohort with full dose ARRY-520 of 1.5 mg/m^2 with Car at 20/27 mg/m^2. One of the first 3 pts had a DLT with non-neutropenic fever, and pneumonia requiring hospital admission. Among 17 pts enrolled to date, 9 remain on study, 4 discontinued for progressive disease(PD), 1 pt was lost to followup, 1 patient died with febrile neutropenia in cycle 4, 2 pts withdrew consent after 1 cycle of therapy. No pts discontinued therapy/withdrew consent due to toxicity. SAE included 4 pts with G3 pneumonia (2 of which were influenza), 2 pts with G1 hypercalcemia requiring hospitalization (due to disease progression), G4 renal failure (due to disease progression), G2 lethargy and 1 pt with G5 febrile neutropenia.
Summary / Conclusion: Conclusions: The combination of ARRY-520 and Car in pts with RRMM is well tolerated with limited hematologic toxicity. An MTD has not been established and enrollment is ongoing in cohort 4 with Car at 20/27 mg/m^2 and ARRY-520 at 1.5 mg/m^2. Updated MTD and safety data will be presented at the meeting.

S580

COMBINING FISH AND GEP SIGNATURES IN MULTIPLE MYELOMA; TOWARDS A MORE ROBUST AND MEANINGFUL HIGH RISK DEFINITION

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Background: Multiple Myeloma (MM) is a heterogeneous disease with different recurring chromosomal aberrations that have been associated with prognosis for survival (e.g. t(4;14), t(14;16)/t(14;20), del13, del17p, add1q). Strategies for combining these markers are emerging [1] and are being evaluated [2]. More recently, gene expression profiling (GEP) studies have identified signatures that distinguish groups of high risk patients, such as the SKY-92 and UAMS-70 signatures [3,4]. Although collectively referred to as high risk signatures they employ different genes and are independent risk markers, similar to how the chromosomal aberrations represent different biological risk markers. It is currently unclear what the best high risk definition is for MM. Here we compare GEP signatures and fluorescent *in situ* hybridization (FISH) markers for this purpose.
Aims: We explore the evidence to combine chromosomal aberrations and high risk GEP signatures towards a more robust and meaningful definition of high risk in MM.
Methods: GEP and FISH data (if available) were analyzed for five clinical cohorts (Table 1). Five high risk GEP signatures (SKY-92, UAMS-70, UAMS-17, UAMS-80, MRCIX-6) have been applied to all five datasets. Associations between markers and overall survival (OS) were investigated in Kaplan-Meier plots with the logrank test P≤0.05 considered as significant.
Results: Univariate associations with survival are shown in Figure 1A. The prognostic value of these FISH markers all failed to reproduce across cohorts while five published GEP signatures did. Figure 1B shows the Kaplan-Meier curves for FISH, GEP, and their combination on the MRC-IX cohort. This analysis indicated that GEP alone offers superior OS prognosis compared to FISH markers currently used. The same results were obtained when using the other high risk signatures, and when applied to the HOVON-65/GMMG-HD4 cohort.
Summary / Conclusion: GEP signatures are superior to FISH markers for high risk stratification in MM. Combining GEP and FISH does not result in better identification of a high-risk group in the MRC-IX and the HOVON-65/GMMG-HD4 cohorts. Our results are perhaps not surprising given the fact that some would classify e.g. t(4;14) as standard risk [1] while others still consider it to predict for worse survival [2].

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A

Cohort	Marker significant on given cohort (Yes: p<=0.05; No: p>0.05; NA: Not Available; *training cohort)										
	t(4;14)	t(11;14)	t(14;16) t(14;20)	add1q	del13	del17	SKY-92	UAMS-70	UAMS-17	UAMS-80	MRCIX-6
HOVON-65/GMMG-HD4 (n=290)	Yes	No	No	No	Yes	Yes	Yes*	Yes	Yes	Yes	Yes
MRC-IX (n=247)	No	No	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes*
TT2 (n=351)	NA	NA	NA	NA	NA	NA	Yes	Yes*	Yes*	Yes	Yes
TT3 (n=142)	NA	NA	NA	NA	NA	NA	Yes	Yes	Yes	Yes*	Yes
APEX (n=264)	NA	NA	NA	NA	NA	NA	Yes	Yes	Yes	Yes	Yes

B

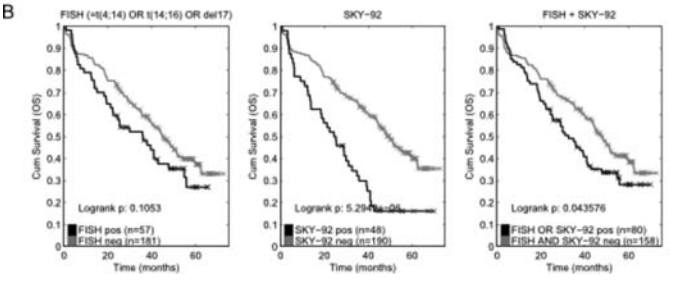

Figure 1. (A) Overview of univariate associations with overall survial; B) Kaplan Meier curves of the independent MRC-IX cohort showing the FISH high risk stratification (left), the SKY-92 GEP high risk signature (middle), and combination of the two (right).

Non-Hodgkin lymphoma - Clinical 1

S581

BRIEF RITUXIMAB, BENDAMUSTINE, MITOXANTRONE (R-BM) INDUCTION FOLLOWED BY RITUXIMAB CONSOLIDATION IN ELDERLY *DE NOVO* ADVANCED STAGE FOLLICULAR LYMPHOMA PATIENTS: A STUDY BY FONDAZIONE ITALIANA LINFOMI

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Background: a previous FIL trial (Vitolo et al, ASH 2011) showed that a brief R-FND (Fludarabine, Mitoxantrone and Dexamethasone) induction chemoimmunotherapy followed by rituximab consolidation achieved high CR and PFS rates in elderly Follicular Lymphoma (FL) patients (pts); bendamustine is a well-tolerated and effective drug.
Aims: These promising results prompted us to modify the original scheme and investigate safety and efficacy of a brief R-BM regimen for treatment of elderly FL lymphoma pts.
Methods: 76 elderly pts (age 65-80) with de novo FL were enrolled (Sept 2009-Nov 2011). Inclusion criteria were: grade I, II and IIIa; advanced (stage III/IV) or stage II disease requiring treatment; "FIT" pts according to comprehensive geriatric assessment (ADL, I-ADL, CIRS). Treatment plan was: 4 monthly courses of R-BM (375 mg/m^{2}Rituximab day 1, 90 mg/m^{2}Bendamustine days 1-2, 8 mg/m^{2}Mitoxantrone day 1) followed by 4 weekly Rituximab infusions as consolidation. Polymerase chain reaction (PCR) for BCL2/IgH rearrangementwas performed on bone marrow samples at diagnosis, after R-BM and at the end of consolidation.
Results: Median age was 71 years (range 65-79); 29 males, 47 females; WHO grading was as follows: I 18%, II 54% and IIIa 28%; 15% had advanced stage II disease, 30% stage III and 55% stage IV; 47% had BM involvement, 20% B symptoms and 7% leukemic dissemination; 59% patients had no comorbidity, 20% one and 21% 2 or more comorbidities. According to FLIPI pts were: 8% at low, 32% at intermediate and 60% at high risk. PCR analysis for BCL2/IgH rearrangement was carried out in 57 patients at diagnosis: 39 (51%) were Bcl-2 positive. Seventy (92%) pts completed the planned treatment: 60/70 in the planned time.Sixpts did not complete the treatment: 1 for progressive disease, 4 for adverse events (2 haematological toxicities with prolonged neutropenia; 1 CMV colitis and 1 for infection and concomitant worsening of pre-existing oral pemfigo) and 1 patient for worsening of performance status. Overall response to treatment was 92% with 58 (76%) complete remissions (CR) and 12 (16%) partial remissions (PR). Response to induction R-BM was as follow: 31 (41%) CR, 40 (52%) PR and 5 (7%) stable (SD) or progressive disease (PD); 29 (72%) of the 40 pts in PR/SD after R-BM converted to CR following further Rituximab consolidation. Twenty-three (60%) of 39 pts Bcl-2 rearranged at diagnosis were evaluable for molecular response during and after treatment: PCR negativity was achieved in 21/23 (91%) pts after R-BM and in 22/23 (96%) pts at the end of treatment; of these, 18/22 (82%) were also in complete remission. A total of 577 courses of R-BM and Rituximab were given. The most frequent severe toxicity (CTC grade 3-4) was neutropenia reported in 18% of the courses. Grade 3-4 extra-haematological toxicities (all resolved) were: 8 neutropenic fevers, 8 grade 3-infections (4 due to bacterial agents), 1 NSTEMI, 1 massive pulmonary embolism. Two deaths were recorded: one pneumonia with worsening of pre-existing pemfigo and one patient with hepatic metastasis of occult carcinoma diagnosed at final restaging after completion of therapy.
Summary / Conclusion: A brief course of chemo-immunotherapy R-BM followed by Rituximab consolidation is safe and effective with a high CR rate in elderly patients with untreated advanced stage FL. In patients with BCL2 rearrangement at diagnosis a high molecular remission was also observed. Data on PFS analysis will be presented with a longer median follow-up.

S582

ROLE OF THE QUANTITATIVE BCL2/IGH@ REARRANGEMENT EVALUATION IN PATIENTS AFFECTED BY FOLLICULAR LYMPHOMA: THE ANCILLARY STUDY OF THE FIL-FOLL05 TRIAL.

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Background: The role of the quantitative assessment of *BCL2/IGH@* fusion gene in patients affected by follicular lymphoma is still matter of debate, even because of the heterogeneity of the adopted therapeutic strategies. Other authors previously reported that 70% of patients with low amount of *BCL2/IGH@* copies achieved complete remission versus 26% of those with higher *BCL2/IGH@* levels, with a significant advantage also on EFS *(Rambaldi A, 2005)*.
Aims: In the present study, we report about significance of the quantitative *BCL2/IGH@* assessment at diagnosis and after treatment. Results from the qualitative assessment have been already reported at ASH 2012 *(Galimberti S, 2012)*.
In the FOLL05 trial, conducted by the Fondazione Italiana Linfomi (FIL), 504 untreated patients affected by advanced follicular lymphoma were randomized to receive R-CHOP, R-CVP, or R-FM (Clinical trial.gov NCT00774826).
Methods: Quantitative PCR assays were performed by the 4 laboratories of the "FIL MRD NETWORK" (Bologna, Pisa, Roma, and Torino, Italy). RQ-PCR was performed according to international guidelines (Ladetto M, 2001; van der Velden, 2007). All samples were tested in triplicate; standard curves were constructed using DNA extracted from DOHH-2 cell line and DNA extracted from mononuclear cells obtained from healthy donors. The sensitivity of these tests was 1:10^{-5}.
Results: At baseline, 415 out of the 504 patients eligible for treatment were assessed for qualitative *BCL2/IGH@* rearrangement; 220 of them (53%) were positive. No significant differences were detected between cases with and without molecular assessment at the enrollment, and between those positive or negative for clinical features, and arm of treatment.
At the enrollment, molecular tumor burden was assessed by quantitative PCR in 105 *BCL2/IGH@* positive cases; the median value was 3 x 10^{-3} copies (range: <1x10^{-5} - 6). After logistic analysis, the *BCL2/IGH@* copy number did not correlate with stage, ECOCG, age>65 year, sex, but it was significantly higher in patients at high FLIPI and FLIPI2 score. For patients with high *BCL2/IGH@* copies, overall response rate was significantly lower than cases with lower tumor burden (76.6% versus 38.9%; P=0.006). When the ROC curve was performed in order to find the *BCL2/IGH@* value that would better predict the relapse, < x10^{-4} copies was the more predictive value. Indeed, 22% of cases showing <1x10^{-4} copies relapsed versus 78% of patients with >x 10^{-4} copies (P=0.033). Moreover, cases displaying values <1 x 10^{-4} copies showed a clear advantage also in terms of PFS (3-year PFS 80% versus 59% for cases with higher tumor burden; P=0.015). On the contrary, the qualitative PCR results at diagnosis did not influence quality of response nor PFS. Concerning the impact of treatment on the *BCL2/IGH@* tumor burden, the mean observed reduction was about 2 logarithms. Seventy-one percent of cases achieved PCR-negativity; no differences in this effect were observed according to the arm of treatment. Nevertheless, an inferior tumor burden reduction in the R-CVP arm, although not statistically significant, was observed.
Summary / Conclusion: In conclusion, this study shows that patients with <1 x 10^{-4} copies of *BCL2/IGH@*at diagnosis would have longer PFS, thus supporting the usefulness of also a quantitative PCR monitoring of these patients.

S583

PRIMARY THERAPY OF WALDENSTRÖM'S MACROGLOBULINEMIA (WM) WITH WEEKLY BORTEZOMIB, LOW-DOSE DEXAMETHASONE AND RITUXIMAB (BDR): FINAL RESULTS OF A PHASE II STUDY OF THE EUROPEAN MYELOMA NETWORK (EMN)

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Background: Bortezomib and rituximab have shown signifcant clinicakl activity andsynergism in preclinical studies.
Aims: In this large, phase II, multicenter trial conducted by EMN in 10 European centers we evaluated BDR in previously untreated, symptomatic WM patients.
Methods: In order to prevent the rituximab-associated "IgM flare", one course of single agent bortezomib was first administered at a dose of 1.3 mg/m2 IV on days 1, 4, 8 and 11. Ten days later, the patients received 4 courses of weekly bortezomib, in order to reduce the incidence of neurotoxicity (1.6 mg/m2 days 1, 8, 15 & 22 every 35 days). In courses 2 & 5, bortezomib was given with dexamethasone 40 mg IV followed by rituximab 375 mg/m2 IV (total of 8 infusions of rituximab). After completion of BDR, patients with CR, PR, MR or SD were followed without further therapy.
Results: From March 2007 to June 2010, 60 patients were enrolled in the study and 59 received at least one dose of therapy. Their characteristics included: age>65 years in 61%, Hb<11.5 g/dL in 81%, platelet counts<100x109/L in 17%, b2-microglobulin>3 mg/dL in 64%, serum monoclonal protein>4 g/dL in 29% and >7 g/dL in 3.4%, lymphadenopathy in 43%, splenomegaly in 29%, while 43% had B-symptoms. Per IPSSWM, 15% were low, 40% intermediate and 45% high risk. Main indications to start treatment were cytopenias in 44%, hyperviscosity in 20%, B-symptoms in 19%, lymphadenopathy in 9% and other symptoms in 8%. Thirty eight (64%) patients completed the planned 5 courses of BDR and 8(13%) received only the first course of bortezomib. On intent-to-treat, responses included CR in 2(3%), PR in 37(64%), MR in 11(19%), SD in 3(5%) and PD in 6(10%) patients. Median time to first response (≥MR) was 3 months and median time to best response was 5.8 months. Despite the significant proportion of patients with high levels of IgM and symptoms of hyperviscosity, plasmapheresis was not required in any patient. Median follow up for all patients is 41 months. Estimated median PFS (progression or death by any cause) is 42 months and 3-year PFS is 71%, 49% and 45% for low, intermediate and high risk patients respectively. Nineteen patients received further treatment on progression and 84% achieved ≥MR. Fifteen patients have died, 9 due to causes unrelated to WM or complications of treatment. The 3-year OS is 82% and for low, intermediate and high risk patients is 85%, 86% & 76% respectively. The 3-year cause specific survival is 91%. Hematologic toxicities included neutropenia(grade≥3 in 15%) and thrombocytopenia (grade ≥3) in 5%. Peripheral sensory neuropathy (≥grade 1) was observed in 46%, grade 2 in 17% and grade≥3 in 7%; neuropathic pain recorded in 20% (grade≥3 in only 1 patient). Other common non-hematologic toxicities included GI toxicity (grade≥3 in 7%) and infections (20%, grade≥3 in 7%). One patient died of septic shock in absence of neutropenia. Three patients (5%) experienced pulmonary toxicity (grade≥3), which was attributed to bortezomib and resolved completely after administration of steroids and 2/3 patients continued treatment as per protocol. The dose of bortezomib was reduced in 51% of patients, primarily because of peripheral neuropathy; 36% of the patients completed the planned 5 courses without any dose reduction of bortezomib.
Summary / Conclusion: This is the largest trial that has evaluated the role of a bortezomib-containing regimen in the frontline treatment of symptomatic, mostly high risk patients with WM and also with a long follow up (median 3.5 years). BDR is active and non myelotoxic. Weekly administration of bortezomib reduces rates of severe neurotoxicity. Induction with single agent bortezomib is effective in the management of patients with hyperviscosity.

S584

MYD88 (L265P) MUTATION IS AN INDEPENDENT RISK FACTOR FOR PROGRESSION IN PATIENTS WITH IGM MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Background: Patients with IgM monoclonal gammopathy of undetermined significance (MGUS) have a risk of progression to Waldenström's macroglobulinemia (WM) or to other lymphoproliferative disorders (LPD) of approximately 2% per year. The level of the IgM paraprotein at the time of diagnosis is the main risk factor for progression (Kyle et al, Clin Lymphoma Myeloma Leuk 2011). Roughly half the patients with IgM-MGUS carry a somatic mutation in the *MYD88* gene [*MYD88* (L265P)] (Xu et al, Blood 2013). In a case-control study, we showed that IgM-MGUS patients carrying *MYD88* (L265P) are at higher risk of progression to WM or to other LPD than patients with wild type *MYD88* sequence (Varettoni *et al*, Blood 2013).
Aims: The aim of this study was to assess the relative contribution of the *MYD88* (L265P) mutation and of the size of IgM paraprotein on the risk of progression to WM or other LPD.
Methods: We analyzed by allele-specific polymerase chain reaction (AS-PCR) bone marrow (BM) samples of 136 consecutive patients with IgM-MGUS, collected at the time of diagnosis. Genomic DNA was extracted from BM mononuclear cells in 92 cases or retrieved from archival Giemsa-stained BM slides in 44 cases. AS-PCR was performed as previously described (Varettoni et al, Blood 2013). Progression-free survival (PFS) was evaluated with the Kaplan–Meier product-limit method. PFS was calculated from the date of diagnosis to the date of progression, or last follow-up for censored cases. The log-rank test was used to compare survival curves. The prognostic effect of *MYD88* mutation and of the size of IgM paraprotein was assessed using a Cox proportional hazards regression model.
Results: The median age of patients was 64 years, 78 (57%) were males and 58 (43%) females. At diagnosis of IgM-MGUS, the median level of serum IgM paraprotein was 11 g/L (range 2-32). Bence-Jones proteinuria was detected in 19% of patients. The median serum free light chain ratio was 1.1 (range: 0.2-170). Patients were followed for a total of 434 persons year (median, 32 months). The *MYD88* (L265P) mutation was detected in 71 of 136 patients (52%). During the period of observation, 11 of 136 patients (8%) progressed to WM (n=9) or to marginal zone lymphoma (MZL) (n=2). At diagnosis of IgM-MGUS, 8 of 9 patients who subsequently progressed to WM and 1 of 2 patients who progressed to MZL carried the *MYD88* (L265P) mutation. In a Cox proportional hazards model, the risk of progression in patients carrying the *MYD88* (L265P) mutation was significantly higher compared with that of patients with *MYD88* wild-type sequence (hazard ratio, HR 6.65; 95% confidence interval, 1.4-31.3, P=0.01). The risk of progression was 16% at 5 years and 56% at 10 years in patients with the *MYD88* (L265P) mutation compared to 2% at 5 years and 16% at 10 years in patients with *MYD88* wild-type sequence (P=0.006) (Figure 1). In multivariate analysis, the *MYD88* mutation and the size of the serum paraprotein were independent prognostic factor for progression, with a HR of 5.54 (P=0.03) and 3.66 (P=0.003), respectively.
Summary / Conclusion: The findings of this study indicate that the *MYD88* (L265P) mutation is an independent predictor of the risk of progression to WM or to other lymphoproliferative disorders in patients with IgM-MGUS, irrespective of the size of the IgM paraprotein.

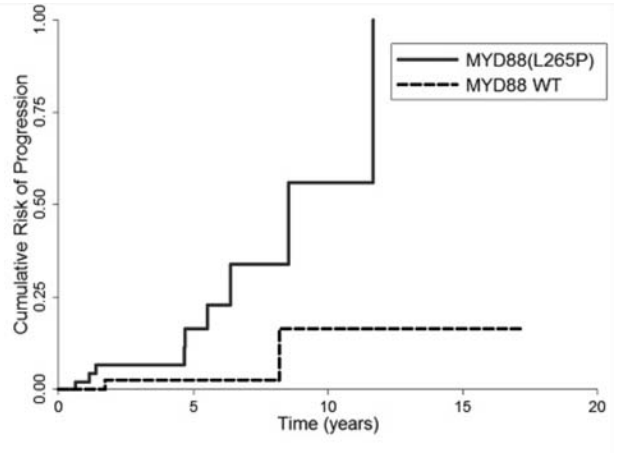

Figure 1.

S585

CHARACTERISTICS AND OUTCOME AMONG 123 PATIENTS WITH HIV ASSOCIATED LYMPHOMA INCLUDED IN THE FRENCH ANRS CO16 LYMPHOVIR COHORT STUDY

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Background: Human Immunodeficiency Virus (HIV) infection is associated with an increased risk of Hodgkin lymphoma (HL) and B-cell non-Hodgkin lymphoma (NHL). Increased risk of NHL is strongly correlated to the severity of the underlying immunodeficiency. Introduction of combined antiretroviral therapy (*cART*) has reduced the incidence of NHL but not the incidence of HL. Outcomes have been reported to be poorer among HIV-infected patients with HL or NHL than among non-HIV-infected patients.
Aims: We carry out a national cohort with the aim to study the characteristics and outcome of HIV-related lymphomas.
Methods: The prospective Cohort of HIV related lymphomas (ANRS-CO16 Lymphovir cohort) enrolled 123 adult patients at diagnosis of lymphoma in 32 centres between July 2008 and April 2012. Investigations were performed after approval of the ethic committee and informed consent. Data collection concerned HIV infection history, clinical, biological and histological presentation, treatment and evolution of lymphoma. Pathological materials were centralized and 91 cases were reviewed. Diagnoses were based on World Health Organization criteria. Each patient was followed every 6 months during 5 years.
Results: Among the 123 patients, 41% (50) were diagnosed with HL and 59% (73) with NHL. Median age were similar in the two groups of patients: 44 years (ranging from 20 to 61) among patients with HL and 48 years (23 to 67) among those with NHL. Gender (male/female) ratio was 6.1 (43/7) among patients with HL, 3.9 (58/15) among those with NHL. HIV infection had been diagnosed for a median of 161 months (0 to 312) among HL patients and 97 (0 to 327) among those with NHL. Patients with HL had a median CD4 T-cell count at diagnosis of lymphoma of 374/mm^3 (range 37-1518), those with NHL, 256/mm^3 (range 7-1322). Patients with HL had a predominance of mixed cellularity subtype (37 out of 40 reviewed cases). All the 44 tested cases for in situ EBV were positive. The histological distribution of the 73 NHL was: diffuse large B-cell lymphoma (DLBCL) 55% (40), Burkitt lymphomas (BL) 22% (16), plasmablastic lymphoma 10% (7), marginal zone /lymphoplasmocytic lymphoma 7% (5), others 6%: PTLD- like lymphoma (2), primary effusion lymphoma (1), follicular lymphoma (1), anaplastic lymphoma (1). Among the 21 reviewed cases of DLBCL, 11 were classified as non-GC and 7 as GC subtype (3 could not be classified). There was a predominance of clinical stages III/IV versus I/II among HL (79%, 37/47) and NHL patients (74%, 52/70). Among patients with DLBCL, LDH level was elevated in 71% (50/70) and performance status altered (2-4 versus 0-1) in 36% (26/72). The median interval between lymphoma occurrence and last follow-up was 21 months (range 0-41). During follow-up, all patients were treated with ARV. Among HL patients, 43 out of 46 were treated with ABVD. Out of 47 patients with DLBCL or BL, 31 received chemotherapies combined with rituximab. At 24 months, overall survival is above 90% among patients with HL and above 75% among those with NHL. There were 10 early deaths (<6 months) from complications of treatment (9) or disease progression (1) and 7 later deaths from disease progression (4), second cancer (2), unknown (1). None of the patients who died of infection during the first 6 months following diagnosis had received rituximab.
Summary / Conclusion: The present study points out the high proportion of HL among HIV infection with lymphoma in the cART era. Although these patients have advanced stage at diagnosis, their prognosis has largely improved. This study also strengthens the heterogeneity of HIV-related lymphomas and the persistence of early deaths among patients with NHL.

Acute myeloid leukemia therapeutics

S586

LOW-DOSE LENALIDOMIDE PLUS LOW DOSE CYTARABINE INDUCE COMPLETE REMISSION THAT CAN BE PREDICTED BY GENETIC PROFILING IN VERY ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Outcome for elderly patients with acute myeloid leukemia (AML) is extremely poor, especially for patients aged more than 70 years. Intensive induction chemotherapy is very often unsuitable. Lenalidomide is an immunomodulatory agent, FDA approved for use in MDS with del(5q) and in multiple myeloma. High-dose lenalidomide was proven to be effective in inducing complete remission (CR) in patients with and without 5q-acute leukemia. However, high-doses are not always feasible in the elderly, due to relevant toxicity. No data regarding concomitant administration of low-dose lenalidomide with other drugs in elderly AML patients have been published, up to now.
Aims: We designed a prospective phase II study to assess the efficacy of the concomitant administration of low-dose lenalidomide and low-dose cytarabine in patients with acute myeloid leukemia (AML) aged more than 70 years.
Methods: 40 patients (median age 76 years, range: 70-85) were consecutively enrolled in the study. Median white blood cell count at diagnosis was 3.2x10^9/l (range: 0,7-46,8x10^9/L), whereas median hemoglobin was 8,9 g/dl and median platelet count was 31x10^9/L. Nineteen out of 40 patients had an intermediate karyotype (14/19 normal), 17/40 an unfavorable karyotype and 4/40 were not evaluable. Seventeen patients had a *de novo* AML, whereas 23 patients had a secondary AML (15 after MDS, 4 after a CMPD, 1 after myelofibrosis, 3 after chemo-radiotherapy for a breast cancer). Patients received concomitant low-dose lenalidomide (10 mg/day orally, days 1-21) and low-dose cytarabine (20 mg twice day subcutaneously, days 1-15). Therapy was repeated every 6 weeks, up to 6 cycles. To identify possible biomarkers associated to sensitivity/resistance, global gene and miRNA expression profiling (Affymetrix Transciptome 2.0) was performed on purified AML cells obtained from 15 patients.
Results: Induction-period mortality was 20%, with 8 deaths occurring during cycle 1. Thirty-two patients completed at least one cycle of therapy and are evaluable for response. Overall CR rate was 38%. Six out of 12 (50%) responding patients are still in morphologic, cytogenetic and FISH CR after a median follow-up of 20 months (range: 6-33). Statistical analysis showed that responding patients had a longer median overall survival than non-responders (491 vs. 64 days, P <0.0001). The CR rate was significantly higher in patients presenting with bone marrow blasts <30% (P=0.04). Interestingly, cytogenetic risk was not predictive of CR. Conversely, by studying the global miRNA and gene expression profile we identified a molecular signature, including 114 genes and18 miRNA associated with the clinical response (CR vs. no CR). Of note, the involved genes belonged to relevant functional categories such as angiogenesis, cell cycle regulation and immune response. Moreover, based on the expression of 5 genes, we developed an algorithm to predict treatment response that was successfully validated in 15/15 (100%) tested cases.
Summary / Conclusion: The administration of concomitant low-dose lenalidomide and low-dose cytarabine induce a high rate of complete remission, that can be predicted by genetic profyling, in a subset of very elderly AML patients with extremely poor-prognosis, not suitable for intensive chemotherapy. The study was registered at EMA with the EUDRACT no 2008-006790-33.
Acknowledgements: Celgene is gratefully acknowledged for providing Lenalidomide for the patients. The study was supported in part by AIL Pesaro Onlus.

S587

PHASE II EVALUATION OF VOLASERTIB (BI 6727) + LOW-DOSE CYTARABINE (LDAC) VERSUS LDAC MONOTHERAPY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML): FOCUS ON GENETIC RESULTS

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Background: The prognosis for patients with AML ineligible for intensive remission induction therapy remains unsatisfactory, and novel therapeutic strategies are needed to improve clinical outcomes. Volasertib (an investigational agent) is a potent and selective cell cycle kinase inhibitor that induces mitotic arrest and apoptosis by targeting Polo-like kinases (Plks). Volasertib has shown antileukemic activity in AML (Bug et al, ASH 2010 and 2011; Maertens et al, ASH 2012).
Aims: The phase II part of this open-label trial was a randomized comparison of volasertib + LDAC versus LDAC in patients with newly diagnosed AML ineligible for intensive treatment. The primary endpoint was objective response (complete remission [CR] or CR with incomplete blood count recovery [CRi]); secondary endpoints included event-free survival (EFS) and overall survival (OS). We present cytogenetic and molecular genetic analyses of updated preliminary phase II data from this ongoing trial.

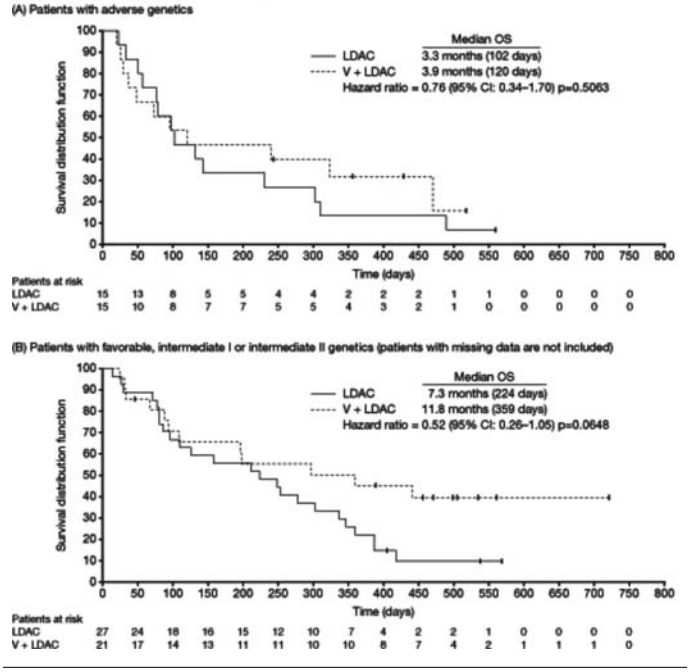

Figure 1. Overall survival by ELN genetic group.

Methods: Eligible pts who had given informed consent received volasertib (350 mg 1-hr intravenously, days 1, 15 Q4W) + LDAC (20 mg bid subcutaneously, days 1–10 Q4W), or LDAC alone until progression/relapse or intolerance. Bone marrow samples were taken at screening for analysis of conventional cytogenetics and molecular genetics (*FLT3* and *NPM1*) in a central laboratory.
Results: 87 patients received volasertib + LDAC (n=42) or LDAC (n=45); as of February 21st 2013, four patients remain on treatment. Genetic groups by European LeukemiaNet (ELN) classification were (n of patients; volasertib + LDAC/LDAC): favorable, 3/5; intermediate I or II, 18/22; adverse, 15/15; missing, 6/3. Response rate (CR or CRi) was significantly higher for volasertib + LDAC versus LDAC patients (31.0% versus 13.3%; odds ratio, 2.91; P=0.0523). Responses with volasertib + LDAC were observed across genetic groups, including patients with adverse cytogenetics. Response by ELN classification was (n of patients; volasertib + LDAC/LDAC): favorable, 2/0; intermediate I or II, 4/4; adverse, 5/2 (including 5/1 with complex karyotype); missing, 2/0. Molecular genetic analysis (volasertib + LDAC/LDAC) showed *FLT3*-ITD in 5/6 patients and *NPM1* mutations in 8/9 patients. Response by molecular genetics was (n of patients; volasertib + LDAC/LDAC): *FLT3*-ITD, 1/2; *NPM1* mutations, 4/4. Median EFS was significantly improved in volasertib + LDAC versus LDAC patients (5.6 months versus 2.3 months; hazard ratio [95% CI], 0.56 [0.34–0.93]; P=0.0237). Median OS was 8.0 months (volasertib + LDAC) versus 5.2 months (LDAC; hazard ratio [95% CI], 0.66 [0.40–1.08]; P=0.0996). OS for both treatments was shorter in patients with adverse versus non-adverse genetics, with a trend for OS benefit with volasertib + LDAC in both groups (Figure 1).
Summary / Conclusion: Responses with volasertib + LDAC were seen across all genetic groups in patients with newly diagnosed AML ineligible for intensive treatment. The increase in response rate with volasertib + LDAC translated into a trend for OS benefit, apparent in both non-adverse and adverse genetic groups. Volasertib combined with LDAC is in phase III development for newly diagnosed AML in patients ≥65 years ineligible for intensive remission induction therapy (POLO-AML-2).

S588

RISK ADAPTED TREATMENT FOR ADULT ACUTE MYELOID LEUKEMIA (AML): FINAL RESULTS OF THE AML-03 TRIAL FROM THE SPANISH CETLAM GROUP

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Background: Improvements on the clinical and biological stratification of AML cases allow provide background for risk-adapted treatment strategies, particularly after complete remission (CR) achievement. This has been the approach of our cooperative group including 20 academic institutions since 1988. Herein we report the final results of our most recent trial.
Aims: To analyse the results of the risk adapted treatment in 862 patients with de novo AML enrolled between 2003 and 2012 into the AML-03 protocol of the Spanish CETLAM group
Methods: Patients received 1 or 2 induction chemotherapy courses of IDICE-G (idarubicin, intermediate cytarabine (IDC), VP-16 and priming with GCSF) followed by mitoxantrone, IDC and priming with GCSF as consolidation therapy. Further treatment was assigned according to the CETLAM risk groups as follows: *Favorable prognosis (FP)* defined as favorable cytogenetics according to MRC (*AML1/ETO* and *CBF/MYH11* AML): autologous stem cell transplantation (ASCT) if leukocyte index (LI) was >20 or high dose cytarabine (HDAC) as in the CALGB schedule if LI≤20. *Intermediate prognosis (IP)*, defined as patients in CR with one induction,≤$50x10^9$ white blood cells at diagnosis, normal karyotype (NK) and noFLT3 internal tandem duplication (*FLT3-ITDwt*) and no *MLL* rearrangement: ASCT. *Adverse prognosis (AP)*, patients not included in FP or IP: ASCT or allogeneic stem cell transplantation (alloSCT) depending on donor availability
Results: Median age at diagnosis was 53 years old (15-71). *FLT3-ITD* was detected in 125 patients (32.1%) and *NPM1* mutation in 151 (38.8%). Four patients died before treatment and 858 patients (99.1%) received induction therapy; CR rate was 76.4% (87.8% with one course), 10.4% were refractory and 12.1% died during induction. Overall survival (OS) and disease-free survival (DFS) of the whole series was 40±2% and 44±2% respectively at 5 years. Cumulative incidence of relapse (CIR) was 39±4% at 5 years. Results according to intention of treatment were: *FP (n=88, 13.7%):* 5-year OS and DFS 81±5% and 72±5%, respectively, CIR 20±5%.There were no differences in OS, DFS and CIR depending if they received HDAC or ASCT; *IP (n=120, 18.7%):* 5-year OS, DFS and CIR were 68±5%, 52±6% and 43±5% respectively. *NPM1* mutation was associated with improved outcome compared with *NPM1wt* (DFS: 55±5 vs 37±5%, P=0.026; CIR: 32±4% vs 48±5%, P=0.032 respectively). *AP (n=399, 62.2%):* 5-year OS, DFS and CIR were 44±3%, 40±3% and 45±3% respectively. Statistical differences were found in CIR when comparing ASCT vs allo-SCT (52±4% vs 38±4%, P=0.006). 32 patients (5.3%) were not evalu-

able due to lost of follow up. We analysed patients with normal karyotype (n=389) and *FLT3* mutation and patients with *FLT3-ITD* had a worse OS and DFS compared with *FLT3wt* (30±5% vs 45±4%, p<0.001; 37±6% vs 48±4%, P=0.006, respectively). Multivariate analysis showed that age>50 y-o, two courses to CR, MRC group, *FLT3-ITD* and double negativity for mutations in *FLT3* and *NPM1* were associated to worse OS and DFS, with high relapse incidence being the main cause for treatment failure

Summary / Conclusion: Age, cytogenetics, molecular findings and courses to CR are important prognostic factors. AML is a biologically and pronostically heterogeneous disease subsidiary to be approached with risk adapted strategies. Currently it is feasible to identify a substantial proportion of patients that may be cured with high-dose cytarabine consolidation or ASCT. In contrast, there are patients with adverse genetic features that need an allogeneic procedure, investigational agents or both

S589

FINAL REPORT OF PHASE II TRIAL OF COMBINATION OF SORAFENIB AND 5-AZACYTIDINE IN PATIENTS WITH FLT3-ITD POSITIVE ACUTE MYELOID LEUKEMIA

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Background: A potential mechanism of resistance to FLT3 kinase inhibitors is high levels of FLT3 ligand (FL) as seen after myelosuppressive chemotherapy. We hypothesized that combining sorafenib with a less myelosuppressive agent, such as 5-azacytidine (AZA), may lead to higher and more durable responses. Both drugs have demonstrated a potential for inducing differentiation in AML cells, providing further rationale for the combination.

Aims: Determine the safety and efficacy of sorafenib plus AZA in pts with AML and *FLT3*-ITD.

Methods: Pts were eligible if they had relapsed or refractory AML, were 18 years of age or older, and had adequate performance status (ECOG ≤ 2) and organ function. Older pts without prior therapy were also eligible, if they were deemed unsuitable to receive chemotherapy. Presence of *FLT3*-ITD was not a requirement. Treatment regimen included AZA 75 mg/m^2 daily for 7 days together with sorafenib 400 mg twice daily for 28 days; cycles were repeated in 4 to 5-week intervals. Overall responses were assessed after the completion of at least one cycle of therapy. Plasma inhibitory activity (PIA) assay was performed using plasma collected on days 1 and 10 of each cycle. Plasma FL concentrations were measured using an ELISA kit.

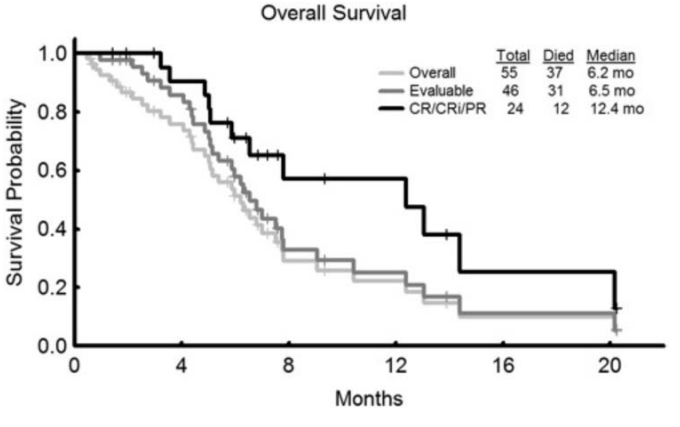

Figure 1.

Results: 55 pts with AML with a median age of 64 years (range, 20-87) were enrolled. They included 26 (47%) pts with diploid cytogenetics, 12 (22%) with chromosome 5/7 or complex cytogenetic abnormalities, 3 (5%) with insufficient metaphases, and 14 (25%) with miscellaneous abnormalities. Prior to the initiation of treatment, *FLT3*-ITD was detected in 51/55 (93%) pts with a median allelic ratio of 0.31 (range, 0-0.93). They had received a median of 2 prior treatments (range, 0-7). 20 (36%) pts had received ≥3 prior regimens and 12 had failed therapy with FLT3 kinase inhibitors (6 with AC220, 2 with PKC412, and 9 with sorafenib, either as monotherapy or with chemotherapy or plerixafor); 4 had failed 2 prior FLT3 inhibitors. 6 pts were inevaluable as they discontinued therapy before response assessment at one month and 3 are too early for response assessment. The overall CR/CRi/PR rate among the 46 evaluable pts is 52%, including 15 (33%) with CRi and 8 (17%) with CR and 1 (2%) with PR (in this pt, bone marrow blast declined from 51% to 6% with normalization of blood counts). Overall, pts have received a median of 3 (range, 1-21) treatment cycles with the median number of cycles to response being 2 (range, 1-4) and the median time to achieving response, 2.1 months (range, 0.9-4.6 months). The median duration of CR/CRi Is 2.3 months (range, 1 - 18.1$^+$ months). Six responding pts have proceeded to allogeneic stem cell transplant. The most common study drug-related adverse events were rash and fatigue with no deaths but one grade 3 cardiomyopathy attributable to study medications. The median survival for the total population is 6.2 months and for the 46 evaluable pts is 6.5 months. The median survival for pts achieving a response is12.4 months. Plasma samples spanning at least one cycle of therapy were available for 22 pts. Among them, 64% achieved FLT3 inhibition to a targeted level of less than 15% of baseline during their first cycle of therapy. Median survival in pts who achieved this degree of inhibition was 238 days, and in pts who did not reach this level was 154 days (P=0.13). Mean FL levels at cycle 1, day 1 and cycle 1, day 10 were 9 pg/mL and 17 pg/mL, respectively. Mean FL levels at cycle 2, day 0 and cycle 2, day 10 were 27 pg/mL and 54 pg/mL, respectively.

Summary / Conclusion:
Combination of AZA and Sorafenib is effective in pts with relapsed and refractory AML and *FLT3*-ITD mutation. There was a trend toward improved survival in pts with adequate FLT3 inhibition during cycle 1. FL levels did not rise to the levels seen in pts receiving cytotoxic chemotherapy.

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SORAFENIB VERSUS PLACEBO IN ADDITION TO STANDARD THERAPY IN YOUNG PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA: RESULTS FROM 264 PATIENTS TREATED IN THE RANDOMIZED-CONTROLLED SORAML TRIAL

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Background: Sorafenib is a multi-kinase inhibitor with activity against several intracellular kinases, which may play a role in the pathogenesis of acute myeloid leukemia (AML). In-vitro data and results from non-randomized clinical trials suggest that sorafenib might be an effective drug for the treatment of AML. So far, no randomized-controlled data are available for treatment of newly diagnosed AML patients up to the age of 60 years. We present the first randomized placebo-controlled efficacy and safety results from the SORAML trial of the Study Alliance Leukemia (SAL).

Aims: To evaluate the efficacy and tolerability of sorafenib added to standard chemotherapy in younger acute myeloid leukemia patients.

Methods: Between March 2009 and October 2011, 276 patients from 25 centers were enrolled in the SORAML trial (NCT00893373). The main eligibility criteria were: newly diagnosed AML, age from 18 to 60 years and suitability for intensive therapy. The treatment plan for all patients included two cycles of induction with DA (daunorubicin 60 mg/m^2 days 3-5 plus cytarabine 100 mg/m^2 cont. inf. days 1-7), followed by three cycles of high-dose cytarabine consolidation (3 g/m^2 b.i.d. days 1, 3, 5). Patients without response after DA I received second induction with HAM (cytarabine 3 g/m^2 b.i.d. days 1-3 plus mitoxantrone 10 mg/m^2 days 3-5). Allogeneic stem cell transplantation was scheduled for all intermediate-risk patients in first complete remission with a family donor and for all high-risk patients with a matched donor. At study inclusion, patients were randomized to receive either sorafenib (800 mg/day) or placebo as add-on to standard treatment. Block randomization at a ratio of 1:1 was performed within cytogenetic and molecular risk strata, allocation was concealed and treatment was double blinded. Study medication was given on days 10-19 of DA I+II or HAM, from day 8 of each consolidation until 3 days before the start of the next consolidation and as maintenance for 12 months after the end of consolidation. The primary endpoint of the trial is event-free survival (EFS) with an event being defined as either failure to achieve a complete remission (CR) after induction, relapse or death. Secondary endpoints were overall survival (OS), CR rate and incidence of adverse events (AE). We present the results of the planned interim analysis (intent to treat) after the occurrence of 50% of EFS events. The O'Brien/Fleming adjusted significance level was set at P=0.0052.

Results: Out of 276 randomized patients, 264 were evaluable for EFS, 132 in each arm. Demographic and disease characteristics were equally distributed between the two arms; the FLT3-ITD incidence was 16%. The median cumulative dose of administered study medication was equal in both arms. The CR rates were 56% versus 60% in the placebo versus sorafenib arm (P=0.622). By the time of analysis, a total number of 100 events had occurred. After a median observation time of 18 months, the median EFS was 12.2 months in the

placebo arm and was not reached in the sorafenib arm, corresponding to a 1-year EFS of 50% versus 64% (P=0.023). The median OS had not been reached in both arms, the 2-year OS was 66% versus 72% in placebo and sorafenib arms, respectively (P=0.367). The most common reported AEs CTC Grade ≥3 were infectious complications including fever and pneumonia, followed by bleeding events, cardiac and hepatic toxicity, hypertension, skin toxicity and headache. The risk for hepatic toxicity (relative risk 6.2, P=0.025) and bleeding events (relative risk 3.6, P=0.016) was significantly higher in the sorafenib arm while the incidence of all other AEs showed no significant differences.
Summary / Conclusion: In younger AML patients, the addition of sorafenib to standard chemotherapy is feasible but associated with a higher risk of liver toxicity and bleeding events. Sorafenib treatment resulted in a marked EFS prolongation; this difference is not significant according to the adjusted significance level of this interim analysis. Results from the final analysis including post-hoc exploration of molecularly defined subgroups are necessary for drawing final conclusions on the efficacy of sorafenib.

Myelodysplastic/Myeloproliferative Neoplasm - Clinical / Biology

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LONG-TERM INTERVENTION EFFECTS ON BONE MARROW MORPHOLOGY IN MYELOFIBROSIS: PATIENTS TREATED WITH RUXOLITINIB AND BEST AVAILABLE THERAPY

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Background: Myelofibrosis (MF) is a myeloproliferative neoplasm associated with splenomegaly, debilitating symptoms, progressive bone marrow (BM) fibrosis, and shortened survival. With the exception of BM transplantation, no therapy has shown a significant impact on disease progression. Ruxolitinib is an oral JAK1/JAK2 inhibitor that has demonstrated improvements in spleen volume, symptoms, and survival in patients (pts) with MF.
Aims: This study was conducted to gain insight into the effects of long-term ruxolitinib treatment on BM morphology in pts with MF and compare them to those seen in a best available therapy (BAT) cohort.
Methods: Trephine biopsies were obtained at baseline, 24 (68 pts) and 48 (18 pts) months (mo) from MF pts treated at MD Anderson Cancer Center who participated in a phase I/II trial of ruxolitinib (NCT00509899). The details of the study design and clinical outcomes have been published previously [Verstovsek, NEJM 2010]. Two of the authors (JT and H-MK) independently quantified BM fibrosis according to the World Health Organization (WHO) grading scale (0-3), and a third author (CB-R) reviewed the findings. Consensus decided discordant scores. Reviewers were blinded to pts characteristics and outcomes. WHO-defined BM fibrosis grading was also determined in prospectively collected specimens within a German, multicenter, observational database from a cohort of 139 pts (160 biopsies) treated with BAT for 24 (97 pts) and 48 (63 pts) mo. BAT included hydroxyurea [HU] (47%), interferon-alpha (7%), or assorted sequential therapies (25%). No active – or only supportive – therapy was given in 21% of the BAT cases. Biopsy intervals in the ruxolitinib-treated pts were defined per protocol; in the BAT cohort, biopsies were mostly performed based on a given patient's change in clinical condition at the discretion of the treating physician.
Changes in fibrosis grade vs. baseline were calculated for all time points and categorized as improvement, stabilization, and worsening. Additional analyses on changes over time in the degree of collagen deposition, amount of osteosclerosis, and BM cellularity were performed only in the ruxolitinib-treated cohort.
Results: At baseline, 21% of the ruxolitinib-treated cases presented with WHO-defined fibrosis grade 1, 53% with grade 2, and 26% with grade 3. At baseline, in this group, accumulation of collagen fibers was observed in 32 cases (47%): 30% with mildly increased (grade 1) and 17% with manifest or intense (grade 2 or grade 3) collagen deposition. About half of these pts had appreciable baseline osteosclerosis (grade 1: 32%; grade 2: 9%; grade 3: 9%). There were no significant differences in the distribution of baseline WHO-defined fibrosis grades between the ruxolitinib and BAT groups (P=0.441 by Cochran–Mantel–Haenszel test).
A higher percentage of ruxolitinib-treated pts showed stabilization or improvement in WHO-defined BM fibrosis at both the 24 and 48 mo time points vs. BAT pts. Worsening in fibrosis was significantly more prevalent in the BAT cohort at both time points.
Summary / Conclusion: This analysis of the effects of ruxolitinib in MF provides evidence that long-term therapy with this JAK inhibitor may meaningfully retard advancement of BM fibrosis. A comparable effect was not seen with long-term BAT. These results expand upon earlier observations using the same approach but with a smaller control cohort, consisting of pts treated with HU alone. Additional research is needed to elucidate the positive impact of JAK inhibition on BM morphology.

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THE INTERFERON SCORE TOWARDS PREDICTION OF RESPONSIVNESS TO INTERFERON ALPHA IN ESSENTIAL THROMBOCYTHEMIA

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Background: Interferon-alpha 2 (IFN) is able to induce hematological response in about 70-80% of ET patients but some of them could be defined as bad responders.
IFN binding its receptor results in tyrosine cross-phosphorylation and autophosphorylation of the JAKs proteins (Tyk2 and Jak1). These phosthyrosines recruit and activate STAT family member such as STAT1 and STAT3. These proteins induce the transcription of SOCSs, whose role is to extinguish cytokine

signaling by inhibition of JAK kinase-activity directly through the KIR-domain, and indirectly promoting the proteasomal degradation of Jak2, by SOCS-box-motif. In summary, IFN induces the expression of SOCSs, which inhibit TPO mediated signaling through Jak2 double inhibition. This allows IFN-α and TPO pathway to cross-talks by means of the JAK-STAT-SOCS cascade.
Aims: In order to identify molecular markers that discriminate responders from non-responders to IFN, we analyzed bone marrow cells transcript levels of specific genes involved in the IFN receptor pathway, which signal cross-talks with the JAK-STAT pathway under TPO receptor. In particular we investigated the mRNA expression of JAK1, TYK2, STAT1, STAT3, SOCS1 and SOCS3.
Methods: We analyzed 60 ET patients treated with 3 million units of IFN-α-2b 5 times a week as induction (3 months), and 3 times a week as maintenance. Two groups of response were identified: *Good-Responders(R)* (n=44), who achieved complete response according to European Leukemia Net criteria, and *Bad-Responders(NR)* (n=17) who failed. The mRNA expression of genes of interest was measured in bone marrow samples from ET patients by RTq-PCR and tested for their predictive value using receiver operating characteristics (ROC) curves. Data were normalized as following: [mRNA normalized copy number (NCN)=mRNA target gene/mRNA GUSB*104]. An IFN score was calculated as an average in log2 of mRNA levels of genes differently expressed between *Good-R* and *Bad-R*.
Results: Main clinical characteristics were similar between the two groups of response. JAK2 V617F mutation was detected in 56,8% of *Good-R* and 58,8% of *Bad-R* (P=0,81) and no difference was found in JAK2V617F allele burden (P=0,17) and mRNA expression (P=0,2). Patients showed a median spleen volume of 500 mL in *Good-R* and 250 ml in *Bad-R* group (P=0.01). *Bad-R* compared with *Good-R* showed higher mRNA expression of JAK1 (134465 vs 44647; P<0.00001), STAT3 (49210 vs 23959; P=0.0002) and SOCS3 (18667 vs 10361; P=0,015). The AUC, using the normalized gene expression values, was 0.88 for JAK1, 0.81 for STAT3 and 0.7 for SOCS3. Average expression in log2 of these three genes was calculated and used as IFN score. The analysis reveled an AUC of 0.9 for this IFN signature (P<0,00001). The optimal cut-off point for IFN score to discriminate between *Good-R* and *Bad-R* was 15,75 and produced a sensitivity of 94,1%, specificity of 88,6% and likelihood ratio of 9.
Summary / Conclusion: We identified this set of three genes whose expression status could be translated into IFN score that showed a significant correlation with response outcome in ET.
Therefore, IFN score could represent a predictive biomarker for responsiveness to IFN and is likely to become a substantial aid to the physician, taking the paradigm of tailored therapy one step further, especially in chronic diseases such as ET.

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DEVELOPMENT AND CHARACTERIZATION OF A MURINE MODEL FOR LEUKEMIC TRANSFORMATION OF MYELOPROLIFERATIVE NEOPLASMS FOR PRECLINICAL THERAPEUTIC STUDIES

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Background: Transformation to acute myeloid leukemia (AML) represents a major clinical consequence of the Philadelphia-chromosome negative myeloproliferative neoplasms (MPNs) (Polycythemia Vera (PV), Essential Thrombocythemia (ET), and Primary Myelofibrosis (PMF)). Leukemic transformation (LT) after MPN occurs in as many as 23% of PMF patients within 10 years of diagnosis, and 4-8% of PV and ET patients develop AML in the first 18 years after diagnosis. The development of a post-MPN AML is associated with a poor clinical outcome, and characterized by a poor response to conventional anti-leukemic therapies. Although somatic mutations in the JAK-STAT signaling pathway occur in the majority of MPN patients, the somatic mutations that drive LT from a pre-existing MPN have not been fully delineated. Recent candidate mutational studies have identified recurrent somatic mutations in a subset of known leukemogenic disease alleles at the time of transformation from MPN to AML, including mutations in *TP53, IDH1/2, TET2* and *SRSF2.* However, the functional contribution of these specific genetic events to LT has not been delineated
Aims: To develop a genetically accurate murine model of LT, in order to further understanding of progression from MPN to AML and to use this preclinical model to test novel therapeutic approaches.
Methods: Expression of the *JAK2*V617F mutation in combination with *Tp53* loss was modeled *in vivo.* Bone marrow (BM) from C57/Bl6 *Tp53*$^{-/-}$ and littermate control mice was infected with *JAK2*V617F-IRES-GFP retrovirus, followed by transplantation of transduced cells into lethally irradiated congenic recipients. Outcomes measured included: survival, peripheral blood counts, organ weights, flow cytometry analysis of bone marrow and spleen derived cells, and morphologic evaluation of bone marrow and spleen.
Results: Transplantation of *JAK2*V617F/*Tp53*$^{-/-}$ cells, but not *JAK2*V617F positive cells was associated with impaired survival; 50% of mice injected with *JAK2*V617F/*Tp53*$^{-/-}$ cells died by day 100, whereas all mice injected with *JAK2*V617F positive cells survived 100 days or longer (P=0.011) (figure1). Mice injected with *JAK2*V617F/*Tp53*$^{-/-}$ cells presented with significant leukocytosis, with a mean WBC of 38.4 in mice engrafted with *JAK2*V617F/*Tp53*$^{-/-}$ cells compared with 11.4 in *JAK2*V617F/*Tp53* wildtype mice (no p value). At the time of sacrifice, all mice engrafted with *JAK2*V617F/*Tp53*$^{-/-}$ cells had increased numbers of blasts in the peripheral blood and bone marrow, as assessed by morphologic evaluation and flow cytometric analysis of CD117 expression. The disease from *JAK2*V617F/*Tp53*$^{-/-}$ cells, but not *JAK2*V617F positive cells, was transplantable into secondary recipients consistent with increased self-renewal *in vivo*. Flow cytometric analysis of spleen and bone marrow derived cells from leukemic mice demonstrated an increased percentage of megakaryocyte-erythroid progenitors (MEPs) and erythroblasts. *In vitro* assays and *in vivo* studies in secondary transplantation studies were carried out using this model. INCB18424 and CYT 387 exposure resulted in dose-dependent inhibition of colony formation *in vitro*. *In vivo* testing of INCB18424 and the HSP90 inhibitor PU-H71 was carried out in secondary recipients and the results of this preclinical trial will be presented.
Summary / Conclusion: The expression of *JAK2*V617F plus *Tp53* loss, a genotype commonly seen in patients who transform to AML after MPN, efficiently models LT *in vivo*. This model can now be utilized to assess the leukemic cell of origin in transformed disease, and to test novel therapeutic strategies which will inform clinical trials in this poor-risk hematologic malignancy.

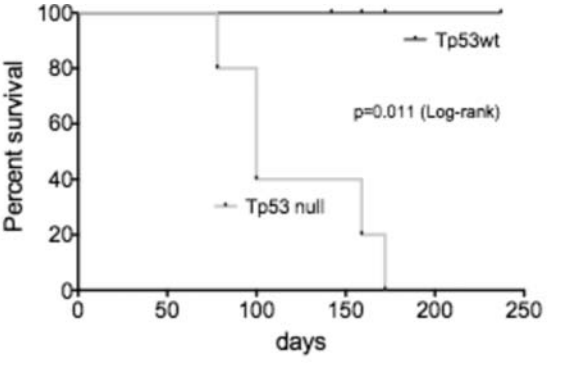

Figure 1.

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INCIDENCE, TREATMENT AND SURVIVAL OF MYELODYSPLASTIC SYNDROMES: A POPULATION-BASED STUDY OF 5,144 PATIENTS DIAGNOSED IN THE NETHERLANDS FROM 2001 TO 2010

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Background: At the beginning of the new millennium, myelodysplastic syndromes (MDS) were formally classified as malignant myeloid neoplasms by the World Health Organization (WHO), and consequently MDS became reportable malignancies to population-based cancer registries as of 2001. To date, studies on incidence and survival in MDS based on data from population-based cancer registries are scarce. Further, studies regarding treatment decisions in the entire MDS population are not available.
Aims: We conducted a nationwide population-based study to assess trends in incidence, initial treatment and survival among newly diagnosed patients with MDS during a 10-year period in the Netherlands.
Methods: Morphology codes of the International Classification of Diseases for Oncology Third Edition (ICD-O-3) were used to identify newly diagnosed patients with MDS from the nationwide Netherlands Cancer Registry (NCR) from 2001 to 2010 with follow-up to 2011. All MDS subtypes according to the ICD-O-3 were included in the NCR, namely refractory anemia (RA), RA with ringed sideroblasts (RARS), MDS with isolated del(5q) (5q- syndrome), refractory cytopenia with multilineage dysplasia (RCMD), RA with excess blasts (RAEB) and MDS, not otherwise specified. Age-standardized incidence rates (ASRs) of MDS were calculated per 100,000 person-years. Relative survival rates (RSRs) were calculated as a measure of disease-specific survival. If initial treatment was started within 9 months after diagnosis, it was recorded in the NCR.
Results: We identified a total of 5,144 newly diagnosed patients with MDS from January 1, 2001 to December 31, 2010 (median age 74 years). Of these patients, 486 (9%) were classified as RA, 583 (11%) RARS, 82 (2%) the 5q-syndrome, 524 (10%) RCMD, 966 (19%) RAEB, while 2,503 (49%) were

unspecified. Interestingly, the proportion of unspecified MDS decreased from 60% in 2001 to 36% in 2010. The reported number of patients diagnosed with MDS increased throughout the study period; however, the annual ASR reached a plateau at 2.8 since 2007. Men had a higher overall incidence than women (3.3 *v* 1.7). The age-specific incidence of MDS during the entire study period increased in parallel with older age, from 0.3 among those aged 50 years or younger to 28.5 among those aged 80 years or older. Of all patients, 4,562 (89%) did not receive treatment or only received supportive care, 348 (7%) received chemotherapy and 74 (1%) received chemotherapy followed by a stem cell transplantation. Survival in MDS did not improve over time. All MDS subtypes had inferior survival compared with the expected survival in the general population (Figure 1A). Five-year RSRs were 53% in patients with RA, 58% in patients with RARS, 48% in patients with the 5q- syndrome, 38% in patients with RCMD, 18% in patients with RAEB, and 39% in patients with an unspecified MDS. Age at diagnosis was an important predictor for survival as RSRs decreased in parallel with older age (Figure 1B). Five-year RSRs were 59% for patients aged 18-49 years, 52% for patients aged 50-59 years, 41% for patients aged 60-69 years, 36% for patients aged 70-79 years, and 29% for patients aged ≥80 years. The RSRs between both sexes were comparable.

Summary / Conclusion: The incidence of MDS in the Netherlands is similar to other Western European countries and increased over time due to improved notification. Morphological assessment of MDS according to the WHO classification seems challenging as almost half of the recorded MDS cases were unspecified. Nevertheless, the proportion of specified cases increased over time, which was presumably due to better knowledge about the WHO classification of MDS. The lack of improvement in patient survival might be explained by the limited availability and use of therapeutic agents. Therefore, more emphasis is needed to improve current treatment strategies and to develop new treatment options in MDS.

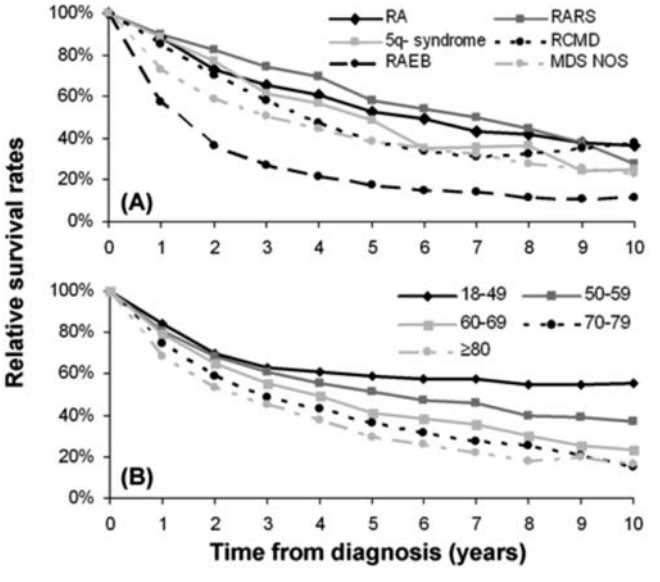

Figure 1. Cumulative relative survival among patients diagnosed with myelodysplastic syndromes in the Netherlands by (A) subtype and (B) age at diagnosis throughout the study period from 2001 to 2010.

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A PILOT PHASE ONE DOSE FINDING SAFETY STUDY OF A THROMBOPOIETIN-RECEPTOR AGONIST, ELTROMBOPAG, IN PATIENTS WITH MYELODYSPLASTIC SYNDROME TREATED WITH AZACITIDINE

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Background: Thrombocytopenia is a common manifestation of Myelodysplastic Syndroms (MDS) and is an independent adverse prognostic factor for survival. Azacitidine (Aza) is today the treatment of choice for patients with high-risk MDS not eligible for stem cell transplantation. In the pivotal randomized studies with Aza, grade 3-4 thrombocytopenia was reported in up to 85% of patients, and the main causes of Aza discontinuation were hematological adverse events. In parallel to the use of growth factors such as Erythropoietin and G-CSF in MDS, stimulating platelet production by a thrombomimetic agent may be an attractive alternative to platelet transfusions. Eltrombopag is a novel thrombomimetic agent that activates the thrombopoietin receptor (TPO-R) and stimulates megakaryopoiesis and thrombopoiesis. Studies with eltrombopag in leukemia cell lines did not show enhancement of leukemia cell proliferation, instead inhibition of leukemia cells was observed in the majority of tested cell lines.

Aims: In this Phase I Pilot Study (ClinicalTrials.gov identifier: NCT01481220) we explored the safety and tolerability of combining eltrombopag with Aza in the treatment of patients with high-risk MDS.

Methods: Patents with high-risk MDS eligible to treatment with Aza according to the labeled indication and thrombocytopenia (Platelet counts $<75x10^9$/L) were included. Cohorts of three patients received Aza in combination with increasing doses of eltrombopag tablets (50-100 mg, 100-200 mg, 200-300mg and a final cohort with 300mg as an unchanged dose) during 3 Aza cycles (13 weeks). Patient monitoring included repeated blood and bone marrow samples.

Results: Eleven patients, with a median age of 71 (range 53-83) have been included. Reported severe adverse events include one bacterial bronchitis (cohort 1, eltrombopag 50-100 mg), recurrent erysipelas (one patient in cohort 3, eltrombopag 200-300 mg), one case of fatal pneumonia with E. coli septicemia (Cohort 3) and one deep vein thrombosis, elevated liver enzymes and progressive disease occurring in a patient with highly proliferative MDS-AML (Cohort 3). This dose-cohort is now being completed with 3 more patients. Platelet counts improved or remained stabile in 9/11 patients despite Aza treatment while two patients remained dependent on platelet transfusion. Median platelet counts were 40, 51 and 64 x 10^9/L at inclusion, after one and after three Aza cycles, respectively. Bleeding symptoms were uncommon. No MDS disease progressions related to study medication were reported. Three more patients are to be recruited to the final cohort and updated results will be presented during the 18th EHA congress.

Summary / Conclusion: The combination of eltrombopag with Aza in high-risk MDS patients with thrombocytopenia is feasible and well tolerated. Improvements in platelet counts and anti-leukemic effect through adding eltrombopag cannot be excluded and needs to be explored in a phase-two study.

Stem cell transplantation - Clinical 2

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TEN-YEAR LONG TERM SURVIVAL AFTER UP-FRONT AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA: RESULTS FROM TWO PROSPECTIVE CLINICAL TRIALS

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Background: Survival of patients with multiple myeloma (MM) has been extended with the introduction of autologous stem cell transplantation (ASCT). More recently, availability of highly effective novel agents has further improved patient outcomes. However, it is still the matter of debate whether a proportion of patients treated with ASCT can enjoy a long term survival, while sustaining prolonged high quality response.

Aims: To identify the variables which were related to long-term survival, we performed a post-hoc analysis of two large prospective clinical trials of ASCT in newly diagnosed MM patients, the first one comparing single versus double ASCT and the second one incorporating thalidomide-dexamethasone (TD) into double ASCT.

Methods: A total of 321 patients were randomly assigned in the first study to receive either a single or double ASCT, as previously described (Cavo M et al, JCO 2007). Three hundred and fifty seven patients were enrolled in the subsequent multicenter phase 2 study incorporating TD from the outset until the second ASCT; details of the protocol were previously reported (Cavo et al, *J. Clin. Oncol* 2009). All the analyses were performed on an intention-to-treat basis.

Results: After a median follow-up of 61 months in the first study, PFS remained significantly longer with tandem versus single ASCT (median 37 vs 25 months, P= 0.012), while OS was similar in the two groups (median 71 vs 67 months). CR was sustained for more than 5 and 10 years in 24% and 12% of the patients, respectively. On multivariate analysis, CR was the most important variable significantly extending PFS and OS; random assignment to double ASCT was an additional variable extending PFS. After a median follow-up of 84 months in the second study, median values of PFS and OS were 47.2 and 109.6 months, respectively. CR was sustained for more than 5 and 8 years in 42% and 9% of the patients, respectively. On multivariate analysis, achievement of CR and absence of t(4;14)±del(17p) were independent variables predicting for longer PFS and OS. Overall, 23% and 20% of patients in the first and second study were alive over 10 or 8 years, respectively (long-term survivors). Median PFS of long-term survivors in the 2 studies were 74 and 87.7 months, respectively, versus 25 and 37 months for the rest of the population (P=0.0000). Median duration of CR were 70 and 78 months in the long-term survivors group for the first and second study, respectively, in comparison with 21 and 49 months in the remaining patients (P<0.001 for both). The 10 and 8-year estimates of OS after relapse or progression in the long-term survivors of the two protocols were 58% and 72%, respectively, in comparison to a median value of 24 and 23 months for the control group (P<0.0001 for both). Sustaining a durable response for more than 42 months was favourably affecting survival after relapse on multivariate analysis. In a logistic regression analysis, independent factors predicting for long-term survival in both the trials appeared to be attainment of CR, sustaining the response for more than 42 months and application of double ASCT.

Summary / Conclusion: Approximately 20-25% of the patients undergoing up-front ASCT can achieve long term survival (8-10 years from start of treatment), with 33% of them remaining relapse free. Attainment of CR and sustaining a durable response as well as application of double ASCT were the leading independent variables predicting for long-term OS.Prolonged survival after relapse was a contributing factor to long-term OS as well and was influenced by the sustenance of a durable response.

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INTENSIFIED CHEMO-IMMUNOTHERAPY FOR ADULT PATIENTS AFFECTED BY NODAL PERIPHERAL T-CELL LYMPHOMAS (PTCLS) AT DIAGNOSIS: FINAL RESULTS OF A PHASE II MULTICENTER PROSPECTIVE TRIAL

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Background: PTCLs have a poor survival with conventional therapy. Recent trials showed: 1) CHOP-alemtuzumab (AL) has a high response rate (ORR); 2) consolidation of response with up-front autologous stem cell transplantation (autoSCT) improves PFS (from 35% to 45% at 3 years); iii) allogeneic stem cell transplantation (alloSCT) is an effective strategy for relapsed disease. Based on these findings, we conducted a prospective phase II study in young (A: ≥18 ≤60 years) and elderly (B: >60 and ≤75 years) patients (pts) at diagnosis. In young pts, the trial tested for the first time a consolidation with up-front allogeneic or autologous stem cell transplantation (autoSCT or alloSCT) based on a genetic stratification. In elderly pts, the trial tests only the role of chemo-immunotherapy.

Aims: The primary endpoint was the treatment efficacy in terms of complete clinical response maintained for at least 6 months.

Methods: Younger pts (A) received 2 courses of CHOP-21 with AL (30 mg), 2 courses of Hyper-C-Hidam (methotrexate, cyclophosphamide, cytarabine). Responding pts received consolidation with up-front autoSCT or alloSCT. Elderly pts (B) received 6 cycles of CHOP-21 with AL (10 mg).

Results: 92 pts were enrolled, 86 fulfilled all inclusion criteria (A: n=61; B: n=25). Clinical characteristics: PTCL-NOS 49%, AILT 24%, ALK-negative ALCL 22%, EATL 5%. International Prognostic Index ≥2, A: 69%; B:100%. For study A, at the end of induction phase, the ORR was 67% (CR, 56%; PR, 11%). Thirty-eight (62%) pts received consolidation: 14 autoSCT, 23 alloSCT and one maintained remission without any further treatment. Twenty-three (38%) did not receive the consolidation for PD (n=18) and deaths for toxicity (n=5). At a median follow-up of 40 months, 30 pts (49%) are alive in CR**.** The cumulative non-relapse mortality (NRM) was 13%. The estimated 4-years OS, PFS and DFS rates were 48.8% (95%CI, 37.6% -63.2%), 44.0% (95%CI, 33.1% -58.5%) and 79% (95%CI, 65.1%>95.5%), respectively. At multivariable analysis, the CR maintained for at least 6 months [48% (95%CI,34.6%>61%)] had a dominant effect on PFS and OS, regardless of patient's age, IPI, extranodal involvement. Transplanted pts had an advantage in OS (HR=0.04, P=0.004 for autoSCT; HR=0.22, P=0.008 for alloSCT). For study B, 13 pts (52%) maintained the response at least for 6 months and 8 pts (32%) are alive at last follow-up. The cumulative NRM was 12%. At median follow-up of 48 months, the estimated 4-year OS, PFS and DFS were 31.5% (95% CI: 17.6%>56.5%), 26.7% (95% CI: 13.6%>52.3%) and 44.4% (95% CI: 24.6%>80.5%), respectively.

Summary / Conclusion: Considering the high-risk population, in younger pts the consolidation with up-front transplantation was associated to a prolonged DFS. In elderly, the chemo-immunotherapy program did not improve the outcome.

S598

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE 8TH DECADE OF LIFE

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Background: Allogeneic stem-cell transplantation (SCT) is a potentially curative therapy for various hematological malignancies. Reduced-intensity conditioning (RIC) allows extension of SCT to older patients, previously considered not eligible for SCT. However, there is still paucity of data on the expected outcome of patients age 70 years and older, and in particular on the long-term outcome after SCT.

Aims: To define SCT outcome in patients age 70 years and older.

Methods: We retrospective analyzed all consecutive allogeneic transplants performed in a single institution between the years 2000-2013. We identified patients age 70 years and above at the day of SCT and analyzed their outcome.

Results: Forty-eight patients older than 70 years were transplanted during the study period (4.2% of all allogeneic transplant recipients during that period); none during the years 2000-4 (0%), 29 during 2004-10 (4.2%) and 19 during 2011-3 (7.2%). The median age at SCT was 71 years (range, 70–76), 32 male, 16 female. Diagnoses included AML/MDS (n=38), myeloproliferative disorders (n=3), lymphoma and CLL (n=5), aplastic anemia (n=1), ALL (n=1). Disease status at SCT was early (n=17), intermediate (n=6), or advanced (n=25) according to CIBMTR criteria. Fourteen patients had a comorbidity score >2 (29%). The donor was an HLA-matched sibling (n=26), matched unrelated (n=20, 7 of them HLA mismatched) or mismatched related donor (n=2). The conditioning regimen was fludarabine with reduced-dose intravenous busulfan (n=27), treosulfan (n=18), cyclophosphamide (n=2) or melphalan (n=1). Thirty-six patients engrafted in a median of 14 days (12-23), 2 died prior to engraftment (4%). With a median follow-up of 15 months (range, 1–50), 15 patients are alive and 33 have died. Fourteen evaluable patients had acute GVHD grade II-IV (30%) and 10 of 29 evaluable patients had chronic GVHD (34%). Ten patients died within 1 year after SCT of treatment-related cause, 7 patients died due to infections (1 before engraftment), 1 due to organ toxicity (before engraftment), 2 due to acute GVHD. The day+100 and 1-year non-relapse mortality (NRM) rates were 11% (95%CI, 5-25) and 24% (95%CI, 14-41), respectively. The 1-year relapse rate was 38% (95%CI, 25-56). In all, the 1-year overall survival (OS) and disease-free survival rates were 49% (95%CI, 34–64) and 38% (95%CI, 23–53), respectively. With current follow-up, 13 patients reached 1 year after SCT disease-free. However, only 5 of these patients remained alive for the next 2 years. Three died of relapse and 5 had late NRM due to myocardial infarction (n=3), chronic GVHD (n=1) and late infection (n=1). There was no apparent plateau on the survival curve due to these late events.

Summary / Conclusion: Allogeneic SCT is increasingly used in patients age 70 years and older. SCT is feasible in a subset of these older patients. RIC regimens are associated with relatively low organ toxicity and GVHD rates in these patients, but infection mortality is relatively high. Late events, some possibly related to patient age and comorbidity rather than to the SCT itself, continue to compromise cure in the late post SCT phase. Larger studies are needed to define patient subsets that are more likely to benefit from SCT in this older age.

S599

HEMATOPOIETIC CELL TRANSPLANTATION (HCT) FOR SEVERE APLASTIC ANEMIA (SAA): IMPACT OF GRAFT SOURCE AND ECONOMIC REGIONS.

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Background: Submitted on Behalf of the International Studies Committee of the Center for International Blood and Marrow Transplant Research (CIBMTR) and the Japan Society for Hematopoietic Cell Transplantation (JHSCT). Bone marrow (BM) is the preferred graft source for treatment of SAA with HCT due to the lower risk of chronic graft-versus-host disease (GVHD) and better survival compared to mobilized peripheral blood stem cells (PBSC). However, this may not apply to all economic regions, as graft failure rate is often higher in regions where patients frequently present later in their disease course and with heavier transfusion load.

Aims: To compare outcomes of HLA matched sibling HCT for SAA with BM and PBSC across different regions according to gross national income per capita (GNI).

Methods: Patients with SAA who received HCT from an HLA-matched sibling donor from 1995 to 2009 and reported to the Center for International Blood and Marrow Transplant Research (CIBMTR, N=1,885) or the Japan Society for Hematopoietic Cell Transplantation (JSHCT, N=560) were analyzed. The study population was categorized by GNI and region/countries into three groups: high income countries (HIC),US and Canada (US-C, N= 504), other HIC (OHIC, N=1,280) and other combined group that included upper middle (UM), low middle (LM) and low income countries (LIC) (UM-LM-LIC, N=661). HIC groups were separated due to greater representativeness of country wide data from US-C in the CIBMTR database.

Results: PBSC was used as the graft source in 72% of HCT in LM-LIC, compared to 17% in HIC and 13% in UMIC. The following demographics differed significantly in the combined UM-LM-LIC group compared to the HIC group: women in 36% (HIC-44%), performance scores <90% in 45% (US-C 32%, OHIC 29%), CMV positive donors/ recipients in 77% (US-C 63%, OHIC 54%), median time from diagnosis to HCT <3 months in 40% (US-C 61%, OHIC 44%), prior use of anti-thymocyte globulin in 6% (US-C 24%, OHIC-23%). Three-year probabilities of overall survival for PBSC and BM graft source were 62% (95% confidence interval [CI],51-72%) and 88% (95%CI, 84-91%, p<0.01) in US-C, 78% (95%CI, 72-83%) and 88% (95% CI, 86-90%, p<0.01) in OHIC, and 64% (95% CI, 55-72%) and 69%(95%CI, 65-74%, P=0.2) in UM-LM-LIC, respectively. Rates of chronic GVHD were generally higher with PBSC irrespective of economic region. Multivariate analysis showed that overall mortality was higher in the following groups (a) UM-LM-LIC (b) PBSC (c) low KPS (<90%) (d) time from diagnosis to transplant > 6 months. Table 1 shows overall mortality and primary graft failure according to graft source and economic region.

Summary / Conclusion: Survival outcomes after HCT for SAA are different according to economic regions and graft sources. BM is associated with better survival than PBSC and outcomes in HIC are better than those in the UM-LM-LIC cohort. Additionally, outcomes in UM-LM-LIC were the same for BM and PBSC, likely due to lower rates of neutrophil engraftment with BM.

Table 1. Multivariate analysis for overall mortality and graft failure.

GNI + Graft Source	N	Overall Mortality			Graft Failure[1]		
		RR	0.95 CI	p	RR	0.95 CI	p
US-C **BM**	419	1.00			1.00		
US-C **PB**	85	2.13	1.35-3.39	0.001	0.83	0.57-1.21	ns
OHIC **BM**	1066	1.00	0.72-1.40	ns	0.74	0.58-0.96	0.021
OHIC **PB**	214	1.52	1.02-2.26	0.041	0.90	0.64-1.27	ns
UM/LM/LIC BM	477	2.26	1.63-3.14	<0.0001	0.52	0.40-0.68	<0.0001
UM/LM/LIC PB	184	2.86	1.92-4.26	<0.0001	0.93	0.68-1.27	ns
Contrasts							
UM/LM/LIC BM vs PB		1.27	0.91-1.78	ns			0.0002
US-C vs **UM/LM/LIC PB**		1.34	0.84-2.14	ns			ns
OHIC vs **UM/LM/LIC PB**		1.89	1.26-2.83	0.002			ns

[1]neutrophil engraftment

S600

OUTCOMES OF UMBILICAL CORD BLOOD TRANSPLANTATION FOR PATIENTS WITH MYELODYSPLASTIC SYNDROME: A NATIONWIDE SURVEY

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Background: For patients with myelodysplastic syndrome (MDS), allogeneic stem cell transplantation (allo-SCT) is the sole curative therapy. However, MDS is a disease that most often develops in older people, with the median age of onset being 70 years, so the potential matched-sibling donors are also elderly. Therefore, there is a substantial need for alternative donors for MDS patients compared to those with other hematological diseases. Cord blood transplantation (CBT) might represent such an alternative, however, there has been concern that the relapse rate of patients who receive CBT is higher than that of patients who undergo bone marrow transplantation from unrelated donors (URBMT).

Aims: We conducted a retrospective study of the CBT outcomes of MDS patients using the data from the Japan Society for Hematopoietic Cell Transplantation Data Registry.

Methods: Patients with MDS with the FAB classification aged 16 years or older who underwent their first CBT between January 1998 and December 2010 were compared to the patients who underwent the first URBMT. We next examined the clinical factors affecting the overall survival (OS) using Fisher's exact test and a Cox proportional hazard model.

Results: There were 431 and 1093 patients with MDS who received a CBT and URBMT. The estimated 5-year OS of the CBT patients was significantly inferi-

or to that of the URBMT patients (32% vs. 46%, P<0.0001). No significant differences were observed between the CBT and URBMT patients in the 3-year cumulative incidence (CI) of non-relapse mortality (NRM); however, the 3-year CI of relapse was significantly worse in the CBT patients than the URBMT patients (The 3-year CI-NRM and CI-relapse of the CBT and URBMT patients was 34% vs. 36% (P=not significant) and 20% vs. 10% (P<0.0001), respectively. The CI of neutrophil engraftment in the CBT patients was 66% at day 30 and 77% at day 100 after SCT. In the CBT patients, the following factors were extracted that predicted a better OS in the univariate analysis; recipients' age at SCT (younger than 50 years), female gender, diagnosis (refractory anemia and refractory anemia with multilineage dysplasia), good performance status (PS, 0 and 1), fewer than 20 Unit RBC transfusions, reduced-intensity conditioning, the development of acute graft-versus-host disease (GVHD) and chronic GVHD and cytogenetic prognostic subgroups according to the international prognostic scoring system. A proportional hazards model showed that the recipients' age at SCT, diagnosis, PS and number of RBC transfusions were the variables affecting the OS.

Summary / Conclusion: Our results suggest that the development of acute/chronic GVHD might improve the OS in CBT patients. However, it is also necessary to carefully select the patients who should receive a CBT, because the OS of CBT may be inferior to that of URBMT patients.

Chronic myeloid leukemia - Biology

S601

CXCR4 ANTAGONIST BKT140 (BL-8040) COOPERATES WITH IMATINIB, EFFECTIVELY ABROGATING STROMA-MEDIATED PROTECTION AND TARGETING CML CELLS IN VITRO AND IN VIVO

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Background: Existence of quiescent leukemic stem cells (LSCs) resistant to tyrosine kinase inhibitors (TKIs) may be responsible for chronic myeloid leukemia (CML) resistance and recurrence. Thus, novel therapies that eradicate LSCs are in need. The bone marrow (BM) microenvironment is believed to have a role in protecting CML cells from TKIs-induced apoptosis. Chemokine receptor CXCR4 and its ligand CXCL12 have a key role in the trafficking and retention of normal and LSCs in the BM niche. Therefore, CXCR4 inhibition may antagonize the survival and spread of CML and LSC cells, restoring their sensitivity to TKIs in the BM microenvironment context.
Aims: To study the role of CXCR4 in CML progression and to evaluiate the effect of CXCR4 antagonist BKT140 (BL-8040) on CML cell survival and sensitivity to rituximab.
Results: Exogenous CXCR4 expression increased the *in vitro* proliferation of K562 cells in response to CXCL12, suggesting the pro-survival role of CXCR4/CXCL12 axis in CML. Accordingly, *in vitro* treatment with CXCR4 antagonist BKT140 (8 μM) directly inhibited the cell growth by 40-60% and induced apoptosis of CML cell lines (K562, LAMA84 and KCL22). Combination of BKT140 with IC50 concentrations of imatinib significantly increased the anti-CML apoptotic effect, achieving 95% reduction in cell viability (p<0.01). To address the impact of BM environment on CML cell growth and sensitivity to imatinib, we established *in vitro* co-culture system with BM stromal cells (BMSCs). We found that murine, as well as primary human BMSCs supported the survival and proliferation of CML cell lines and primary CML cells and protected them from imatinib-induced apoptosis. Furthermore, BMSCs remarkably increased the expression of proto-oncogene BCL6 in CML cells in response to imatinib treatment, suggesting the possible role of BCL6 in stroma-mediated TKI resistance. Moreover, exogenous expression of CXCR4 in K562 cells elevated the basic level of BCL6, which was further increased by imatinib treatment in the presence of BMSCs. Interestingly, BKT140 treatment in co-culture system with BMSCs increased the percent of cycling (G2/M+S) CML cells from 22% to 30%, respectively (P<0.01). However, the combination of BKT140 with imatinib in the co-culture with BMSCs remarkably decreased the percent of proliferating CML cells to 9% and induced cell apoptosis. These results may indicate that CXCR4 blockade may abrogate the stroma-mediated CML quiescence and increase the sensitivity to TKIs. Notably, combination of imatinib with BKT140 decreased BCL6 mRNA levels in CML cells co-cultured with BMSCS. To further explore the role of CXCR4 inhibition in vivo, we established a xenograft bioluminescent model of CML. Luciferase-transduced K562 cells were intra-peritoneally injected into NOD/SCID mice and tumor burden was quantified using bioluminescence imaging. BKT140 (100 μg/injection), administered intra-peritoneally, effectively reduced disease burden.
Summary / Conclusion: Taken together, our data indicate the importance of CXCR4/CXCL12 axis in CML growth and CML-BM stroma interaction. CXCR4 inhibition with BKT140 antagonist efficiently synergized with imatinib, overcoming the protective effect of the BM stroma. These results provide the rational basis for CXCR4-targeted therapy in combination with TKI to override drug resistance and suppress residual disease.

S602

PRECLINICAL AND CLINICAL EFFICACY OF KPT-330-MEDIATED XPO1 INHIBITION IN PH+ LEUKEMIAS

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Background: Because ABL tyrosine kinase inhibitors (TKIs) fail to induce long-term response in blast crisis chronic myeloid leukemia (CML-BC) and Philadelphia chromosome-positive (Ph^+) acute lymphoblastic leukemia (ALL), novel therapies targeting pathways dysregulated in either a BCR-ABL1 kinase-independent or –dependent manner, are necessary. The karyopherin exportin1

(XPO1/CRM1) regulates cell proliferation and survival by facilitating nuclear export of several factors, including tumor suppressors (e.g. p53, p21, FOXO and IκB) and leukemia-relevant proto-oncogenes (e.g. hnRNP A1, ABL1 and SET), the expression and/or activity of which have been found altered in BCR-ABL1$^+$ leukemias. Thus, it is possible that XPO1 activity plays an important role in Ph$^+$ leukemogenesis.
Aims: To determine whether XPO1 controls Ph$^+$ leukemogenesis, identify the molecular mechanisms, and assess the clinical relevance of the selective inhibitor of XPO1-mediated nuclear export KPT-330 for Ph$^+$ leukemias refractory to TKI-based therapies.
Methods: *Preclinical:* CD34$^+$ bone marrow (BM) and peripheral blood (PB) progenitors from CML (n=10), ALL (n=9) and healthy (n=8) individuals, and myeloid precursor 32Dcl3 cells were used for immunoblots, Annexin-V staining, confocal microscopy, clonogenic and PP2A phosphatase assays. KPT-330 was used *in vitro* at 0.5-1μM, (12-72 h) whereas it was given twice/week at 15 mg/kg to SCID mice bearing a 32D-BCR/ABL-driven leukemia, used as a CML-BC model. *Clinical:* A 37-year-old male with accelerated phase CML (CML-AP), refractory to 9 prior therapies, including TKIs and several investigational agents, received KPT-330 (16.5 mg/m^2) for compassionate use, after refusing BM transplant.
Results: Compared to normal progenitors, significantly higher XPO1 protein levels were found in CML and ALL blasts. Increased XPO1 expression depended, at least in part, on BCR-ABL1 activity, as it was significantly reduced by imatinib treatment. Accordingly, XPO1 levels were augmented by BCR-ABL1 expression in 32Dcl3 cells. KPT-330 treatment induced apoptosis and decreased clonogenic potential of CML and ALL but not normal progenitors, and increased survival of leukemic mice (P=0.002), 50% of which were alive after 16 weeks of treatment, while all untreated animals died (median survival=5 weeks). Notably, 60% of the surviving KPT-330-treated mice were also BCR-ABL1-negative by RT-PCR, and no drug toxicity was observed in healthy animals. Mechanistically, KPT-330 altered cellular localization of tumor suppressors (p21, p53, FOXO3A and IκB), hnRNP A1 and SET, an inhibitor of the PP2A tumor suppressor phosphatase which, reportedly, is not only inhibited in Ph$^+$ leukemias but, when restored, impairs leukemogenesis and decreases BCR-ABL1 activity/expression. Accordingly, KPT-330 rescued PP2A activity in 32D-BCR/ABL cells, and decreased BCR-ABL1 expression and activity in CML and Ph$^+$ ALL progenitors. Finally, KPT-330 was administered to a CML-AP patient with hyper-leukocytosis and severe bone pain. After a single oral dose, bone pain, immature myeloid cells in PB smears, WBC count (>300,000 to 7,000 cells/μL), splenomegaly (13 to 4 cm below costal margin) and lactate dehydrogenase (513 to 264 IU/L) were reduced. After 7 days, WBC count increased to 37,000 cells/μL with reappearance of leukemic blasts; however, the patient declined dose escalation.
Summary / Conclusion: XPO1 activity is necessary for the enhanced survival of Ph$^+$ leukemic blasts, and KPT-330 may represent an effective treatment for TKI-refractory Ph$^+$ leukemias.

S603

THE CML EPIGENOME SHOWS DYNAMIC CHANGES IN THE CD34+ COMPARTMENT IN PATIENTS WHO ACHIEVE COMPLETE CYTOGENETIC RESPONSE ON TYROSINE KINASE INHIBITORS

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Background: CML is thought to originate from acquisition of a BCR-ABL1 fusion gene in a pluripotent stem cell. Despite this consistent event, the clinical course is remarkably heterogeneous. Genetic alterations play a key role in the biological and clinical aspects of CML yet they do not entirely explain the disease pathogenesis. Growing evidence from other leukaemias including acute myeloid leukaemia highlights the interplay between genetic and epigenetic events in shaping the cancer landscape.
Aims: The aim of this work was to perform the first integrated epigenomic study in CML looking at the combined genome-wide DNA methylation and transcriptome profiles of CD34+ cells collected at different stages of the disease in the same patients. We hoped to identify disease-specific and/or stage-specific aberrant epigenetic programming in CML cells as a prelude to or consequence of the t(9;22).
Methods: CD34+ cells were purified from the blood of 29 patients with BCR-ABL1-positive CML in chronic phase (CML-CP) before treatment. These patients all achieved complete cytogenetic response (CCyR) within 12 months of starting imatinib at 400mg/day; they underwent granulocyte colony-stimulating factor (G-CSF) mobilization of Ph-negative peripheral blood stem cells after a median of 23 months. Using the Illumina Infinium HumanMethylation450 and HumanHT-12 v4 Expression BeadChips we compared for each patient the pre-treatment genome-wide DNA methylation and gene expression profile at diagnosis with the corresponding sample obtained in CCyR. Analogous signatures were obtained from CD34+ cells collected from healthy transplant donors treated with G-CSF. Further comparisons between our dataset and other publically available leukemia and solid cancer 450K array data were made using MARMAL-AID, a tool developed in house.
Results: Unsupervised hierarchical clustering of all samples using Ward's method identified differentially methylated probes (DMPs) associated with CML. The three groups formed two major clusters, one from CML-CP samples and the other from the controls and CML-CCyR. In the latter cluster CML-CCyR were distinct but closer to normal CD34+ cells than to the diagnostic samples. In contrast, the CML-CP gene expression signature was closer to healthy controls than to CML-CCyR. We examined the overall gene expression patterns for genes identified as displaying significant differential expression and DNA methylation changes. Separating the methylation probes into those associated with gene promoters or gene bodies we found a significant (P= 7.4e-13) anti-correlation with gene expression in the methylation probes associated with the promoters but not with gene bodies. We identified 3,859 DMPs which distinguished CML-CP from healthy controls. These were enriched in gene bodies (P=1e-16) and intergenic regions (P=1e-16) but depleted in promoter regions (P== 1e-16). The DMPs associated with CML were also enriched above background for associations with CpG Islands (P= 1e-16). CML-CCyR showed global methylation patterns much closer to that of healthy controls with 89% of DMPs returning to within 20% of these controls. Despite this restoration towards normal inter-individual variability was apparent which was not related to clinical parameters including time on imatinib, Bcr-Abl1 transcript levels or the time to MMR. Using MARMAL-AID we were able to define a signature common to leukaemia and solid cancers but in addition we identified a DNA methylation signature unique to CML. On KEGG analysis these probes were significantly enriched for genes related to the JAK-STAT (P= 9e-5) and MAPK (P= 6e-5) pathways.
Summary / Conclusion: We showed a consistent pattern of DNA methylation and gene expression in untreated CML patients, which reverted towards normal at the time of CCyR in the same individuals. The use of publically available reference methylome datasets for normal tissues and cancer types confirmed our identification of a CML-specific DNA methylation signature.

S604

THE ROLE OF RHO KINASE IN THE SURVIVAL OF CHRONIC MYELOID LEUKAEMIA CELLS

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Background: Chronic myeloid leukaemia (CML) is characterised by expression of an oncogenic tyrosine kinase BCR-ABL that drives uncontrolled proliferation. Inhibition of BCR-ABL with tyrosine kinase inhibitors (TKI) controls the disease in the majority of patients, but in some, TKI resistance develops. Even optimal responders harbour minimal residual disease believed to be the result of persistence of leukaemic stem cells (LSC) despite treatment. CML LSC are not dependent on BCR-ABL for survival and it is therefore hypothesised that alternative pathways regulate their survival and maintenance. The Rho-associated serine/threonine kinases (ROCK) have emerged as central regulators of the actomyosin cytoskeleton. Their function is to promote actin filament bundling and myosin-driven contraction, leading to changes in cell morphology, motility, growth and survival. ROCK is increasingly recognised as an important contributor to cancer and genome sequencing has revealed three ROCK1 activating mutations in human breast and non-small cell lung cancer cell lines. Cells transformed with the oncogenes KITD814V, BCR-ABL and FLT3N51 showed constitutive ROCK activation leading to myeloproliferative disease in mice. Treatment with ROCK inhibitors resulted in cell death and increased lifespan in these mice (1). Our in-house microarray of CML versus normal stem cells, before and after TKI exposure (nilotinib, imatinib, dasatinib), has shown up-regulation of the ROCK pathway in CML LSC that was not fully normalised by TKI treatment. These results suggest that ROCK pathway may represent a critical survival pathway in persistent CML cells.
Aims: We aimed to investigate the role of ROCK in the survival of CML cells, including LSC to ascertain whether the addition of ROCK inhibitors to TKI would result in enhanced CML cell kill, with ultimate applicability in CML TKI resistance or LSC persistence.
Methods: A TKI sensitive human CML blast crisis cell line KCL22 was treated for 24-96h with no drug/TKI(nilotinib)/commercial ROCK inhibitor (H1152)/nilotinib+H1152 at a range of concentration combinations. Viable cell counts and Annexin V staining were analysed by the Chou-Talalay method to ascertain combination indices (CI). Flow cytometry analysis of proteins downstream of ROCK, including phospho-myosin light chain (pMLC) and analysis of DNA content for cell cycle progression were performed on the same cells to investigate the biochemical effects of combination treatment.
Results: We established the IC50 for nilotinib (10 nM) and H1152 (2μM). Cell viability counts showed synergistic cell kill between the two compounds at 24 & 48h. Combining 2 μM H1152 with 10nM nilotinib resulted in 12% increased apoptosis versus best single agent (P=0.0091 using one-way ANOVA with repeated measurements). Flow cytometry showed concentration-dependent inhibition of pMLC, with 88% inhibition at 24h with 2μM H1152. BCR-ABL inhibition alone resulted in G1 arrest and ROCK inhibition alone in G2/M-phase

arrest. Combination of the two reverts this M phase block back to a more normal cell cycle profile.
Summary / Conclusion: The ROCK pathway appears to offer a legitimate and promising potentially novel target for CML patients persistent CML with drug resistance and LSC persistence despite TKI treatment. Investigation is ongoing into the optimal use of the two drugs in combination for the benefit of CML patients.

Reference
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S605

NEOPLASTIC STEM CELLS OF PH+ CHRONIC MYELOID LEUKEMIA (CML) EXPRESS THE ALPHA-CHAIN OF THE IL-2 RECEPTOR (CD25)
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Background: Chronic myeloid leukemia (CML) is a myeloid neoplasm characterized by the Philadelphia (Ph) chromosome and the related oncoprotein, BCR/ABL. During the past few years, considerable effort has been made to characterize neoplastic stem cells (NSC) in patients with CML.
Aims: The aim of the present study was to identify new specific markers and targets expressed in CML NSC.
Results: We found that CD34+/Lin-/CD38- CML NSC express the IL-2R-alpha-chain CD25 in almost all patients (31/33=95%). CML NSC were also found to express IL-1RAP, DPPIV (CD26), Siglec-3 (CD33), KIT (CD117) and IL-3RA (CD123). Highly purified NSC were found to express BCR/ABL and to engraft irradiated NOD-SCID-IL-2Rg$^{-/-}$ (NSG) mice with BCR/ABL+ cells, whereasCD34+/CD38-/CD25-/CD26- cells from the same patient failed to express BCR/ABL, and engrafted NSG mice with BCR/ABL-negative cells. To define signaling-molecules contributing to CD25 expression in CML NSC, we employed primary murine hematopoietic cells infected with BCR/ABL-p210 in combination with a retrovirus encoding for STAT5A or STAT5B. Enforced expression of STAT5A/STAT5B resulted in enhanced CD25 expression in leukemic cells. However, STAT5-signaling in CML NCS is regulated by both, BCR/ABL as well as cytokines present in the microenvironment. Correspondingly, BCR/ABL alone was unable to induce a significant expression of CD25 in leukemic cells in mice. Moreover, imatinib did not inhibit expression of CD25 in CML NSC. However, the multikinase inhibitor ponatinib was found to suppress expression of activated STAT5 and expression of the STAT5 target-gene CD25 in CML cells.
Summary / Conclusion: Our data show that CD25 is a novel marker of CML NCS. Application of CD25 may assist in detection, enumeration, and enrichment of NSC in BCR/ABL+ CML.

Drug responsiveness in acute leukemias

S606

DRUG RESPONSE PROFILING TO IDENTIFY NEW TARGETS IN REFRACTORY LEUKEMIA
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Background: Acute lymphoblastic leukemia (ALL) is a genetically highly complex disease. Despite enormous recent genomic profiling efforts that identified underlying lesions, the use of individual genetic lesions as predictive markers for treatment response has been so far very limited, and functional correlations of oncogenic lesions with drug response profiles are ill defined for childhood acute lymphoblastic leukemia (ALL).
Aims: We have decided to pursue a reverse approach, namely to obtain phenotypic information with extended functional drug response profiling and to correlate this with other diagnostic, including genetic, information. In particular, preclinical testing of new therapeutic agents should be designed to evaluate multiple combinations to detect synthetic lethality directly in the relevant clinical samples.
Methods: Based on a collection of ALL xenografts that we established from patients enrolled on the ALL-BFM-2000 and ALL-REZ-BFM 2002 treatment protocols, we developed an automated-imaging based platform for large scale *in vitro* drug testing. This approach enables us to evaluate a large number of compounds very effectively directly on primary ALL cells that are maintained on bone marrow mesenchymal stromal cells, which recreates a standardized serum free natural microenvironment.
Results: Using this platform, we provide evidence for activity profiles of a selection of bioactive molecules and new therapeutic agents in primary samples from distinct ALL subgroups.
We obtained patient specific response patterns by comparing samples from distinct subtypes of precursor B-cell ALL including samples with translocation t(17;19) - a highly resistant subtype - and with t(1;19) - a subtype that is associated with good outcome, as well as T-ALL samples. This approach identifies classes of compounds with a high degree of activity across multiple samples, such as inhibitors of mTOR or chromatin remodelling. Using this platform we detected reproducible activity of the new NOTCH inhibitor I3 which blocks transcription by interfering with the NOTCH transcriptional complex in the low micromolar range (1μM) specifically in T-ALL samples in which the cleaved intracellular fragment of Notch1 (ICD) could be detected by immunoblotting. We identified I3-responsive cases that were not shown to have a mutation in NOTCH1 or FBXW7 but in which Notch1 ICD was strongly detected, indicating that such a functional screening platform has the potential to capture samples with deregulation of the NOTCH pathway that would not be predicted based on mutation analysis.
Summary / Conclusion: Our data indicate presence of specific drug response profiles in genetically distinct ALL subtypes and suggest that our platform will be useful for *in vitro* ALL drug profiling. We expect to derive relevant functional information directly from individual patient samples, which may mirror perturbations in relevant cellular programs. We will evaluate this system for the identification of novel anti-leukemic compounds, for the definition of predictive profiles of clinical response and will evaluate synthetically lethal drug combinations to identify new alternatives for the treatment of refractory ALL.

S607

TARGETING COMPONENTS OF THE ALTERNATIVE NHEJ PATHWAY SENSITIZES KRAS-MUTANT LEUKEMIC CELLS TO CHEMOTHERAPY
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Background: Activating KRAS mutations are detected in a substantial number of hematologic malignancies. Using a conditional *KRAS^{G12D}* knock-in mouse model, we and others have demonstrated that expression of oncogenic Kras in hematopoietic progenitor cells causes an arrest at the DN2/3 stage during T-cell differentiation followed by the development of an aggressive T-cell lymphoblastic leukemia/lymphoma with long disease latency. Interestingly, 50% of analyzed leukemia samples harbored Notch1-mutations, likely acquired during the Kras^{G12D}-mediated block in differentiation *(Kindler et al., Blood 2008*). These additional mutations may be acquired by replicative stress or increased reactive oxygen species (ROS) production. Alternatively, oncogenic RAS may directly affect DNA repair pathways, thereby causing misrepair and, due to concomitant resistance to apoptotic cell death, accumulation of genomic changes. Recent studies indicate that DNA damage response and repair (DDR)

can be disturbed within a defined malignant background. For example, leukemic cells harboring FLT3-ITD or BCR-ABL oncogenes preferentially use the alternative non-homologous end-joining pathway (alt-NHEJ) for double strand break (DSB) repair *(Sallmyr A. et al., Blood 2008; Fan J et al., Blood 2010)*. Mechanism of DDR in context of mutated KRAS are currently poorly understood.
Aims: The goal of this study was to investigate the impact of mutated KRAS on DDR and to identify potential therapeutic targets in the case of altered DNA repair.
Methods: The T-ALL cell line CCRF-HSB2 and the AML cell line U-937 were lentivirally transduced to express mutant $KRAS^{G13D}$. In an alternative approach we suppressed KRAS expression in Nomo-1 AML cells, harbouring an endogenous *K-Ras^{G13D}* mutation. Additionally, Lck-Cre mice were crossed with Lox-stop-Lox-$KRAS^{G12D}$ mice. Using the described *in vitro* and *in vivo* models, we examined the functional consequences of oncogenic KRAS expression on DDR.
Results: Expression of oncogenic KRAS correlated with increased resistance to genotoxic stress, elevated levels of DNA damage and delayed repair kinetics of DSBs as revealed by γH2AX-staining and the neutral comet assay, respectively. In addition, using an *in vitro* plasmid religation assay we observed increased DNA misrepair in the context of mutated KRAS. Sequencing analysis of the religated DNA ends demonstrated a preferred use of DNA-microhomologies accompanied by large deletions. These findings have previously been shown to be strongly associated with the recruitment of the alt-NHEJ DNA repair pathway. Interestingly, immunoblot analysis demonstrated increased expression of DNA ligase-3α, PARP1 and XRCC1, all crucible components of an alt-NHEJ pathway, in KRAS-mutant cell lines compared to KRAS-wt control cells. To address the question whether the aberrant use of the alt-NHEJ pathway in KRAS-mutant cells represents a therapeutic target, we treated KRAS-mutant and –wt cells with the PARP1-inhibitor NU1025 or downregulated the expression of DNA ligase-3α using shRNA. Inhibition of PARP1 or knockdown of DNA ligase-3α significantly reversed resistance to apoptotic cell death upon treatment with cytotoxic agents. Of note, this effect was only observed in cells expressing oncogenic KRAS suggesting specific vulnerability of KRAS-mutated cell.
Summary / Conclusion: Here, we show that mutant KRAS shifts the balance between the classical NHEJ DNA repair pathway towards the highly error-prone alt-NHEJ pathway. We further show that inhibition of components of the alt-NHEJ pathway including PARP1 and DNA ligase 3α is able to specifically sensitize KRAS-mutant cells to genotoxic agents. These data suggest that targeting the alt-NHEJ pathway represents a promising therapeutic approach in cells harboring otherwise non-druggable KRAS mutations.

S608

DYNAMICS OF EXPANSION OF TYROSINE KINASE INHIBITOR-RESISTANT MUTANTS AS ASSESSED BY DEEP SEQUENCING OF THE BCR-ABL KINASE DOMAIN: IMPLICATIONS FOR ROUTINE MUTATION TESTING

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Background: In Philadelphia-positive (Ph+) acute lymphoblastic leukemia (ALL) patients (pts), efficacy of tyrosine kinase inhibitor (TKI)-based therapies is often compromised by selection of resistant mutations in the BCR-ABL kinase domain (KD). Currently, the gold standard for BCR-ABL KD mutation screening is conventional Sanger sequencing (SS). However, more sensitive approaches are desirable to allow more timely and rational therapeutic intervention.
Aims: A Deep sequencing (DS) strategy based on the Roche 454 next-generation sequencing technology was set up in order to: study the dynamics of expansion of different types of BCR-ABL KD mutations in Ph+ ALL patients developing resistance to TKI-based therapies; test the ability of DS to highlight emerging clones harboring TKI-resistant mutations.
Methods: 29 Ph+ ALL pts who had developed resistance to TKI-based (imatinib, dasatinib, nilotinib) therapies were selected for this retrospective analysis. All the pts were known to have developed TKI-resistant BCR-ABL mutations on treatment, as assessed by SS. To reconstruct the dynamics of mutation emergence, longitudinal re-analysis of samples from relapse backwards (n=97; 1-3 months sampling interval) was performed on a Roche GS Junior instrument. DS runs were designed so as to enable high sensitivity mutation calling (minimum target sequence coverage 4,000 reads). However, to minimize the likelihood of false positive results, data were analyzed filtering out all variants with <1% abundance.
Results: DS could successfully detect all the mutations (n=85) previously identified by SS (>15% abundance). In addition, DS revealed that both those samples that had been scored as apparently wild-type by SS and those samples already known to harbor mutations as assessed by SS might be carrying one or more 'lower level' mutations (<15% abundance). In the latter cases, clonal analysis showed complex textures with the same mutation alone and also in combination with other(s) ('compound' mutations) in distinct subclones. Some lower level mutations were silent or apparently irrelevant from a clinical standpoint (passenger mutations?). In more than half of the cases, however, known TKI-resistant variants could be recognized that corresponded either to 'withdrawing' mutants not (yet) entirely de-selected by the switch in TKI or to outgrowing mutations anticipating an imminent relapse. Lower level mutations were confirmed with independent methods (ASO-PCR, RFLP). Notably, in 16/29 (55%) pts with molecularly detectable disease but not yet evidence of cytogenetic or hematologic relapse, DS could identify emerging mutations 1 to 3 months before they became detectable by SS. In the remaining 13 pts, however, outgrowth of the TKI-resistant mutation (T315I=7, Y253H=2, E255K=2, E255V=1 and F317L=1) was so rapid that not even a strict monthly monitoring could have allowed to pick them up before they became dominant.
Summary / Conclusion: Now that multiple options are available, BCR-ABL KD mutation monitoring is a precious tool to maximize the efficacy of TKI-based regimens as induction or salvage therapy of Ph+ ALL. DS proved as reliable as SS for the detection of mutations with >15% abundance. As a key advantage, DS added precious quantitative and qualitative information on the full repertoire of mutated populations, that SS underestimated in more than half of the samples analyzed. TKI-resistant mutations leading to patient relapse were not necessarily preexisting at diagnosis or at the time of switchover to another TKI, underlining the importance of regular monitoring of pts. Although the majority of mutations were found to arise and take over very rapidly, a monthly monitoring by our DS approach would have allowed to identify them earlier than SS actually did - and well in advance of clinical relapse - in half of the pts. DS technologies would enable higher sensitivity mutation calling: further studies are warranted to determine the optimal lower detection limit to aim to in order to exclude both transient mutant subclones that will never take over and sequencing errors.
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S609

PROGNOSTIC RELEVANCE AND KINETICS OF P-LOOP MUTATIONS IN PATIENTS WITH BCR-ABL/PH+ ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Background: Clinical resistance to TKI is associated with the presence of BCR-ABL TKD mutations in approximately 80-90% of patients with Ph+ALL, with a preponderance of p-loop and T315I mutations. The frequency of mutations depends on the specific TKI administered due to the different sensitivity of individual mutations to TKI inhibition. While p-loop mutations may be susceptible to second generation TKI their impact and that on stem cell transplantation (SCT) on long-term treatment outcome are not known.
Aims: This retrospective analysis examines the prognostic relevance of p-loop mutations detected by direct sequencing in patients with Ph+ALL in relation to disease history, prior therapy and the type of salvage treatment. We also examined the kinetics of p-loop mutations, the impact of 2nd generation TKI on long-term survival, the achievement of SCT and the eradication of mutated clones by specific therapy.
Methods: 63 ALL-pts. who developed p-loop mutations at some point during their course of treatment were identified. Frontline-treatment was given according to GMALL studies 05/93 and 06/99 (n=21), 07/03 (n=6), GMALL elderly studies (n=31), TKI alone (n=1) or other TKI-based regimens (n=4). 37 pts. (58%) were treated with an imatinib based regimen before the first occurrence

of the p-loop mutation. Median age at diagnosis was 61 yrs.(range 15-80), 51% male. Minimal residual disease (MRD) was serially assessed by quantitative RT-PCR, mutational analyses was performed by direct sequencing.
Results: The P-loop mutation was detected during frontline therapy prior to relapse in 11 pts., at first, second and third relapse in 41 pts. (65%), 10 (16%), 7 (11%), respectively. Median time between initial diagnosis and the detection of the p–loop mutation was 9.5 mo. (range 2.1–37.8) for pts. who received IM during initial therapy vs.12.04 mo. (3.6 – 98 mo) with a IM-free regimen. Following detection of a mutation, 80 % of pts. received TKI based therapy (imatinib n=17 (26%), nilotinib n=6 (10%), dasatinib n=28 (44%). 14 (22%) pts underwent allogeneic transplantation. Median OS from first detection of a p-loop mutation was 5.3 months. 2 are in ongoing CR and alive (f.-up 1465 d, 58 d). All transplanted pts. died due to progressive disease (n=6) or TRM (n=8). 6 pts. developed compound mutations,a switch to a different TKD mutation was noted in 26 pts.
Summary / Conclusion: The outcome of Ph+ALL pts. who develop a TKD p-loop mutation is dismal, despite the availability of potent 2nd generation TKI and allogeneic SCT. This highlights the importance of preventing resistance mutations during front-line therapy, and the need to identify mutant clones earlier using more sensitive techniques than direct sequencing.

S610

A PERSONALIZED MEDICINE EX VIVO TEST TO PREDICT CLINICAL RESPONSE TO FIRST LINE INDUCTION THERAPY WITH IDARUBICIN AND CYTARABINE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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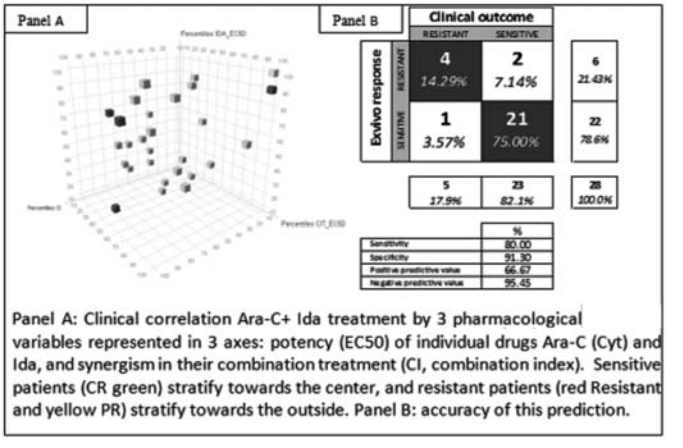
Background: Complete remission (CR) after induction therapy is the first treatment goal in acute myeloid leukemia (AML) patients. Several clinical and biological features, like cytogenetics and molecular alterations, may advance the probability to achieve CR. However, to date, there is no test accurately predicting the response to specific drug combinations allowing the use of specific induction therapies for individual patients.
Aims: The aim of this study is to determine the ability of the Vivia's Personalized Medicine Test (PM) to predict the CR rates after induction chemotherapy with cytarabine (Ara-C) and idarubicin (Ida) in patients diagnosed with AML, using an *ex vivo* drug sensitivity test, based on the analysis of leukemic cells death.
Methods: This non-interventional and prospective study included samples from adult patients over 18 years of age who were diagnosed with de novo AML in Spanish centres from the Programa Español de Tratamientos en Hematología (PETHEMA) group. Marrow samples were collected at diagnosis, sent to the Vivia laboratories, and incubated for 48 hours in well plates containing Ara-C, Ida, or the combination Ara-C+Ida, each at 8 different concentrations to calculate dose responses. Annexin V-FITC was used to quantify the drug-induced apoptosis. Pharmacological responses are calculated using pharmacokinetic population models, which essentially performs the fitting of each dose response across all patient samples simultaneously. Induction response was assessed according to the Cheson criteria (2003). Patients attaining a CR/CRi were classified as responders. The remaining patients were considered as resistant. Patients dying during induction response assessment were non-evaluable.
Results: Seventy patient samples were used to calculate the dose response curves for Ara-C alone, Ida alone, and Ara-C plus Ida. For clinical correlation, 28 patients with a median age of 53 years (range 33 to 71), were included in the study. Twenty-three patients (82%) achieved CR after Ida+Ara-C, and the remaining 5 (18%) were resistant. Correlations of the PM test are showed in Figure 1, Panel B. Four of the five (80%) patients who fail to achieve CR were predicted as resistance in the *ex vivo* test. Twenty-one of the 23 patients (91%) who achieved CR showed good *ex vivo* sensitivity to Ida+Ara-C predicting for CR. Overall, 25 patients (89%) had an accurate prediction of their response to treatment. Panel A shows the 3D representation of the 3 key pharmacological variables that predict clinical response: the potency of both Ara-C (labeled Cyt EC50) and Ida (Ida EC50) used alone; and the synergism between them in the combination (labeled CI). The variables are represented not in absolute but in relative values, as percentiles, orienting the best scenario (high drug potency or high synergism) towards the zero axis point. This means that patients whose pharmacological parameters are positioned towards the center of the 3D graph would be predicted sensitive, while patients positioned towards the outside of the graph would be predicted resistant. Indeed, sensitive patients colored green appear towards the center of the graph, while resistant patients colored red and yellow appear towards the outside of the graph.
Summary / Conclusion: This study shows that this novel PM Test is able to predict the clinical response to Ida+Ara-C induction. We are increasing the number of patients in this ongoing study, and we are planning a PM Test-adapted European Clinical Trial.



Panel A: Clinical correlation Ara-C+ Ida treatment by 3 pharmacological variables represented in 3 axes: potency (EC50) of individual drugs Ara-C (Cyt) and Ida, and synergism in their combination treatment (CI, combination index). Sensitive patients (CR green) stratify towards the center, and resistant patients (red Resistant and yellow PR) stratify towards the outside. Panel B: accuracy of this prediction.

Figure 1.

Bleeding and Thrombosis

S611

THALIDOMIDE FOR THE TREATMENT OF SEVERE RECURRENT EPISTAXIS IN HEREDITARY HEMORRHAGIC TELANGIECTASIA: INTERIM ANALYSIS OF A PROSPECTIVE TRIAL

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Background: Hereditary hemorrhagic telangiectasia (HHT; OMIM 187300 and 600376), also known as Rendu-Osler-Weber syndrome, is an autosomal dominant disease that leads to multiregional angiodysplasia. Recurrent severe epistaxis is the most common presentation of HHT, frequently leading to severe anemia. In the management of HHT epistaxis, multiple approaches, including surgical options, have been tried, but all approaches are largely palliative with variable and temporary results. Since angiogenesis has been implicated in the pathogenesis of HHT, it has been suggested that anti-angiogenic substances may be effective in the treatment of vascular malformations.
Aims: The aims of our ongoing open label, phase II, prospective, non-randomized, single centre study are to evaluate the effectiveness of thalidomide in reducing epistaxis and to identify the lowest effective dose of the drug in patients with HHT refractory to standard therapy as well as to evaluate specific biological and clinical parameters predictable for response and side effects profile (EudraCT 2011-004096-36, ClinicalTrials.gov Identifier: NCT01485224).
Methods: HHT patients with at least one episode of overt bleeding/week requiring at least one blood transfusion during the last three months and refractory to mini-invasive surgical procedures are enrolled. Thalidomide, supplied for compassionate use, is administered at a starting dose of 50 mg/day orally. In the event of no response, thalidomide dosage is increased by 50 mg/day every 4 weeks until complete (cessation of nose bleeding) or partial response (reduction in the severity of epistaxis less than complete response) to a maximum dose of 200 mg/day. After the achievement of complete/partial response patients are treated for 16 additional weeks. Monthly follow-up evaluates the epistaxis severity score according to well defined criteria (Am J Rhinol Allergy 2009;23:52-58) and the transfusion need, with adverse events being reported.
Results: Eighteen patients for whom informed consent was obtained, 11 M and 7 F, aged 44-80 years (median 60), with mutations in either *ACVRL1* (15 cases) or *ENG* gene (3 cases) have been enrolled so far (median follow-up 28 weeks, range 2-64). Treatment was effective in all 15 evaluable patients. Seven patients responded within 4 weeks of starting the drug: complete response was observed in one case, and partial response has been obtained in 6 cases. Eight patients achieved a good, partial response after 8 weeks of treatment. As a consequence, thalidomide therapy significantly increased hemoglobin levels (P=0.04), abolished or greatly decreased the need for red blood cell transfusions and improved the quality of life. Only nonserious, drug-related adverse effects were observed during treatment, including constipation and drowsiness. In no patient thalidomide had to be discontinued. Ten patients completed the treatment: 6 remained stable, off of thalidomide, without the loss of response during a median follow-up of 21 weeks, range 8-44, whereas 4 patients relapsed in 4 weeks after the end of treatment. No correlation was found between genetic or clinical features and time to response or response duration.
Summary / Conclusion: These results strongly support the hypothesis that low-dose thalidomide is safe and very effective for the treatment of epistaxis in patients with severe HHT who did not benefit from other available modalities of treatment, allowing for a rapid and often durable clinical improvement.

S612

EXTRACELLULAR HISTONES-INDUCE MICROCIRCULATORY THROMBOSIS AND ACUTE RESPIRATORY FAILURE

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Background: Extensive cell damage associated with coagulation activation and the development of respiratory failure are common features in critically illness but the underlying pathophysiological mechanisms remain unclear. During cell death, nuclear breakdown products and histones in particular, are released into the circulation and cause toxicity through thrombin generation, platelet aggregation, endothelial damage and cytokine release. The release of extracellular DNA and histones from neutrophils during neutrophil extracellular trap (NET) formation also facilitates thrombus formation and has shown relevance in animal models of deep vein thrombosis, transfusion-related acute lung injury, cancer, trauma and sepsis.
Aims: These recent discoveries led us to hypothesize whether extracellular histones serve as mediators of respiratory failure after extensive cell death.
Methods: To investigate this further, we used *in vivo* experimental models and clinical samples from patients with severe non-thoracic trauma.
Results: Markers for endothelial damage (sTM) and coagulation activation (TAT) significantly increased immediately after trauma or histone-infusion in mice. Cardiac function was impaired in histone-infused mice with increased pulmonary pressure and right ventricular size. Pathological examination showed that lungs were the predominantly affected organ with severe edema, multicocal alveolar hemorrhage, microvascular thrombosis, cytokine surge, pulmonary capillary congestion and significant neutrophil infiltration. In fact, histones directly induced morphological changes of neutrophils, resulting in the formation and deposition of NETs in the pulmonary microcirculation, suggesting that NET formation may serve as a mechanism for neutrophil congestion and thrombosis in lungs. Clinically, circulating histone levels surged significantly immediately after injury to levels that were toxic to cultured endothelial cells (≥50 μg/mL). The high levels were significantly associated with the incidence of acute respiratory failure and SOFA scores, as well as sTM (median: 4.6, quartile: 3.6, 5.5 ng/ml) and TAT levels (median: 81.2, quartile: 33.7, 110.3 ng/mL).
Summary / Conclusion: This work has elucidated a new mechanism for respiratory failure in critically ill patients and proposes future translational intervention with rapid assays to monitor circulating histone levels and anti-histone therapies to reduce thrombosis and improve the pulmonary microcirculation. These findings have wider relevance when histones are released from damaged cells or neutrophils during NETosis, suggesting that anti-histone therapy could potentially protect against the development of respiratory failure and improve overall outcome in many critical illnesses.

S613

CLINICAL COURSE OF CEREBRAL VENOUS THROMBOSIS IN ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Venous thromboembolism (VTE) occurs frequently in patients with acute lymphoblastic leukemia (ALL). Reported incidence varies between 2 and 36%. Remarkably, cerebral venous thromboses (CVT) form a relatively large proportion of VTE and represent up to 50% of events.
Aims: To explore the clinical course of a large number of CVT episodes that occurred in a well-defined cohort of adult patients treated for ALL. We analyzed clinical characteristics, prodromal symptoms, radiological characteristics, time relationships with treatment components of ALL, clinical outcome of CVT and impact of CVT on ALL treatment.
Methods: CVT incidence was assessed in 240 adults (16-59 years) treated for newly diagnosed ALL in the Dutch-Belgian HOVON-37 multicenter study (1999-2005; NTR228 on www.trialregister.nl). Patients generally received 3 cycles of combined chemotherapy before stem cell transplantation assessment. CVT was defined as an intraluminal filling defect or presence of a thrombus in one of the cerebral veins or sinuses, detected with magnetic resonance venography or computed tomographic venography. For patients with CVT, we systematically extracted clinical data from patient records and re-evaluated imaging results. We conducted a nested case-control study to explore relevant prodromal symptoms. Associations were expressed as odds ratios (OR) with corresponding 95% confidence intervals (CI).
Results: 9 of 240 patients experienced CVT (4%; median age 33 years (range 17-49), 56% female, 5 with B-ALL and 4 with T-ALL). CVT was preceded by headache in 8 of 9 patients, while only 5 of 18 matched controls without CVT reported headache (OR 20.8; 95% CI 2-212). Seizures occurred in 8 of 9 patients with CVT and in none of 18 controls (OR 280; 95% CI 9-9236); 6 patients with CVT presented with focal neurological deficits versus 2 of 18 controls (OR 16; 95% CI 2-121). Median time between symptom onset and CVT diagnosis was 1 day (range 0-6). CVT was located in the superior sagittal sinus in 8 of 9 patients. 7 of 9 patients had cerebral parenchymal lesions (5 hemorrhagic infarcts, 2 non-hemorrhagic infarctions). All CVT events occurred during Cycle I of remission induction treatment; in 8 of 9 patients during or shortly after L-asparaginase therapy (*E.coli*) and in all patients after at least one dosage of intrathecal methotrexate (Figure 1). CVT formed 38% of all VTE during Cycle I. Two patients underwent endovascular thrombolysis and mechanical thrombectomy in addition to anticoagulant therapy; both died in the acute phase due to transtentorial herniation. After 12 months, three additional patients had died due to ALL-related causes. The surviving patients did not experience enduring neurological morbidity due to their CVT. Two of nine patients with CVT had recurrent VTE, none a recurrent CVT. CVT occurrence was adversely associated with attainment of complete remission on protocol (adjusted OR 0.12; 95% CI 0.03-0.51).
Summary / Conclusion: Although CVT is a rare manifestation of VTE, our study indicates it is much more common among adult ALL patients, with an adverse impact on ALL treatment outcomes and a high mortality rate in the first year after the thrombotic event. CVT often occurred during L-asparaginase and methotrexate therapy in Cycle I of remission induction treatment. Presence of

headache and seizures were strongly associated with CVT. Close monitoring of headache during ALL treatment may contribute to earlier detection of CVT and could improve its outcome.

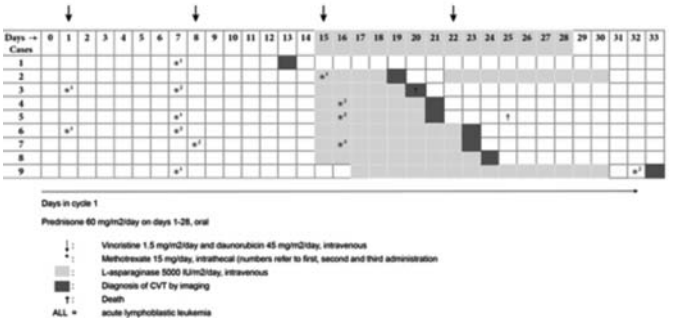

Figure 1. Time relationship between cerebral venous thrombosis (CVT) and ALL treatment component in HOVON-37 ALL study Cicle I. Bleeding &

S614

EVALUATION OF SEVERE THROMBO-HEMORRHAGIC SYNDROME (THS) BY ASSESSING LEVELS OF ACTIVATED FVII-ANTITHROMBIN COMPLEX (FVIIA-AT) AND TISSUE FACTOR (TF) MRNA IN ACUTE PROMYELOCYTIC LEUKEMIA (APL) PATIENTS

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Background: The onset of APL is characterized by a severe thrombo-hemorrhagic syndrome (THS) responsible of a high rate of hemorrhagic deaths, mainly due to intracerebral hemorrhages. The pathogenesis of the THS is complex and TF expressed by APL cells may have an important role. Differentiation therapy with all-trans retinoic acid (ATRA) downregulates TF expression by APL blasts and produces a simultaneous correction of the hypercoagulation and THS. However, the rate of early deaths due to the THS is still relevant. Therefore, characterizing the THS and identifying predictive markers remains a critical issue.
Aims: To prospectively evaluate in newly diagnosed APL patients the rate of hemorrhage and thrombosis in the first month after diagnosis, in relation to plasma levels of FVIIa-AT, a new parameter reflecting the degree of TF exposure and activity, thrombin-antithrombin (TAT) complex, D-dimer, and cellular TF mRNA.
Methods: Fifty-four patients (29M/25F) diagnosed with APL admitted to the Divisions of Hematology (Bergamo, Brescia, Rome, Vicenza) were enrolled from January 2000 to February 2009. All patients received induction therapy with Idarubicin + ATRA (GIMEMA AIDA 2000 protocol) and were prospectively monitored for thrombo-hemorrhagic episodes for 4 weeks. Blood samples from 26 of these patients were obtained at the onset (T0) of the disease, and on days 7, 14 and 28 (T7, T14, T28) of remission induction therapy. Twenty-five healthy subjects acted as controls.
Results: At T0, 14.8% of patients presented with early major hemorrhages, including 3 fatal intracranial bleeding and 5 non-fatal major bleeding; while 7.4% had thrombosis (1 fatal and 3 non-fatal events). Two more patients developed deep vein thrombosis at T7 and T14, respectively. Of the 26 patients included in the laboratory study, 3 had thrombosis at diagnosis. At T0, before starting therapy, FVIIa-AT, TAT and D-dimer levels were significantly higher in APL patients compared to controls (P<0.05). FVIIa-AT progressively decreased at T7 and T14, and dropped significantly at T28 (P<0.05 vs T0); TAT and D-dimer also decreased starting from T7 to T28 (P<0.05 vs T0). In addition, in peripheral blood mononuclear cells (PBMC) isolated from 9 APL patients, TF mRNA, initially elevated compared to healthy subjects (P<0.05), significantly decreased from T7 to T28. Interestingly, the 3 patients with thrombosis showed lower plasma levels of FVIIa-AT compared to those without (120±50 vs 332±75 ng/ml), while TAT and D-dimer were not different between the two groups. This might suggest FVIIa-AT as a more sensitive marker of the consumption coagulopathy compared to the other markers.
Summary / Conclusion: Our study confirms a significant rate of thrombo/hemorrhagic events in APL patients. Plasma FVIIa-AT complex level paralleled TF mRNA expression and may therefore be a useful surrogate tool for the TF activity measurement in the peripheral blood. In addition, the study shows that, among hypercoagulation markers, which are overall elevated at the onset of APL, the 'low' plasma FVIIa-AT complex level is capable to distinguish patients at the highest risk of severe THS. Although the small size of the study did not allow to calculate the predictive value of this marker, FVIIa-AT complex may be a promising simple candidate biomarker to test for the predictive risk of lethal/severe THS in larger prospective studies.

S615

DEFERASIROX PREVENTS CARTILAGE DAMAGE FOLLOWING HEMARTHROSIS IN HEMOPHILIC MICE, DEMONSTRATING IN VIVO THE ROLE OF IRON IN BLOOD-INDUCED CARTILAGE DAMAGE AND PROVIDING A POTENTIAL TARGET FOR THERAPY.

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Background: Joint bleedings upon trauma or major joint surgery and in hemophilia result in iron-mediated synovitis and cartilage destruction.
Aims: It was evaluated whether deferasirox, an iron chelator, was able to prevent the development of hemophilic synovitis and cartilage damage.
Methods: Hemophilic mice were randomly assigned to oral treatment with deferasirox (30 mg/kg) or its vehicle (control) (30mg/kg). After two months of pretreatment, the right knees were punctured to induce hemarthrosis. The mice were sacrificed after another five weeks of treatment. Post-mortem, knee joints were isolated, sectioned for histology and stained with hemotoxylin-eosin and safranin O. Blood-induced synovitis and cartilage damage were determined by two blinded observers.
Results: Treatment with deferasirox resulted in a statistically significant (P<0.01) decrease in plasma ferritin levels as compared to the control group (823 ng/mL ±56 and 1220 ng/mL ±114, respectively). Signs of hemophilic synovitis, as assessed by the Valentino score (score 0-10), were not different (P=0.52) when comparing the control group to the deferasirox group: score 1 (12.4% vs. 7.7%), 2 (16.7% vs. 11.5%), 3 (12.5% vs. 38.5%), 4 (29.2% vs. 19.2%), 5 (16.7% vs. 11.5%), 6 (4.2% vs. 7.7%), and 8 (8.3% vs. 3.8%). However, deferasirox treatment resulted in a statistically significant (P<0.01) reduction in cartilage damage, as assessed by the Glasson score (score 0-6), when comparing the control group to the deferasirox group: 2 (4.2% vs. 65.4%), 3 (4.2% vs. 26.9%), 4 (20.8% vs. 7.7%), 5 (54.2% vs. 0%), and 6 (16.7% vs. 0%).
Summary / Conclusion: Treatment with deferasirox prevents cartilage damage following the induction of a joint hemorrhage in hemophilic mice. This in vivo study demonstrates the role of iron in blood-induced cartilage damage. Moreover, these data indicate iron chelation to be a potential treatment option to limit the development of hemophilic arthropathy.

POSTER SESSION II

Acute lymphoblastic leukemia - Clinical

P616

MINIMAL RESIDUAL DISEASE BEFORE AND AFTER TRANSPLANTATION FOR CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA: IS THERE ANY ROOM FOR INTERVENTION?

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is considered of benefit for approximately 10% of the patients who are at very-high risk at frontline therapyand for the majority of the patients after relapse. Nevertheless, relapse remains the most frequent cause of treatment failure after transplantation. During the last decade, the prognostic role of minimal residual disease (MRD) has been shown in frontline protocols.and in relapse protocols, even when MRD was used to stratify patients and tailor risk-adapted therapy. MRD was shown as a relevant prognostic factor also in the transplantation setting with higher levels of MRD before HSCT being associated with higher risk of relapse and lower event-free survival after transplant. Pre-emptive interventions after HSCT were also attempted based on MRD at HSCT.
Aims: To assess the impact of MRD before and after HSCT on outcome, and to evaluate the impact of possible interventions in a series of consecutive ALL patients transplanted in a single Institution.
Methods: MRD was monitored by real-time quantitative PCR of clonal patient-specific rearrangements of Immunoglobulin and T-cell receptor genes. PCR-MRD targets were tested for specificity and sensitivity for each patients with the aim to select 2 targets with a sensitivity of at least 10^{-4} and a quantitative range of at least 10^{-4} for one target and at least 5×10^{-4} for the second target. RQ-PCR analysis was performed and interpreted according to the guidelines developed within the European Study Group for MRD detection in ALL (EuroMRD ALL).
Results: Eighty-two children and adolescents who underwent allogeneic transplantation for ALL in remission (period 2001-2011,median follow-up 4.9 years) had been assessed for MRD before and at 1,3,6,9 and 12 months after transplantation. Five-year-EFS and CIR were 77.7%(SE5.7) and 11.4%(SE4.4), respectively, for patients with pre-transplant MRD$<1\times10^{-4}$(68%), *versus* 30.8% (SE9.1) (P-value <0.001) and 61.5%(SE9.5) (P-value<0.001), respectively, for those with MRD$^3 1\times10^{-4}$(32%). Pre-transplant MRD$^3 1\times10^{-4}$ was associated with a 9.2-fold risk of relapse (CI 3.54-23.88; P-value<0.001) compared with patients with MRD$<1\times10^{-4}$. Patients who received pre-transplant additional chemotherapy to reduce MRD had a 5-fold reduction of the risk of failure (hazard-ratio0.18, 95% CI 0.05-0.66, P-value=0.01). Patients who experienced MRD positivity post-transplant did not necessarily relapse (5-year-EFS4 0.3%, SE9.3), but had a 2.5-fold risk of failure (CI 1.05-5.75; P-value=0.04) if any level was detected in the first 100 days and 7.8-fold (CI 2.2-27.78; P-value=0.002) if detected after 6 months. Anticipated immunosuppression-tapering according to MRD may have improved outcome, nevertheless all patients with post-transplant MRD$^3 1\times10^{-3}$ ultimately relapsed, regardless of immunosuppression discontinuation or donor-lymphocyte infusion (Figure 1).

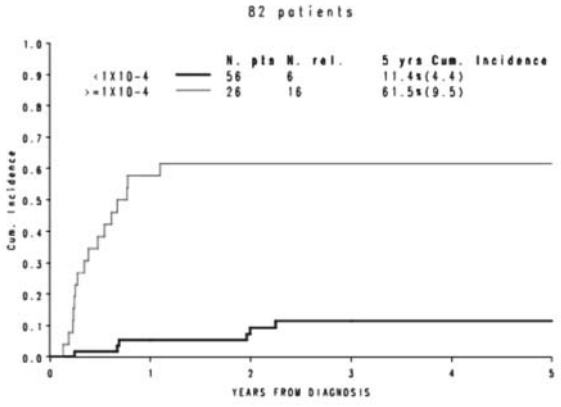

Figure 1. Cumulative incidence of relapse according to MRD level at transplantation.

Summary and Conclusions: MRD before transplantation has the strongest impact on relapse, additional intensified chemotherapy improves ultimate outcome, modulation of immunosuppression may reduce the risk of relapse and MRD positivity after transplantation does not necessarily imply relapse, mostly if detected early at low levels.

P617

A COHORT STUDY OF POST-RELAPSE/RESISTANCE SURVIVAL IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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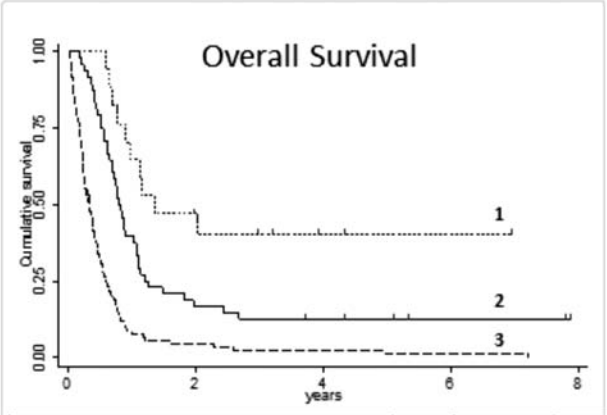
Background: Although outcome of adults with Philadelphia-negative (Ph-) acute lymphoblastic leukemia (ALL) improved significantly in the last two decades, long-term survival of refractory/relapsed (R/R) patients (pts.) remains unsatisfactory. However, this is best analyzed in unselected patient series from clinical studies, allowing to define limits and potential of current therapeutics, and offer a proper comparator to newer treatment modalities.
Aims: To analyze the long-term survival and prognostic factors for survival in an unselected cohort of R/R Ph- ALL pts. belonging to a prospective clinical study.
Methods: Between 2000-2008, 404 ALL pts. (100 Ph+ and 304 Ph-) were enrolled into NILG trial 09/00. Among 304 Ph- ALL pts., 161 (53%) had R/R ALL (median age 35 years [range 16-66], 98 male, 102 B-lineage [SR 45/HR 57], 59 T-lineage [SR 19/HR 40]).
Results: Among 161 R/R pts., 18 were refractory and 143 relapsed. One-hundred and twenty-seven pts. (89%) relapsed during 1st line chemotherapy and 16 after allogeneic stem cell transplantation (SCT) in 1st CR. The median time to relapse was 9.4 months (range 0.6-57.8); 129 pts. (90%) developed a BM or combined relapse, 9 (6.5%) an isolated CNS relapse and 5 (3.5%) an isolated extra-medullary relapse. A total of 110 pts. (77%) had an early relapse (within 18 months from CR) and 33 (23%) a late relapse. Data about salvage therapy were available for 160 pts.: 143 (89%) received re-induction therapy and 17 did not. The response to salvage therapy was evaluable in 138 pts., of whom 59 (43%) achieved a 2nd CR. The allogeneic SCT realization rate was 45% (n=65), 34 (52%) pts. receiving SCT in 2nd CR, 30 with active disease and 1 with unknown disease status. The estimated 5-year survival of the whole population was 8%, 12% and 7% in refractory and relapsed pts., respectively, with no significant differences according to site of relapse. However, pts. with late relapse had a better 5-year survival, 12% *vs.* 5.5% in refractory or early relapsing disease (P=0.005). Among pts. receiving any type of salvage therapy, those entering 2nd CR had a significantly better 5-year survival, 18% *vs.* 3% in refractory pts. (P=0.0000). Estimated 5-year survival was significantly better in transplanted pts. (20%) compared with non-transplanted pts. (1%, P=0.000), especially when SCT was performed in remission (32% *vs.* 7%, P=0.008) and in pts. suffering from late relapse (40%) (Figure 1). In univariate analysis survival was favorably affected by age ≤40 years, late relapse, achievement of 2nd CR, and remission status at SCT. Multivariate analysis confirmed that response to salvage therapy and realization of allogeneic SCT were the most significant prognostic factors for survival.



Groups		n	OS % at 5 years	p
Failures (n=161)				
1.	Late relapse, allo SCT	17	40	*0.0000*
2.	Refractory/early relapse, allo SCT	48	12.5	
3.	Refractory/relapse any time, NO allo SCT	96	1	

Figure 1.

Summary and Conclusions: The long-term outcome of patients with R/R ALL is highly unsatisfactory. This analysis confirms the need to obtain a second remission and increase the number of pts. that can benefit from allogeneic SCT, which remains the only curative option for a proportion of late relapsing pts. New drugs are required to improve CR rate and SCT feasibility in primary refractory pts. and the larger group of early relapsing pts.

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A SEQUENTIAL USE OF TKI, CHEMOTHERAPY AND TRANSPLANT IS ASSOCIATED WITH HIGH COMPLETE REMISSION RATES, DISEASE-FREE AND OVERALL SURVIVAL IN ADULT PH+ ALL. RESULTS OF THE GIMEMA 0904 PROTOCOL

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Background: The clinical scenario of Ph+ ALL, considered the most aggressive form of leukemia, has changed profoundly following the introduction of 1st and 2nd generation tyrosine kinase inhibitors (TKI). In adult Ph+ ALL, TKI have been administered as part of the backbone of induction chemotherapy together with conventional drugs or, as in two earlier GIMEMA studies, alone in combination with steroids. The use of TKI has enabled high rates of complete hematological remissions (CHR), also in elderly patients. In the GIMEMA 0904 protocol, adult Ph+ ALL patients (≤60 years) were initially treated in induction and consolidation with Imatinib together with chemotherapy. This combination was associated with unacceptable toxicity and the protocol was amended. Imatinib was given as single agent, in combination with steroids, as induction therapy, while chemotherapy was added as consolidation.
Aims: Aim of the study was to verify in adult Ph+ ALL the feasibility and efficacy of a sequential scheme based on an induction phase with Imatinib plus steroids, followed by a consolidation with chemotherapy plus Imatinib and, when applicable, by a transplant procedure.
Methods: The steroid pre-phase was started from day -6, to allow central molecular screening, up to day 31. Imatinib (600 mg) was administered from day 1 to day 50. Patients who achieved a CHR received as consolidation therapy a cycle of HAM, without discontinuing Imatinib. HAM+Imatinib was planned also for non-responsive cases, followed by a further cycle of chemotherapy. Eligible patients received an allogeneic or autologous stem cell transplant (allo-SCT, auto-SCT). *BCR-ABL1* transcript levels were normalized to the number of *ABL1* control gene and expressed as a percentage of *ABL1*.
Results: From July 2007 to April 2010, 51 patients have been enrolled; 23 were males and 28 females. The median age was 45.9 years (range: 16.9-59.7) and the median WBC 28.0 × 10^9/L (range: 1.4-597). Thirty-nine patients had a p190 fusion transcript, 7 a p210 and 5 had both. Two patients went off-study for medical decision and toxicity, respectively. After the steroid pre-phase, 38 patients (79%) had a blast reduction ≥75%. At the end of induction (day 50), 47 patients (96%) had achieved a CHR, 1 had a partial response and 1 did not respond. After HAM, also the latter 2 cases obtained a CHR. No deaths in induction were recorded. Of the 43 patients who received HAM, 23 underwent a SCT procedure (20 allo-SCT, 3 auto-SCT), while 20 did not: so far, 3 relapses have occurred in the transplanted group (13%) and 8 in the non-transplanted group (40%). Disease-free survival (DFS) and overall survival (OS) at 36 months are 50.5% and 69.1%, respectively. SCT had no significant impact on DFS, while a trend was observed for overall survival OS (P=0.06). *BCR-ABL1* transcript levels decreased during induction therapy, with a highly significant reduction (P<0.0001) between the onset and the end of induction. A further, non-significant, reduction was induced by HAM. Interestingly, a log reduction >1.3 at day 50 (end of induction) was associated with an improved DFS (P=0.03) and a decreased cumulative incidence of relapse (P=0.004).
Summary and Conclusions: A sequential approach with Imatinib alone in induction, consolidated by chemotherapy plus Imatinib and followed by a SCT, is a feasible strategy for the management of adult Ph+ ALL patients. We confirm the very high CHR rates following induction with a TKI as single agent with steroids and no deaths in induction. This sequential therapeutic approach is associated with promising DFS and OS rates.

P619

ACUTE LYMPHOBLASTIC LEUKEMIA IN THE ELDERLY: PROGNOSTIC FACTORS AND COMORBIDITIES IMPACT

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Background: The prognosis of acute lymphoblastic leukemia (ALL) is poor in the elderly, with a 5-years overall survival less than 10% for patients above 60 years old. Age is one of the most important prognostic factors.
Aims: The objective is to study the impact of comorbidities in elderly patients treated for ALL and to correlate it with clinical, biological characteristics and outcome.
Methods: Between 1995 and 2012, 92 consecutive patients aged of 60 years or more with newly diagnosed ALL were referred to our institution (Institut Paoli Calmettes, Marseille, France). Clinical, biological characteristics and treatment strategies were retrospectively collected. Comorbidities have been assessed according to 3 different scoring systems: the Charlson Comorbidity Index (CCI), the Adult Comorbidity Evaluation 27 (ACE27) and the Hematopoietic Cell Transplantation Specific Comorbidity Index (HCT-SCI).
Results: Median age was 69 years old (range 60-89). Median leukocyte and platelet counts were 11 G/L and 47 G/L respectively. Performance status at diagnosis was 0 or 1 in 42%, 2 or more in 41% and unknown in 17%. Comorbidities indexes were 0 or 1 in 79% for CCI, 95% for ACE27 and 72% for HCT-SCI. 21% of patients had a history of previous malignancy. The most frequent phenotype was B ALL (84%), including 53% of B2 ALL according to EGIL classification and 12% of Burkitt leukemia. Other phenotypes were biphenotypic (10%), T ALL (2%), undifferentiated (1%) or unknown (3%). Most frequent cytogenetics were translocation (9;22) in 41% of cases, and normal karyotype in 14% of cases. Treatment was induction chemotherapy for 86 patients (94%), palliative care for 4 patients (4%) or Dasatinib for one patient. In patients treated with induction chemotherapy, 25% were admitted in intensive care unit. Only 9.5% received allogenic hematopoietic stem cell transplantation. Complete remission rate after induction is 71.5%. Relapse free survival and overall survival are 10 and 12 months respectively. Induction mortality is 10%. Three months mortality is 14% including 3 patients with non relapse mortality, without any correlation between age or comorbidity. Outcome does not differ according to performance status, phenotype or cytogenetics. We did not demonstrate any impact of comorbidities indexes on overall survival for the whole population. However, in Philadelphia chromosome-positive ALL, median overall survival was 23 months for patients with CCI=0 as compared with 13 months for patients with CCI=1 or more. The use of tyrosine kinase inhibitors in Ph positive ALL after 2004 has a significant prognostic value (overall survival 23 *versus* 12 months, P=0.04). 18 patients (20%) are long term survivors, including 5 bone marrow transplant recipients (Figure 1).

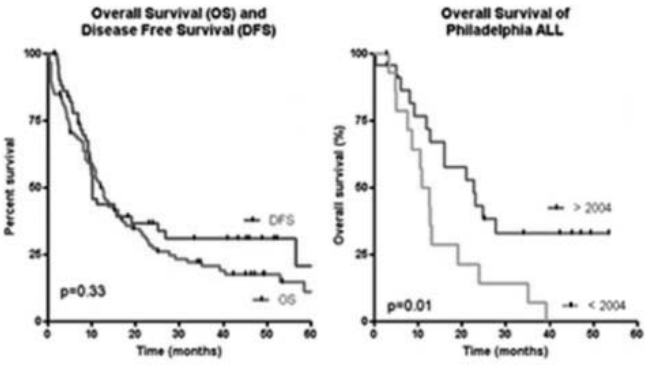

Figure 1.

Summary and Conclusions: Impact of comorbidity scoring in patients with ALL seems limited to Philadelphia positive ALL. This may be related to the low dose intensity of ALL induction regimens and a low incidence of comorbidities in our cohort. The impact in Philadelphia positive ALL may be correlated with the use of tyrosine kinase inhibitors that significantly improve short term survival, which allow time to develop underlying comorbidities related complications. Introduction of new drugs in the treatment of elderly ALL, such as Asparaginase, may favors the impact of comorbidities scores in the next years.

P620

A PHASE I, DOSE-FINDING STUDY OF THE ORAL, DUAL PI3-KINASE/MTOR INHIBITOR BEZ235 IN ADULT PATIENTS WITH RELAPSED OR REFRACTORY ACUTE LEUKEMIA

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Background: Phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling plays a central role in cell proliferation, growth, and survival and metabolism. Aberrant PI3K signaling promotes cell proliferation, survival, and drug resistance in various malignancies including Philadelphia chromosome positive (Ph+) chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL), B-precursor ALL other than Ph+ ALL, T-ALL and acute myeloid leukemia (AML). BEZ235 is a potent dual pan-class I PI3K and mTOR complex C1 and 2 inhibitor and an attractive agent for relapsed or refractory leukemias.
Aims: This phase I, open label, dose escalation study was designed to determine the dose-limiting toxicity (DLT), maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) of the investigational drug BEZ235 in pts. with advanced acute leukemia. Secondary objectives are characterization of tolerability and preliminary evidence of anti-leukemic activity of BEZ235.
Methods: Inclusion criteria of this ongoing study are: adult pts. with relapsed or refractory AML, ALL or CML-BP considered ineligible for intensive treatment. Pts. with a fasting blood glucose >160mg/dl or an HbA1c >8% were excluded. BEZ235 is administered as a single oral agent starting at 400 mg twice daily (BID) during 28d cycles, dose escalation was based on a "rolling-six"design. Pts. were evaluable for safety and DLT if they completed the first 28-day treatment cycle and received BEZ235 for at least 21 days. Dose escalation was followed by an expansion phase at the RP2D. All pts. gave informed written consent, the study was approved by the Ethics Committee of the University of Frankfurt.
Results: 18 pts. (11m, 7f), median age 65.5 years (range 30-82), have been enrolled to date including 11 with AML, 5 with B-lin ALL, one with T-ALL and one with CML in myeloid blast phase (CML-BP). 3 pts. were refractory and 15 had relapsed, 11 of them after allogeneic stem cell transplant (SCT). Pts. were evaluated at two BEZ235 doses, 6 at dose level 1 (400 mg BID) and 13 at dose level -1 (300 mg BID). AEs of all grades observed in more than 15% of pts and considered at least possibly related to study drug included diarrhea (13 [72%]), stomatitis/mucositis (11 [61%]), decreased appetite (7 [39%]), nausea/vomiting (9 [50%]), abdominal cramps (3 [17%]) and perianal pain (3 [17%]). Grade 3/4 treatment-related AEs included hyperglycemia (2 [11%]), mucositis and diarrhea (one each). No DLTs were observed, but BEZ235-related AEs in the 400 mg BID cohort necessitated treatment interruptions in 3 of 6 pts. (stomatitis, GI toxicity) and dose reductions to 300 mg BID in 2 pts. This dose level was considered incompatible with prolonged administration and enrollment was continued at 300 mg BID (dose level -1), which was well tolerated and selected as the RP2D. Enrolment in the expansion cohort at 300 mg BID is ongoing. Ten of 18 pts. evaluable for efficacy completed cycle 1, 8 pts. discontinued prematurely because of disease progression after a median of 8 days (5-18) of treatment. Clinical responses were observed in 3 of 18 pts.: one CR ongoing after 6 mos. and one hematologic improvement (both in Ph neg. B-precursor ALL) and stable disease of 4 mos. duration in an AML patient. Median time to progression is 28 days (5-112).
Summary and Conclusions: No formal DLTs and MTD were defined, but 400 mg BID was considered too poorly tolerable for prolonged administration. 300 mg BID was generally well tolerated and defined as the RP2D. Single-agent activity in AML was poor, indicating that the PI3K pathway is not a sole "driver pathway" in the majority of pts. Evidence of anti-leukemic efficacy was observed in ALL, with one patient in ongoing CR 6 months after starting BEZ235. Combination therapy may enhance the efficacy of dual PI3K–mTOR inhibitors in advanced leukemias.

P621

COMPARABLE OUTCOME OF HAPLOIDENTICAL HEMATOPOIETIC CELL TRANSPLANTATION AS POST-REMISSION THERAPY IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: INTERIM ANALYSIS OF PROSPECTIVE STUDY

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Background: Although allogeneic hematopoietic cell transplantation (alloHCT) has been generally adopted as a post-remission therapy (PRT) in adult acute lymphoblastic leukemia (ALL), little has been known about the implication of donor difference, especially the feasibility of third-party source such as haploidentical familial donor (HFD).
Aims: We performed a prospective trial assessing the feasibility of donor-specific alloHCT protocol, including HFD as stem cell source for PRT in adult ALL.
Methods: Patients over 16 years old with ALL were enrolled in this prospective trial in Asan Medical Center, Seoul, Korea, if he/she was in first (CR1) or second (CR2) complete remission and agreed with alloHCT as PRT. Those who had a matched sibling donor (MSD) received stem cell from bone marrow (BM) or peripheral blood (PB) with conventional (CON) conditioning (age <55) consisting of busulfan 3.2mg/kg/day×4 days plus cyclophosphamide 60mg/kg/day×2 days or nonmyeloablative (NMA) one (age ≥55) consisting of fludarabine 30mg/m2/day×6 days, busulfan 3.2mg/kg/day×2 days, and thymoglobulin 3mg/kg/day×3days. When MSD was unavailable, PB stem cell from unrelated donor (UD) with full-match or acceptable mismatch and HFD was considered in sequence with NMA conditioning irrespective of the age of patient. Mononuclear cells were infused for PB stem cell; neither T-cell depletion nor CD34+ selection was used for HFD-derived stem cell. Target number of infused nucleated cells was 3.0×10^8/kg for BM, 5.0×10^8/kg for PB source. Cyclosporine and methotrexate were used as graft-*versus*-host disease (GVHD) prophylaxis.
Results: Fifty patients (male:female=27:23) with median age of 31 years (range, 16-62) who received alloHCT from Jan 2010 until Aug 2012 were included in this interim analysis. Immunophenotype (B-/T-/mixed) was 40:4:6, 19 patients (38%) were Philadelphia-positive (Ph), and 38 ones (76%) were high-risk by UKALL/MRC criteria. Patients in 'advanced status' at the initiation of conditioning was 10; CR1 by salvage chemotherapy (n=1), CR2 (n=1), and relapse (REL; n=8) which was confirmed by bone marrow examination just before conditioning. ECOG performance status of all patients was ≤2. Donor groups were MSD (n=13)/MUD (n=20)/HFD (n=17, parent [9]/off-spring [3]/sibling [5]), and BM (n=12)/PB (n=38) was adopted as stem cell source. Eleven patients received CON and the other ones received NMA as a conditioning. Distribution of immunophenotype, Ph status, and risk group was not significantly different between donor groups, except the proportion of advanced status was higher in HFD group (35%; REL[5], CR2[1]). All patients achieved granulocyte engraftment over 1×10^3/μL in a median 13 (range, 11-24) days, and 47 (94%) patients also achieved platelet engraftment over 5×10^6/μL in a median 16 (range, 9-89) days. Three patients in HFD group experienced secondary engraftment failure; one finally relapsed and died, the others are alive in their remission status after donor lymphocyte infusion (DLI). In terms of complication, donor difference did not affect the incidence/severity of GVHD (acute: 34% [8% for grade≥3], chronic: 36% [22% for extensive]), infection, and organ dysfunction. Among 42 patients in CR1/CR2 at the initiation of conditioning, 2-year RFS rate (78.0% as a whole) was likely to be higher in HFD group than non-HFD (MSD+UD) group (83.3% *vs.* 68.4%, P=0.615). When events were defined as death/relapse/engraft failure/performance of DLI, 2-year EFS rate (52.2% as a whole) was also likely to be higher in HFD group (66.7% *vs.* 44.8%, P=0.565). Among all patients including those in REL, 2-year OS rate (57.3% as a whole) was nearly in statistical significance (59.5% *vs.* 58.7%, P=0.053). When patients were grouped by disease status at the initiation of conditioning, the difference of 2-year OS rate was significance (80.6% *vs.* 25.0%). All of those in REL finally died in their relapse/refractory status, and the median OS was 12.9 months.
Summary and Conclusions: In this study, AlloHCT strategy considering HFD as third-party stem cell source was feasible and the outcome was satisfactory as PRT in adult ALL. Except for the risk of engraftment failure, HFD may be considered as a feasible stem cell source for patients in their CR1/CR2 with ALL, although salvage alloHCT was not effective for those in REL irrespective of stem cell source. (Clinicaltrias.gov, NCT01037764).

P622

DISTRIBUTION OF MLL GENE REARRANGEMENTS IN A LARGE COHORT OF INFANT ACUTE LYMPHOBLASTIC LEUKEMIA AND INFANT ACUTE MYELOID LEUKEMIA PATIENTS

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Background: *MLL* rearrangements are found in the vast majority of infant ALL and about one half of infant AML cases.
Aims: To evaluate the distribution of *MLL* rearrangements among infants (<365 days) with acute leukemia (AL).
Methods: 175 infants (117 ALL cases, 57 AML cases and 1 acute undifferentiated leukemia case) were included in the current study. 11q23/*MLL* rearrangements were detected by chromosome banding analysis (CBA), fluorescence in situ hybridization (FISH) and reverse-transcriptase PCR (RT-PCR). In 57 cases genomic DNA breakpoint was detected in *MLL* and translocation partner genes by long-distance inverse PCR (LDI-PCR).

Results: 11q23/*MLL* rearrangements were revealed in 74 ALL patients (63.2%). Among this group *MLL-AF4* was detected in the majority of cases (52.7%), less frequently were found *MLL-MLLT1* (18.9%), *MLL-MLLT3* (13.5%), *MLL-MLLT10* (6.8%), *MLL-EPS15* (6.8%) and others. Children under 6 months of age with ALL had significantly higher incidence of *MLL* rearrangements in comparison with older infants (84.0% *vs.* 47.8%, P<0.001). *MLL*-positive patients more frequently had BI-ALL and less frequently BII-ALL than infants without these rearrangements (P<0.001 for both). Fusion gene transcripts were sequenced in 35 *MLL*-rearranged ALL cases. Depending on breakpoint position within *MLL* and partner genes we detected 7 different types of *MLL-AF4* fusion gene transcripts, 3 types of *MLL-MLLT1*, 3 types of *MLL-MLLT3*, 2 types of *MLL-EPS15*. The most common fusion site within *MLL* gene in ALL patients was exon11, revealed in 20 cases (57.1%). It was confirmed by LDI-PCR, that in addition to common breakpoint location in *MLL* gene (21 out of 41 cases in intron 11), allowed to reveal less frequent breakpoint sites like intron 10 (6 cases), intron 9 (5 cases), intron 12 (1 case), intron 7 (1 case). *MLL* rearrangements were found in 28 AML cases (49.1%). In AML patients the most common *MLL* rearrangements were *MLL-MLLT10* (32.1% of cases) and *MLL-MLLT3* (28.6%). Other ones were detected less frequently. In AML frequency of *MLL* rearrangements was similar in children younger and older than 6 months (P=0.904). Among *MLL*-positive cases AML M5 was detected significantly more often and AML M7 significantly less frequent than in *MLL*-negative patients (P=0.024 and P=0.001, correspondingly). The most common breakpoint location within *MLL* gene in AML patients was intron9, detected in 6 out of 15 cases (40.0%). Additional chromosomal abnormalities were revealed in 7 out of 21 *MLL*-positive AML patients with known karyotype (33%), while complex karyotype was detected in 5 cases (24%). Application of LDI-PCR allowed to verify rare *MLL* rearrangements, including *MLL-AFF3* (1 ALL case), *MLL-MYO1F* (2 AML cases), *MLL-SEPT6* (1 AML case), *MLL-SEPT9* (1 AML case). In 4 ALL and 3 AML patients *MLL* rearrangements with concurrent 3'-deletion of *MLL* gene were found. 3'-deletion of *MLL* was not associated with breakpoint position in *MLL* gene and type of translocation partner gene. None of the patients with 3'-deletions had reciprocal fusion gene. Based on LDI-PCR data we assessed several mechanisms of fusion gene formation. Reciprocal translocations were detected in 43 cases, 3-way translocations in 6 cases, inversions in 6 cases, combination of inversion and insertion in 2 cases.

Summary and Conclusions: In the current study we described some specific features of *MLL* rearrangements in a large cohort of infant AL patients.

P623

MRD LEVEL ANALYSIS IN ADULT ALL PATIENTS. ANALYSIS OF THE CZECH LEUKEMIA STUDY GROUP–FOR LIFE (CELL)

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Background: Ninety-two patients with adult ALL were treated in two major centres in the Czech Republic in 2007–2012. Median age at diagnosis was 37.8 mos. Patients ≤55 yrs. of age were treated according to GMALL 07/2003 protocol, treatment of older patients was not standardised. MRD levels were monitored in bone marrow at clearly defined timepoints.

Aims: To evaluate prognostic impact of MRD load at different timepoints of the therapy on overall suvival (OS).

Methods: The search for PCR target was performed in 85 pts. PCR marker was found in 78/85 (92%) pts. In 30/85 (35.5%) pts. a fusion gene was found (BCR/ABL in 24 pts., MLL/AF4 in 6 pts.), Ig/TCR rearrangements were monitored in 48/85 (56.5%) pts.. The search for Ig/TCR was undertaken in the group without fusion gene only; the success rate for Ig/TCR detection was 87% (48/55 pts.). MRD negativity (MRD^{neg}) was defined as whatever value $<10^{-4}$ when outside of quantitative range.

Results: Patients reaching MRD^{neg} after the first course of chemotherapy (day 26) had 3 yrs. OS 78%, n=30 (*vs.* 52%, n=37) with median survival not reached (*vs.* 52 mos.) (P=0.036), median time to relapse was not reached (NR) (*vs.* 34 mos.) (P=0.012). Patients reaching MRD^{neg} after the second induction therapy (day 46) had 3 yrs. OS 77%, n=44 (*vs.* 46%, n=24) with median survival not reached (*vs.* 30 mos.) (P=0.023), median time to relapse was NR (*vs.* 34 mos.) (P=0.066). Patients who were MRD^{neg} at the beginning of consolidation therapy (after previous 2 induction cycles, week 11) had 3 yrs. OS 80%, n=40 (*vs.* 48%, n=15) with median survival not reached (*vs.* 30 mos.) (P=0.007), median time to relapse was NR (*vs.* 10 mos.) (P<0.001). Of 34 pts. who were indicated to alo-HSCT, 24 pts. were MRD^{neg} prior to HSCT. Three-year-OS in MRD^{neg} group was 82% (*vs.* 45%), median survival NR (*vs.* 24 mos.) (P=0.034), median time to relapse was NR (*vs.* 10 mos.) (P=0.005). All 4 pts. who remained MRD^{pos} after the alo-HSCT relapsed in median of 7 mos (P<0.001); median OS was 22 mos. (P=0.005). The mean time to MRD negativity was significantly longer in BCR/ABL+ ALL (105 days) than in other types of B-ALL (44 days) and T-ALL (56 days) (P=0.002).

Summary and Conclusions: MRD load after the first induction cycle and at the beginning of consolidation therapy has prognostic significance regarding OS and relapse free survival. MRD load prior to alo-HSCT is of prognostic impact as well. MRD^{pos} status within 3 months after the HSCT defines the group with especially unfavorable prognosis. MRD response in BCR/ABL+ ALL is significantly slower than in other adult ALL subgroups.

P624

FLUDARABINE, ARA-C AND LIPOSOMAL DAUNORUBICIN (FLAD) PLUS HEMOPOIETIC STEM CELL TRANSPLANT (HSCT) AS SALVAGE THERAPY IN PATIENTS WITH RELAPSED-REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Patients with relapsed or refractory acute lymphoblastic leukaemia (ALL),have a poor outcome, that is even worse if relapse occurs early or if patients have been previously transplanted and poor risk karyotype is present. Therapeutic options are generally uneffective and badly tolerated and clinical trials with new drugs are lacking. Usually in such patients the achievement of a second remission is followed by hemopoietic stem cell transplant (HSCT). As is known, the absence of active disease before transplantation and the low toxicity of the previous salvage treatment positively affect HSCT outcome. The use of daunoxome (a formulation consisting of daunorubicin as a citrate salt entrapped within liposomes) may reduce drug-induced cardiotoxicity and increase delivery of daunorubicin to leukemic cell. We previously showed that the association of fludarabine, Ara-C and Daunoxome (FLAD) is well tolerated and effective in both poor risk AML and ALL and may allow to deliver BMT in complete remission in a group of patients.

Aims: The aim of this study is to review our overall experience with FLAD as salvage therapy of ALL and to evaluate its usefulness as bridging therapy to stem cell transplant.

Methods: The regimen consisted of three-days treatment with a 30-minute infusion of Fludarabine 30 mg/sqm followed 4 hours later by a 4 hour infusion of Ara-C 2 g /sqm and a 60 minute infusion of DNX 100 mg/sqm. Patients in CR or PR after FLAD induction received a further identical consolidation course and underwent HSCT if aged 60 or less and an HLA matched or haploidentical donor was available. Thirty-five patients with refractory (n. 12) or relapsed (n. 23, 4 after allogeneic BMT) ALL have been included in the trial. Median age was 34 years (range 13-76) and patients had received a median of 3 prior regimens (range 1-7).

Results: Three patients died of infection during therapy (8%). FLAD was well tolerated by most patients; eleven cases of fever with seven sepsi were observed; none cardiac complications and severe mucositis were recorded. Sixty per cent of relapsed and 66% of refractory ALL achieved CR. Six out of 11 ALL patients who were refractory to HYPERCVAD regimen achieved CR. Neither disease status before FLAD, nor karyotype or age affected the probability of obtaining a CR. Fourteen patients out of 30 aged 60 or less (46%) underwent HSCT (13 in CR, 1 in PR). In recent years we have observed that the percentage of patients who can receive salvage HSCT is more than doubled (31% and 72% before and after 01-1-2010, respectively).Donors were 6 HLA identical siblings, 7 haploidentical siblings, and 1 matched unrelated donor. Median DFS was 7 months (range 2-109); median OS was 8 months (range 1-120).

Summary and Conclusions: Our experience in a series of patients with very severe prognosis shows that FLAD is a feasible and effective salvage treatment for patients with relapsed and refractory ALL.The high remission rate, the low toxicity of the regimen with fast haematological recovery and the progress in haploidentical HSCT allows an increasing proportion of these patients to benefit of BMT in CR, thus improving survival.

P625

POOR OUTCOME OF TCF3-PBX1 DESPITE ITS ASSOCIATION WITH FAVORABLE RISK FACTORS STRESSES THE NEED FOR INTENSIFIED REMISSION THERAPY FOR PATIENTS OF THIS GENETIC SUBGROUP IN ADULT ACUTE LYMPHOID LEUKEMIA

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Background: Adult Acute lymphoblastic leukemia (ALL) is a genetically heterogeneous disease with very poor survival. It involves many genetic abnormalities, many of which lead to formation of fusion oncogenes (FGs) which have an established role in leukemogenesis (*Harrison & Foroni*, 2002). Although role of FGs in prognostic stratification and drug selection is well characterized in pediatric ALL, the role of FGs in adult ALL is not well established (Moorman *et al*., 2007) except BCR-ABL and MLL-AF4.
Aims: Therefore, objective of this study was to study the association of FGs with disease biology and clinical pattern.
Methods: We employed RT-PCR and interphase FISH to study the distribution of five most common FGs (BCR-ABL, MLL-AF4, ETV6-RUNX11, TCF3-PBX1 and SIL-TAL1) and their association with clinical characteristics and treatment outcome in 208 adult ALL patients from 2002-2010. Written informed consent was obtained from patients. All patients were treated with MRC UKALL XII protocol.
Results: Overall survival (OS) and relapse free survival (RFS) were 26.17 and 11.147 months, respectively. FGs were detected in 78.8% (164/208) patients. Subjects with SIL-TAL1 (35.36%) showed lymphadenopathy (which is rare in adult ALL), frequent organomegaly, low platelets count and poor survival. BCR-ABL positive patients (20.3%) presented with high total leukocyte count (TLC), prominent splenomegaly, low platelets count (P-value <0.001), poor survival (OS & RFS 9.3 & 6.3 months, respectively) and 10% less chances of 4-6 week remission as compared to those with negative BCR-ABL status. MLL-AF4 (12.19%) was associated with elevated TLC, organomegaly, frequent CNS involvement, and a poor clinical outcome (OS=8.8 months). Surprisingly, ETV6-RUNX1 was least represented with frequency of 4.8% (10/208), and was mostly associated with low TLC, less common organomegaly, high CR rates and higher OS (30.2 months). However, long term survival of ETV6-RUNX1 was negatively affected by frequent late relapses. Frequency of TCF3-PBX1 was higher (16.3%, 34/208) than reported worldwide (3%). This genetic subgroup was associated with younger age (59%), lower TLC (82%), platelet count higher than 50×10^9/l (12/17, 71%), less common hepatomegaly (2/17, 12%), less common splenomegaly (3/17, 18%), early CR (11/17, 65%), indicating a favorable prognosis. However high relapse rate (13/17, 76.1%) and shorter OS (11.6 months) were observed in TCF3-PBX1 positive patients.
Summary and Conclusions: High relapse rates and shorter OS despite favorable prognosis evident by clinical features at presentation and high 4-week CR in 65% of TCF3-PBX1 positive patients highlight the need for differential genetic diagnosis at presentation and intensified remission induction treatment in this sub-group of patients (Foa *et al*., 2003). Our data is in accordance with recent reports (Burmeister *et al*., 2010). This, along with lower frequency of ETV6-RUNX1, higher *MLL-AF4* frequency (9.7%) and association of SIL-TAL1 with lymphadenopathy (which can help in differential diagnosis of this genetic subgroup of adult ALL in low-resourced countries) in our patients, reflect ethnic differences in disease biology and treatment outcome in adult ALL (Burmeister *et al*., 2010). Overall high percentage of poor prognostic FGs in study subjects partially explains the overall poor outcome of adult ALL in our country. Therefore, we recommend the molecular testing for ALL FGs in routine clinical settings at diagnosis and its implication in prognostic stratification and drug selection during various phases of treatment. It also indicates the need for high-throughput molecular analysis of different ALL genotypes for elucidating the mechanism of leukemogenesis and its implication in finding novel targeted therapies for adult ALL.

P626

CLINICAL OUTCOME OF T- ACUTE LYMPHOBLASTIC LEUKEMIA/ LYMPHOMA (T-ALL/T-LBL): THE BOLOGNA EXPERIENCE

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Background: Precursor T cell ALL/LBL occurs most frequently in late childhood, adolescence, and young adulthood, with a 2:1 male predominance; it comprises 15 and 25 percent of childhood and adult ALL, respectively, and 2 percent of adult non-Hodgkin lymphoma. In adult patients prognosis is poor, due to the high incidence of relapse even after allogenic stem cell transplantation.
Aims: To identify new targets, to optimize therapy, to reduce toxicity and to improve clinical outcome.
Methods: We retrospectively analysed and currently report clinical outcome results about 52 newly diagnosed and younger than 60 years T-ALL patients (median age 30 years, range 14-73 years) treated, from 2006 to 2012 according to standard chemotherapy regimen, including adapted pediatric-like schedule, BFM protocol, and adult schedules. After induction, all the patients underwent consolidation for at least one course. All the patients shared the same strategy for intensification, that consisted, when available, in allogenic or autologous stem cell transplantation. Detailed data about cytogenetics and molecular biology will be provided on site. Durations of complete remission (CR) and overall survival (OS) were estimated according to the Kaplan-Meier method. The CR duration was calculated from start of CR to first evidence of disease recurrence.
Results: Informed consent was obtained; after a single induction course 41/52 patients obtained a CR (78.8%) and 2 patients a partial remission (PR) (3.8%) for an overall response rate (ORR) of 82.6%. Seven patients (13.4%) had resistant disease, and 2 (3.8%) died during induction. After a median follow-up of 22.7 months, 19 patients (36.5%) are still in CR. The median CR duration and OS were 12.3 and 23.15 months, respectively. The most common grade 3 adverse events included gastro-intestinal toxicities (*i.e.* nausea, vomiting, mucositis and diarrhoea) and liver dysfunction.
Summary and Conclusions: Our analysis confirms, in line with literature data, that, despite intensive chemotherapeutic treatments and stem cell transplantation, T-ALL and T-LBL adult patients still show a bad prognosis. The relatively satisfying induction remission rate is followed, in most cases, by a high relapse rate. Therefore, a molecular stratification approach, based on gene-expression profile analysis (Ferrando *et al*., Cancer Cell 2002) should be routinely performed, in order to identify new targets, to optimize therapy, to reduce toxicity and to improve clinical outcome.
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P627

ENCOURAGING SURVIVAL IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA - RESULTS OF RUSSIAN ACUTE LYMPHOBLASTIC LEUKEMIA (RALL) STUDY GROUP

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Background: T-cell ALL is a heterogeneous disease regarding biological features and long-term outcome. We have evaluated the characteristics and 3-years survival rates of T-ALL patients treated within Russian prospective multicenter trial ALL-2009 (ClinicalTrials.gov public site; NCT01193933).
Aims: The main principle of the protocol (ASH, 2012, poster 2572) was no aggressive but non-interruption treatment with prolonged L-asparaginase (Σ=590.000 IU), and autologous HSCT with BEAM conditioning followed by maintenance in T-cell ALL pts without HLA-identical donors.
Methods: From Nov, 2008, till Jan, 2013, 29 centers enrolled 203 ALL pts: 63,6%=B-lin, 35,3%=T-lin,1,1%=biphenotypic. Median age in T-ALL pts was 28 y (15-54); male gender prevailed - 24f/46m, L -24,5*10^9/l (0,8-313), b/m blast - 75,5%(0,1-99), LDH -995 UI (131-11000). 32 (47,1%) pts had early T-ALL (T-I/II), 31 (42,9%) - thymic (T-III), 7 (10%) - mature (T-IV). Cytogenetics was available in 50% of pts (n=35) and 43% of them (n=15) had normal karyotype (NK). T-I/II was characterized by more frequent bone marrow involvement (P=0,01), T-IV - mediastinum involvement (P=0,05). The analysis was performed in Jan, 2013. Induction and follow-up data were available in 58 pts.
Results: CR was achieved in 100% of T-III (26/26) *vs* 72% in T-I/II (18/25) and 71,4% (5/7) in T-IV (P=0.01). Late responses were more frequent in early T-ALL (CR after the $1^{st}/2^{nd}$ phase n=13/5) comparing to T-III (22/4) and T-IV (4/1). Induction death occurred only in early T-ALL pts - 16% (P=0.01). Primary resistance and even progression during induction was registered in T-I/II (12%) and T-IV (28,6%). 15 pts were transplanted (3-allo, 12-auto), non of them relapsed so far, comparing with 21,4% relapses in no transplanted group (P=0.05). Comparing 3-years overall and disease-free survival in T-cell-subgroups we did not find any differences so far: OS=67,8%(T-I/II), 72,4%(T-III), 71,4%(T-IV) and DFS=79,4%(T-I/II), 71,9%(T-III), 100%(T-IV). In a multivariate analysis no variable from the list (age, gender, T-ALL type, initial WBC, LDH, b/m blasts, karyotype, time to CR, L-asparaginase cessation, HSCT) was found that significantly influenced survival.
Summary and Conclusions: Our data demonstrate that early T-all is characterized by lower CR rate, more frequent refractory (even progressing) disease and higher early death rate. Thymic ALL is the most favorable subtype regarding induction results. Regardless these differences the survival rates were quite similarly good. We assume that the analyzed group is small, but promising long term results in different T-ALL categories suggest that ALL-2009 protocol approach - no aggressive but non-interruption treatment - is highly effective, nontoxic and reproducible.

P628

FLOW CYTOMETRIC INVESTIGATION OF CENTRAL NERVOUS SYSTEM INVOLVEMENT IN CHILDHOOD ACUTE LEUKEMIAS

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Background: Central nervous system (CNS) involvement is one of the important risk factors in childhood acute leukemia (AL). Tumor cells detection in cerebrospinal fluid (CSF) is one of the main signs of CNS lesion. Traditionally blasts presence in CSF is assessed by conventional cytomorphology (CM) of cytospin slides. However, sensitivity of this method is relatively low. Flow cytometry (FC) having a higher sensitivity could provide better diagnostic applicability for CSF blasts detection.
Aims: To compare results of tumor cells detection in CSF of children with AL by flow FC and CM.
Methods: 183 samples from 52 boys and 31 girls aged from 5 months to 15 years with different types of acute lymphoblastic leukemia (ALL) (77 patients), acute myeloid leukemia (AML) (5 patients) and acute biphenotypic leukemia (1 patient) were investigated. 17 positive samples obtained by traumatic lumbar puncture were excluded from analysis because tumor blasts were also detected in peripheral blood. Comparison between FC and CM data was performed in 166 samples. Among these samples 61 was taken at the time of initial diagnostics, 34–during AL follow-up, 17–at relapse and 54–during relapse monitoring. Monoclonal antibodies panels were constructed according to immunophenotype of tumor cells in bone marrow.
Results: In 24 out of 166 samples (14.5%) tumor cells were detected by CM. In all these cases blasts were also found by FC, while FC allowed finding blasts in other 35 samples. Thus the total number of FC-positive samples was 59 out of 166 (35.5%). This frequency was significantly higher than rate of CM-positive cases ($p < 0.0001$). Among initial diagnostics samples there were 20 FC-positive and only 10 CM-positive patients (32.8% *vs.* 16.1%, P=0.0585). At relapse 9 (52.9%) patients were FC-positive, while 6 (35.3%) were CM-positive (P=0.4897). In both B-lineage and T-lineage ALL, analyzed separately, FC detected blasts in CSF frequently than CM (P=0.0098 and P=0.0002 respectively). Absolute blast count in 1 mL in CSF samples, positive by both methods was significantly higher than in samples, positive only by FC (median=418, range 8-158171 and median=34, range 5-2762 respectively, p=0.0002). Thus FC allows detecting tumor cells in CSF much more frequently than conventional CM, which could be explained mainly by higher FC sensitivity. Moreover FC is applicable also for qualitative and quantitative monitoring of CNS lesion. Nevertheless prognostic impact of FC CSF investigation is questionable. Among 13 patients in whom discordant results were obtained in initial diagnostics samples and at relapse, only for one patient risk stratification could have been changed. For all other patients there were other risk factors, that decreased significance of FC leukemic blast detection in CSF.
Summary and Conclusions: Flow cytometry allows more frequent detection of tumor blasts in CSF of children with AL, while prognostic significance of these findings is still unclear and needs to be confirmed in large prospective trials.

P629

FOUR YEARS EXPERIENCE WITH HYPER-CVAD TREATMENT OF ADULT T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA IN SWEDEN. POPULATION-BASED DATA

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Background: Hyper-CVAD (fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with cycles of high-dose methotrexate and cytarabine) has shown promising results in adult T-cell Acute Lymphoblastic Leukemia (T-ALL). The protocol was introduced as standard treatment of T-ALL in Sweden in October 2002 and was recommended until September 2006 in the national guidelines from the Swedish Adult ALL Group.
Aims: Assessment of efficacy of Hyper-CVAD in adults with T-ALL in a population based cohort.
Methods: 24 patients with T-ALL diagnosis were prospectively reported to the Swedish Adult Acute Leukemia Registry between October 2002 and September 2006. Missing data were complemented retrospectively. Hyper-CVAD was recommended to all patients without severe comorbidity. Allogeneic stem cell transplantation (SCT) was recommended for patients with high-risk disease: white blood cell count $>100\times10^9$/L, complete remission (CR) achievement after more than two courses, high minimal residual disease level, and relapsed disease (after CR2 achievement). In patients without high-risk factors maintenance therapy was given with per oral mercaptopurine and methotrexate for two years including reinduction courses: daunorubicine, vincristine and prednisolone every second month (1st year) and cytarabine, thioguanine and prednisolone every third month (2nd year).
Results: Totally 24 patients were reported. Four patients were treated with palliative intention, and were excluded from further analysis, as was one with sarcoma and secondary T-ALL. The remaining 19 patients (15 men and 4 women) were treated according to Hyper-CVAD protocol. The median age at diagnosis in this group was 32 years (range 18-72). CR was obtained in 17/19 (89%) patients and in additionally one after salvage with nelarabine. 5 year overall survival (OS) and leukemia free survival were 34% (95%CI: 10-56) and 26% (95%CI: 7-46) respectively. Allogeneic SCT was performed upfront in four patients (including one without CR achievement) with two patients becoming long-term survivors. 14/15 (93%) of patients not transplanted in CR1 completed the Hyper-CVAD protocol (8 courses). One patient received two courses only due to agranulocytosis and invasive candidiasis. 5 year OS and leukemia free survival for the 15 patients were 30% (95%CI: 5-55) and 20% (95%CI: 0-40) respectively. 12/15 (80%) patients relapsed after median 9 months (range 2-23) from CR1 achievement. Six of them received allogeneic SCT in CR2 with three still alive. All six relapsed and not transplanted patients died after median 2.5 months (range 1-6) from relapse. Age >35 years influenced survival negatively (HR 5.1, 95%CI: 1.55-16.7) and there were no long-term survivors in this older group (N-7) except one (aged 72) who died after 91 months in CR1 compared to 58% (95%CI: 30-86) OS at 5 years in 12 younger patients. 5 year OS in all transplanted patients (N-10: sibling donor-4, unrelated-5, cord blood-1) was 50% (95%CI: 19-81) compared to 17% in nine non-transplanted (HR 2.76, 95%CI: 0.86-8.84). Only 1/7(14%) of patients aged >35 years received allogeneic SCT (in CR2) compared to 9/12 (75%) in the younger patients (P=0.01, chi-square test).
Summary and Conclusions: The results of treatment with Hyper-CVAD without any subsequent SCT were discouraging especially regarding patients >35 years although the number of patients was small. The protocol is no longer recommended in Sweden. The efficacy of upfront allogeneic SCT cannot be assessed in this study but the procedure emerges as a possible treatment after relapse.

P630

POST INDUCTION TREATMENT OF PHILADELPHIE POSITIVE CHROMOSOME ALL: TKI BASED-INTENSIVE CHEMOTHERAPY OR ALLOHSCT ?

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Background: Allogenic Hematopoïetic Stem Cells Transplantation (AlloHSCT) represents the standard treatment of Philadelphie chromosome Positive Acute Lymphoblastic Leukemia (Ph+ALL)[1]. Recently, Tyrosine Kinase Inhibitor (TKI)-based strategy combined with intensive chemotherapy had improved the outcomes of patients treated for a Ph+ALL [2]. Post remission consolidation and maintenance therapy comprising TKI instead of alloHSCT has not been evaluated in patients with Ph+ALL. However, in some studies in which transplantation has not been performed, excellent overall survival (OS) and disease free survival (DFS) were reported.[3]
Aims: The aim of our study was first to compare alloHSCT and maintenance TKI treatment in patients with Ph+ALL and secondly, to show that when transplant is not a possible option, targeted therapies can permit long-term complete molecular response (mCR).
Methods: We retrospectively analysed all the patients treated for a Ph+ALL between March 2004 and July 2012 in our department, with a TKI (Imatinib or Dasatinib) chemotherapy strategy combination (Hyper-CVAD or GRAALL recommendations chemotherapy).
Results: Eighteen patients with a median age of 53 years (range: 29-71) were treated with Imatinib (n=13) or with Dasatinib (n=5) based chemotherapy. Eight patients received chemotherapy and TKI treatment (chemo group) without transplant because of age (n=7) or no donor (n=1) and 10 patients underwent alloHSCT (5 sibling, 3 unrelated donor and 2 umbilical cord blood). Patients in the chemo group were significantly older (median age: 63 years *vs* 46 years, P<0,001). Median time from diagnosis to HSCT was 6 months (range 4-12). Median white blood cells count in alloHSCT group at diagnosis was higher than in chemo group (median10,46 $10.^9$ /L, range1,23- 240,39 *vs*4,98 $10.^9$ /L, range:2,63- 214,5). No patient had central nervous system involvement in the chemo group and only one in the alloHSCT group. All but one patient in the chemo group had high LDH level (>440 UI/L). Additional cytogenetic abnormalities were found in 3 patients (deletion of chromosome 7 for 2 of them, one in each group). After induction chemotherapy, mCR was reached in eleven patients (4 in the alloHSCT group and 7 in the chemo group with a median duration of6,8 months; range1,3- 17). Only one patient in the chemo group never reached a mCR. Relapse occurred for six patients: 5 in the alloHSCT group in a median duration of 8 months from the transplant (range 6-21 months) and

17 months from diagnosis (range 12-26 months) and 1 in the chemo group in a median duration from the diagnosis of7,5 months. Five patients died secondary to relapse (4 in the alloHSCT group and 1 in the chemo group). One patient developed a fatal Pneumocystis infection and one died secondary to glioblastoma. The median OS is 56 months (45 months in the alloHSCT group and no reached in the chemo group). With a median follow up of 30 months (range 9-83), the estimated 30 months-OS is 70%, 62% and 70% (respectively for all patients, alloHSCT and chemo group) (Figure 1).

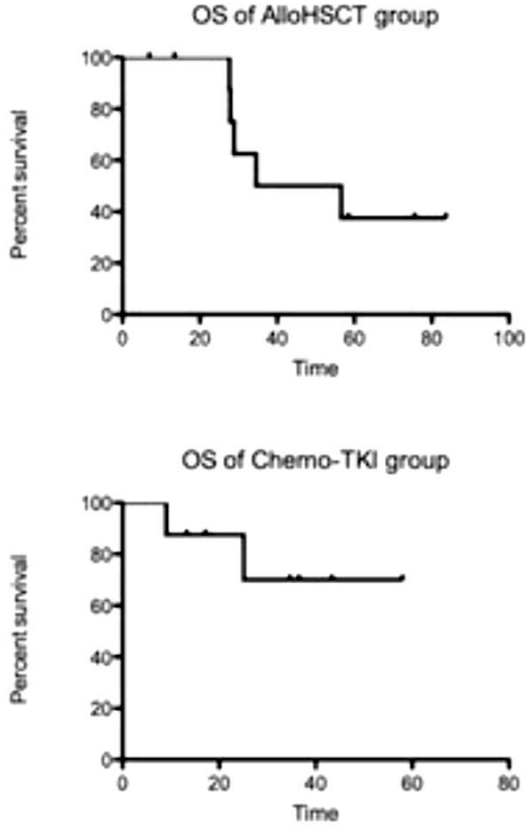

Figure 1.

Summary and Conclusions: AlloHSCT is still the standard post-induction treatment of Ph+ALL patients. However, the outcomes of intensive chemotherapy have improved at the TKI era. Although this study concern a small sample size, our results show that good outcomes can be achieved without alloHSCT and suggest the choice of maintenance treatment instead of reduced intensity conditioning alloHSCT in patients with advancing age.

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ADULT MIXED-PHENOTYPE ACUTE LEUKEMIA ACCORDING TO THE WHO 2008 CLASSIFICATION–A SINGLE CENTER EXPERIENCE

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Background: Mixed-phenotype acute leukemia (MPAL) is a rare disease (2-5% of all leukemias) and represents a heterogeneous category of acute leukemias that show expression of a combination of antigens of different lineages. For many years, the diagnostic criteria were based on the scoring system proposed by the European Group for the Immunological classification of Leukemias (EGIL). More recently, in 2008, the WHO adopted new criteria for the diagnosis of MPAL which are based on the expression of strictly specific T-lymphoid (cytoplasmic CD3) and myeloid (MPO) antigens, the later by flow cytometry or cytochemistry and/ or clear evidence of monocytic differentiation; B-cell lineage assignment in MPAL relies on the strong expression of CD19 together with another B-cell associated marker, or in cases with weak CD19, on the expression of at least 3 B-lineages markers.

Aims: To describe the clinical features and response to therapy in 13 cases of MPAL.

Methods: A total of 403 patients were diagnosed with Acute Leukemia in our department between January 1998 and December 2010. We identified 13 patients who met the WHO 2008 criteria for MPAL. Data regarding their clinical features at diagnosis, morphology according to the French-American-British (FAB) classification, immunophenotype characteristics by flow cytometry and karyotype analysis were collected. Overall survival (OS) and disease-free survival (DFS) were obtained by the Kaplan-Meier method. Statistical analysis was performed using SPSSv20.

Results: MPAL represented 3.2% of the Acute Leukemias diagnosed in our Department over a 13-year period. Of the 13 patients analyzed, there was a predominance of the male gender (11; 84.6%); median age was 39.0 years (15.2–64.7). At diagnosis, median leukocyte count was 11.8×10^9/L (0.23–88.1), median platelet count was 88.5×10^9/L (9–255) and median lactic dehydrogenase was 409 IU/L (193–766). Morphology was compatible with acute lymphoblastic leukemia (ALL) in 61.5% and acute myeloid leukemia (AML) in 38.5%. Flow cytometry analysis revealed "B+myeloid" (n=6), "T+myeloid" (n=3) and "B+T" (n=4). Cytogenetic analysis showed normal karyotype (n=5), complex karyotype (n=2), t(9;22) (n=1), del22q (n=1); 4 patients didn't have metaphases. Seven patients received ALL therapy, 4 patients AML and 2 received a combination of ALL+AML therapy. ALL treatment induced a complete remission (CR) in 57.1% and AML therapy in 75%; both patients submitted to combination therapy achieved CR. Five patients were submitted to allogeneic stem cell transplantation in 1st CR (n=4) or 2nd CR (n=1). Nine patients died, 7 of progressive/resistant disease and 2 of infectious complications. Median overall survival (OS) and disease-free survival (DFS) were 22.1 and 16.4 months, respectively; estimated 5-year OS and DFS of 30.8% and 33.3%. A subgroup analysis revealed, a 5-year OS of 20% for "B+myeloid", 33.3% for "T+myeloid" and 50.0% for "B+T" (P=0.459) and a 5-year DFS of 33.3% for both "B+myeloid" and "T+myeloid", and 50.0% for "B+T" (P=0.764). Analysis according to induction therapy showed, a 5-year OS of 42.9% for "ALL", 25.0% for "AML" and 0% for combined treatment (P=0.586) and a 5-year DFS of 50.0% for "ALL", 33.3% for "AML" and 0% for combined treatment (P=0.130).

Summary and Conclusions: We presented a small series of MPAL which is often described as having a poor prognosis. In our series, we observed a 5-year OS of 30.8% and a 5-year DFS of 33.3%.

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INTEGRATING ALTERNATIVE DONORS ALLOWS BEST OUTCOMES IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN HIGH RISK ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: A SINGLE CENTRE STUDY

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Background: Hematopoietic stem cell transplantation (HSCT) from an HLA-matched allogeneic donor is the best option for patients (pts) with high and very-high risk adult acute lymphoblastic leukemia (ALL). However, only 30% of pts have an HLA identical sibling donor. The remaining pts need to find an alternative donor in suitable times preceding relapse but it is not codified as a standard procedure.

Aims: The aim of our retrospective analysis is to provide data on HSCT from any donor (HLA identical sibling, matched or partially mismatched unrelated volunteers, haploidentical donors, cord blood) in adult patients affected by high risk ALL treated in any stage of disease in our centre.

Methods: Seventy-eight pts with ALL received a HSCT between 1999 and 2012: 30 (38%) of them were B-ALL, 20 (26%) T-ALL, 8 (10%) biphenotipic-ALL, and 20 (26%) Philadelphia positive ALL. The median age at transplant was 36 years (range 19-68). Twenty-nine pts (37%) underwent HSCT in first complete remission (CR1) because of high or very high clinical risk at diagnosis or because of molecular failure after induction; 6 and 3 pts (8% and 4%) were in CR2 and CR3 respectively; 40 pts (51%) were in chemo-refractory persistence of disease. Seventeen pts (22%) received graft from an HLA identical sibling, 19 pts (24%) from an unrelated donor, 38 (49%) from an haploidentical donor and 4 (5%) from umbilical cord blood. We offered alternative donors to allow prompt HSCT mainly in refractory cases, but also in CR (8 and 4 patients received an haplo-HSCT respectively in CR1 and CR2). Conditioning regiments were mainly at reduced intensity (RIC 61 pts, 78%) and in few cases fully myeloablative (MAC 17 pts 22%).

Results: After a median follow-up of 901 days, 28 (36%) pts are alive, all of them in CR. Median overall survival (OS) is 254 days (95% CI 142-366). In univariate analysis significant factors for OS were CR *vs.* not CR at HSCT (OS at 3y 50% *vs.* 9%, P<0.01), HLA identical sibling donor *vs.* any alternative (OS at 3y 46% *vs.* 24%, P<0.01), and conditioning RIC *vs.* MAC (OS at 3y 33% *vs.* 14% P=0.02). There are not differences in outcome among transplants from unrelated or haploidentical donors. Relapse incidence and TRM are 49% and 40% respectively at 3 years. The most significant factor for TRM is HLA iden-

tical sibling *vs.* any alternative (TRM at 5y 7% *vs.* 50%, P<0.01). Significant factor for relapse is CR *vs.* not CR at HSCT (31% *vs.* 68% 3y, P<0.01). In multivariate analysis the only significant factor for overall survival, relapse and TRM consists in CR at transplant (P<0.01), whereas the type of donor is not statistically significant for OS, TRM and relapse.
Summary and Conclusions: These data report our single centre activity on high risk ALL: the best prognosis belongs to pts in CR at HSCT, mainly in CR1. An HLA-matched donor is the best source in terms of OS and TRM. Unfortunately the largest part of patients in need for a transplant lacks an identical sibling: our data show that HSCT from an alternative source is feasible and do not negatively affect outcome in multivariate analysis. Every effort should be directed to promptly recognize patients at high risk and to offer them a transplant as soon as possible in complete remission, considering alternative donors in a strategic decision-making algorithm.

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OBESITY, INSULIN RESISTANCE AND ADIPOKINES IN SURVIVORS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA.
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Background: Obesity is a common complication of treatment for acute lymphoblastic leukemia (ALL) in children. The adipokines, leptin and adiponectin play important role in regulation of energy metabolism, obesity and insulin resistance.
Aims: In order to assess the prevalence and risk factors for obesity and to explore the links between obesity, insulin resistance and adipokines in survivors of childhood ALL,
Methods: seventy-three children and adolescents (31 females and 42 males) were studied 3.4±2.9 (mean±SD) years after completion of ALL-Berlin-Frankfurt-Münster (BFM)-95 chemotherapy protocol. Informed consent and IRB approval were obtained for the study. Body mass index (BMI), levels of fasting blood glucose, serum insulin, leptin, adiponectin and lipids, and HOMA-IR (Homeostasis Model of Assesment-Insulin Resistance) for insulin resistance were determined at the last follow-up visit. Clinical data and changes in BMI and blood chemistry at diagnosis of ALL and during chemotherapy were retrieved from their medical files.
Results: The mean age of 73 ALL survivors was 13.9±4.8 years at the last visit and 7.6±4.6years at the diagnosis of ALL. Of them, 34.2% were overweight (BMI>85th -90th percentile) or obese (BMI>95th percentile). The proportion of overweight/obese children was not significantly different between girls and boys, risk groups and with and without parental obesity or cranial irradiation (1200 or 1800Gy). It was 23.3% at diagnosis and did not change significantly during the whole chemotherapy period. Of the 49 children who were lean or normal weight at diagnosis of ALL, 24.5% became overweight and 4.1% became obese at the time of this study. Leptin level (8.10±8.62 ng/mL) and leptin:adiponectin ratio (2.3±3.48) were significantly higher in ALL survivors than (3.16±2.24 ng/mL) in age matched healthy controls (P:0.02) while the levels of adiponectin, insulin and HOMA-IR were not significantly different between these two groups (P<0.05). HOMA-IR was significantly correlated with both serum leptin level (r:0.57) and leptin/adiponectin ratio (r:0.51),(P:0.001). However, the levels of leptin, adiponectin, insulin and HOMA-IR were not significantly different between lean/normal weight and overweight/obese ALL survivors. Fasting blood glucose was elevated in 7.5% and lipid profile was abnormal in 16.4% of 73 survivors.
Summary and Conclusions: Obesity is increased in survivors of childhood ALL independently of cranial irradiation, gender and parental obesity. Elevated leptin level in association with insulin resistance and dyslipidemia after treatment of childhood ALL may induce susceptibility to diabetes and cardiovascular disease.

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ZAP70 GENE EXPRESSION MAY BE A PROGNOSTIC FACTOR FOR ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS
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Background: Zeta-chain-associated protein (*ZAP- 70*) gene is normally expressed in T and natural killer cells that acts quickly after T cell activation to propagate signal. It has been described that *ZAP-70* contributes to the B-cell development at early stages of B-cell differentiation and *ZAP-70* gene expression is associated with poor prognosis in chronic lymphocytic leukaemia (CLL) but its role in adult acute lymphoblastic leukemia (ALL) has not been well established.
Aims: This study was to investigate the expression of *ZAP-70* in newly diagnosed patients of adult ALL and to explore the correlation between *ZAP-70* expression and outcome.
Methods: BM samples from 73 newly diagnosed ALL patients were analyzed. CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD22, CD34 and other leukocyte surface markers were analyzed by cytofluorimeter. Of the 73 patients, 33 were females and 40 were males, with a median age of 23 years (range: 14 to 73 years). Among them, 52 out of 73 patients were B- ALL, 16 out of 73 patients were T-ALL and 5 out of 73 patients were biphenotypic ALL (BAL). 17 patients had the BCR-ABL translocation (Ph positive) confirmed by cytogenetics. ALL patients received 1 cycle of standard induction of VDLPfollowed by 6-8 courses of Hyper-CVADtherapy alternating with high-dose MTX and Ara-C (HD MTX–Ara-C) therapy. The patients were subdivided into high and low *ZAP-70* expression groups according to the median expression of *ZAP-70* mRNA in their pre-treated bone marrow cells. Comparisons were performed using X^2 test and independent-samples T test.
Results: *ZAP70* expressions were detected in 69.8% ALL patients.There were no statistical differences of *ZAP70* gene expression levels in FAB subgroups but in immunophenotyping groups, *ZAP70* gene expression levels of T-ALL patients were significantly higher than that of B-ALL and biphenotypic ALL (P<0.01). The expressions levels of *ZAP70* gene were not correlated to peripheral white blood cell (WBC) counts, hemoglobin level, platelet counts and percentage of bone marrow blast cells at presentation. There was no statistically significant relation between *ZAP-70* expression and BCR-ABL translocation. There was no statistically significant relation of complete remission (CR) rates between high and low *ZAP70* expressions in *de novo* ALL patients and T-ALL subgroup (*P* >0.05), but in common B-ALL subgroup, there was a correlation of CR rate between high and low *ZAP70* expressions (*P*=0.041). Adult ALL patients with high levels of *ZAP70* had a significantly higher relapse rates than those with low levels (48.3% *vs* 21.7%, *P*<0.05).
Summary and Conclusions: Our results showed *ZAP-70* expression correlates with an increased relapse rate in adult ALL patients and a worse CR rate in common B-ALL subgroup. Therefore the expression of *ZAP-70* may provide useful information for clinical decision.

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HIGH FREQUENCY OF BCR-ABL IN PEDIATRIC ACUTE LYMPHOID LEUKEMIA WITH POOR OUTCOME IDENTIFIES THE IMMEDIATE NEED FOR INCORPORATION OF IMATINIB AND OTHER TYROSINE KINASE INHIBITORS IN TREATMENT PROTOCOLS
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Background: Acute lymphoblastic leukemia (ALL), a neoplasm of lymphoid origin, is the most common cancer in children. It is a genetically complex disease involving many fusion oncogenes (FGs) which have important implications for prognosis, drug selection and treatment outcome (Xu *et al.*, 2012). The frequency of various FGs can vary in different ethnic groups (van-Dongen *et al.*, 1999). Pakistan has much lower cure rates of pediatric ALL which may be partially due to adverse genetic factors associated with the disease.
Aims: This study was designed to find out frequencies of prognostically important FGs in pediatric ALL and their association with disease biology and treatment outcome, which can provide a genetic clue of low cure rates in Pakistani pediatric ALL patients.
Methods: From 2002 to 2011, we analysed 204 pediatric ALL patients using interphase FISH and RT-PCR (van-Dongen et al, 1999) at presentation, for FGs and their association with clinical features as well as treatment outcome. Written informed consent was obtained from patients/guardians. All patients were treated per UKALL-2003 protocol.
Results: Overall relapse-free survival was 9.446 months (95% C.I.). Five most common FGs, *i.e.* BCR-ABL, ETV6-RUNX1, MLL-AF4, TCF3-PBX1 and SIL-TAL1, were detected in 183/204 (88.1%) patients, with frequencies of 47.05%, 17.2%, 16.2%, 7.4% and 1.97%, respectively. ETV6-RUNX1 was underrepresented in our pediatric ALL population. The highest early response (94.4%) was seen in this genetic subtype followed by SIL-TAL1 (71.4%) and patients with no FGs (66.7%).The highest relapse-free survival (RFS) was documented in ETV6-RUNX1 (14.167 months) followed closely by those 10.4% cases in which no gene was detected (13.1 months). RFS in BCR-ABL, MLL-AF4, TCF-PBX4 and SIL-TAL1 was less than 10 months (7.994, 3.559, 5.500 and 8.080 months, respectively). Lymphadenopathy was most common in MLL-AF4 (70.6%)
BCR-ABL was detected in 47.05% (96/204) patients. Frequency of occurrence was directly proportional to age (6.7% in less than 2 year age group, 35.5% in the 2-7 year age group and 57.8% in the older than 7 group). Total leukocyte count (TLC) was higher when compared to patients with other oncogenes and

organomegaly was not common (). BCR-ABL positivity was associated with low remission rates (64.4% late remissions) and shortened survival (43.73±4.24 weeks) (Table 1).

Table 1. Clinical characteristic and treatment outcome of Pakistani pediatric ALL patients.

Clinical and laboratory parameters	BCR-ABL (%) N=96	ETV6-RUNX1 (%) N=35	MLL-AF4 (%) N=33	SIL-TAL1 (%) N=15	TCF3-PBX1 (%) N=4	FGs not detected (%) N=21
FG Frequency	47.1%	17.2	16.2%	7.4%	1.97%	10.4%
Male	75.6	61.2	70.6	42.9	0	76.2
Female	24.4	38.8	29.4	57.1	100	23.8
Age						
<2	6.7	55.5	29.4	0	0	0
2-7	35.5	38.9	11.8	28.6	50	41.6
8-15	57.8	5.6	58.8	71.4	50	58.4
WBC						
<30,000	53.4	94.4	58.9	57.1	50	76.2
>30,000	46.6	5.6	41.1	42.9	50	23.8
Hepatomegaly						
No	51.1	83.3	47.2	42.9	0	76.2
Yes	48.9	16.7	52.8	57.1	100	23.8
Splenomegaly						
No	77.8	83.3	52.8	71.4	0	58.3
Yes	23.3	17.7	47.2	28.6	100	41.7
Lymphadenopathy						
No	66.7	61.1	29.4	72	100	84.3
Yes	33.3	38.9	70.6	28	0	16.7
Platelets						
<50,000	31.1	33.3	52.9	85.7	100	23.8
>50,000	68.9	66.7	47.1	14.7	0	76.2
CR						
<4weeks	28.9	94.4	23.5	71.4	50	66.7
>4weeks	64.4	5.6	82.4	14.2	50	19.0
No remission	6.7	5.6	13.6	14.2	0	14.3
RFS (months)	7.994	14.17	3.559	8.08	5.5	13.1

Summary and Conclusions: We are the first group from Pakistan reporting the correlation of molecular markers with disease biology and treatment outcome in pediatric ALL. Poor prognostic FGs namely BCR-ABL, MLL-AF4, SIL-TAL1 and TCF3-PBX1 were collectively detected in 72.4% of patients may be the one of the reasons for already reported low cure rates of pediatric ALL (Asim *et al.*, 2011) in our country. We found the highest reported frequency of BCR-ABL FGs in Pakistani pediatric ALL, in consistent with various other reports from Pakistan (Iqbal & Akhtar, 2006; Iqbal *et al.*, 2007; Faiz et al*.,* 2011; Awan et al, 2012) and the rest of the world which (Siddiqui *et al.*, 2010). It was associated with late responses and poor survival. The study has a specific clinical implication after FDA approval of imatinib for pediatric BCR-ABL positive ALL and other tyrosine kinase inhibitors (TKIs) under clinical trials on the way of rapid FDA approval. We recommend immediate incorporation of imatinib in pediatric ALL treatment protocols and provision of other TKIs for imatinib resistant BCR-ABL positive pediatric ALL in our population. Further large scale studies are required to find out the possible reasons for high BCR-ABL frequency among Pakistani pediatric ALL patients.

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SAFETY OF ASPARAGINASE ERWINIA CHRYSANTHEMI IN A COMPASSIONATE-USE TRIAL: A SUBANALYSIS OF THE ADOLESCENT/YOUNG ADULT PATIENT POPULATION

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Background: L-asparaginase (L-ASP) is an important component of multiagent chemotherapy for treatment of acute lymphoblastic leukemia (ALL) in children and young adults. The adolescent/young adult (AYA) population is usually defined in the literature as patients aged 16–39 years. NCCN guidelines recommend that AYA patients with ALL be treated with "pediatric-inspired" protocols that include L-ASP as an integral component of their multiagent chemotherapy regimen. Hypersensitivity reactions occur in 10%–30% of patients treated with *E coli*–derived asparaginases, often resulting in their discontinuation. In those patients, it is recommended that *Erwinia chrysanthemi* (ERC), an L-ASP immunologically distinct from *E coli*–derived L-ASP, be initiated.
Aims: This large compassionate-use trial in patients with ALL or lymphoblastic lymphoma who developed a grade ≥2 hypersensitivity reaction to an *E coli*–derived L-ASP was conducted to evaluate the safety of ERC.
Methods: This study met institutional review board approval, and all patients provided written consent. The ERC safety information for the entire study population was previously reported at the American Society of Hematology Annual Meeting in December 2012. Patients were excluded if they had a history of pancreatitis, previous allergic reaction to ERC, or were pregnant. Here, we report a safety analysis of a subset of 147 AYA patients, a population in which little ERC safety information has been presented.
Results: 1368 patients were treated with ERC from which 940 patients completed adverse events (AEs) and/or case report forms. The 147 AYA patients were primarily male (68.7%), had nonrelapsed disease (71.4%), B-lineage ALL (71.4%), received intramuscular ERC (87.8%), and had a median age of 18 years (range 16–39 years). 72.8% completed their planned ERC course. Discontinuation was due to allergic reaction (3.4%), other AEs (9.5%), other reasons (6.1%), and unknown reasons (8.2%). 45.6% of AYA patients had a least 1 treatment-emergent AE. Hypersensitivity occurred in 13.6%, hyperglycemia in 6.1%, pancreatitis in 3.4%, thrombosis in 3.4%, and sepsis in 3.4%. Grade 3/4 AEs with a >2% incidence included hyperglycemia (6.1%), hypersensitivity (2.7%), and dehydration (2.7%). There were 9 deaths; 4 disease progression, 3 infection, 1 renal impairment, 1 unknown. One death was considered possibly related to the study drug.
Summary and Conclusions: The safety profile of ERC in this subset of AYA patients was consistent with the profile reported in the entire study population. This compassionate-use trial permitted the completion of L-ASP in 72.8% of AYA patients with hypersensitivity reactions to *E coli* formulations.

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ASSESSMENT OF COAGULOPATHY DUE TO PEG-ASPARAGINASI IN CHILDREN WITH ALL

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Background: Background. Treatment with L-Asparaginase (L-Asp) or PEG-L-Asparaginase (PEG-L-Asp) in patients with acute lymphoblastic leukemia (ALL) is associated with coagulation disturbances, being deep venous thrombosis the most common clinical consequence, which should weighted with hemorrhagic risk. Classical coagulative parameters hardly identify the coagulative imbalance, being only antithrombin (AT) plasma levels the only reliable parameter for thrombotic risk. Whole blood rotation thromboelastometry (ROTEM®), using FIBTEM test, has been reported to be a good tool to identify hypofibrinogenemia and hemorrhagic tendency.
Aims: To evaluate whole blood rotation thromboelastometry profiles in children with ALL treated at our institution with PEG-L-Asp according to AIEOP-BFM ALL protocol in order to identify strategies for monitoring and treating complications.
Methods: Children treated with PEG-L-Asp for ALL consecutively referred to the Pediatric Hemato-Oncology Clinic of the Padua University Hospital, from June to December 2012, were enrolled. After informed consent, samples were obtained at predefined time points during the induction and reinduction phases of chemotherapy. Maximum Clot Firmness (MCF, normal value 9-25 mm), *i.e.* the maximum amplitude in millimeters reached in FIBTEM thromboelastogram, which is used to assess the specific role of fibrinogen in whole blood clot formation following inhibition of the platelets by Cytocalasin D, was performed. Moreover, blood counts, AT and fibrinogen plasma levels were measured on the same samples.
Results: wo hundred twenty two sets of thromboelastometry measurements were collected from 24 ALL patients (age range 20 months - 17 yrs; M/F 13/11). Ninety-two MCF measurements (41%) resulted below the lower limit of MCF normal value (<9mm). A significant linear correlation was found between MCF values in FIBTEM and fibrinogen plasma levels measured with Clauss method (P<0.001). No influence of reduced AT plasma levels on thromboelastometry determination was observed. Replacement with i.v. fibrinogen concentrates was suggested for MCF values <2 mm. AT values below 50% were found in 50 determinations (23% of tested samples). AT was supplemented in 12 cases. Coagulation abnormalities were found up to 20 days after PEG ASP administration. In two children undergoing prolonged drug injection, a cumulative effect of drug toxicity in fibrinogen plasma level was not observed; in these patients AT was only moderately reduced. Classical tests were not always consistent with the ROTEM profile. No major hemorrhagic complication was reported in our cohort. One 6-year old boy presented cerebral venous thrombosis, while in a severe hypocoagulable state but in presence of recovering platelets count.
Summary and Conclusions: The MCF value in FIBTEM was more accurate than the Clauss assay to define discoagulopathy and to identify the threshold for supplementation. The determination of fibrinogen function by means of ROTEM® can be considered a complementary test to the conventional clotting profile used for the management of AsP-induced coagulopathy in children with ALL. Larger studies are needed to evaluate the role of thromboelastometry to guide fibrinogen replacement therapy in acquired hypofibrinogenemia due to Asp treatment.

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EFFECTS OF PROPHYLACTIC CRANIAL RADIOTHERAPY ON GROWTH HORMONE SECRETION OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: With the recent developments in the treatment of acute lymphoblastic leukemia (ALL) life expectancy is prolonged and complications due to therapy are increased. Combined chemotherapy may be applied in combination with distinct doses and schema of cranial radiotherapy (CRT) according to the risk groups of patients. CRT damages the hypothalamus-hypophysis axis and affects the secretion of growth hormone (GH) at first and the other anterior hypophysis hormones also. At high doses of CRT, GH deficiency might occur in the long term follow-up, at lower doses the secretion pattern of GH may change.
Aims: We aimed to demonstrate whether prophylactic doses of cranial radiotherapy raise the neurosecretory dysfunction of growth hormone in children with ALL.
Methods: Thirty-two children (14 girls and 18 boys), diagnosed as ALL and applied prophylactic cranial therapy were enrolled to the study. Physical findings, weight and height measurements, Tanner stagings and bone age evaluations were performed to all patients. In order to interpret the functions of anterior and posterior hypophysis morning basal IGF-1, IGFBP-3, thyroid hormones, cortisol, prolactin and dansity of urine were measured. Gonodotropins were not tested because none of our patients had the signs of early or late puberty. In order to evaluate the growth rate; weights and heights of the patients were measured after the first year. Even though the growth rate of some patients were normal, GH stimulation tests with clonidine were done to all cases, due to the fact that GH deficiency and neurosecretory dysfunction might be present pharmacologically. Second GH stimulation test with L-dopa were applied to patients with peak GH levels of <10 ng/mL. If the level was low in the second test, patients were accepted as GH deficient. For patients with low growth rate, low IGF-1/IGFBP-3 according to age and sex, but normal GH levels, night GH secretion pattern were tested. Patients with inadequate response to night secretion test were accepted as neurosecretory dysfunction.
Results: In our study, 25% of the patients were found to have complete and 21,7% incomplete GH deficiency. In 9.3% of cases there was GH-neurosecretory dysfunction (GH_NSD). There was no statistically significant relationship between the time passed after radiotherapy and GH axis dysfunction degree. IGF-BP3 was found to be more reliable than IGF-1 in estimating GH deficiency/GH-NSD.
Summary and Conclusions: Prophylactic CRT affects the GH axis negatively in ALL patients, so their close follow-up is precious and mandatory.

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THE IMPACT OF RISK STRATIFICATION BY EARLY BONE-MARROW RESPONSE AND FCM-MRD IN CHILDHOOD LYMPHOBLASTIC LEUKAEMIA (A SINGLE INSTITUTION EXPERIENCE IN AZIZA OTHMANA HOSPITAL, TUNIS, TUNISIA).

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Background: In a previous retrospective analysis of 58 children with ALL treated according to the EORTC 58951 protocol between 2001 and 2003, patients were stratified by age, WBC at diagnosis, immunophenotype, karyotype and steroids response in peripheral smears after 7 days of corticosteroid and one IT dose of MTX. 3-year EFS, DFS and OS were unsatisfactory: 46%, 49,8% and 55,4%. In order to improve our results, the stratification strategy was extended by additional use of early response to therapy: blast cell count in the BM was examinated on D7 and D19 during remission induction, and determination of MRD measured by FCM in the BM at the end of induction (D35). All patients were assigned to a DXM based induction. Patients whose blast cells were ≥25% in BM (M3 marrow) at D7 and or at D 19, or whose MRD was positive at the end of induction, had their treatment one step upgraded.
Aims: This study aimed to assess the prognostic value of morphological assessment of bone marrow blasts during remission induction and the prognostic significance of minimal residual disease (MRD) detected by a simplified flow cytometric assay after remission induction in children with acute lymphoblastic leukemia (ALL).
Methods: Patients under 25 years of age with previously untreated ALL were treated according to the EORTC 58951 protocol between January 2006 and December 2010. The diagnosis of ALL was based on morphological and immunophenotypical criteria. Immunophenotype was determined with standard techniques,Chromosome analysis used standard techniques. We performed a prospective study of an early treatment response as assessed by morphological examination of BM blasts on D7 and on D19, and MRD determination by CMF, based strategy. Patients were assigned to 3 risk groups: The average 1 risk (AR1) group consisted of all patients with B-cell lineage ALL, NCI standard risk with an M1/M2 bone marrow on day 7. B-cell lineage ALL patients NCI high-risk or T-cell lineage ALL patients were classified as average 2 risk group AR2. All patients with gonadal or CNS involvement, were also included in the AR2 group, and patients with a day-7 M3 marrow and day-19 M1/M2 were assigned to AVR2 group. And very high risk (VHR) group consisted of blast counts in peripheral blood ≥1×109/L at completion of the prephase, t(9;22), t(4;11) or another MLL rearrangement, near-haploidy (<34chromosomes), or acute undifferentiated leukemia (AUL), or patients with a day-19 M3 marrow, or failure to achieve complete remission or minimal residual disease (MRD) >10-2 at completion of induction.
Results: 162 pediatric patients (100 males and 62 females) were enrolled, median age was 8 years (18months to 25 years), 40,7% aged ≥10 years at diagnosis, 29% with a WBC ≥50 Giga/l. Immunophenotyping was done for all patients, 110 (67,9%) B-ALL, 37 (22,8%) T-ALL, 4 (2,5%) undifferenciated, 7 (4,3%) biphenotypic leukemia and immunophenotype was no contributive in 4 cases. 116 (71,6%) patients had M3 marrow responses on day 7. 31 (19,1%) were steroid poor responders at day 8 in in the peripheral blood (pB). On day 19, 16 (9,9%) patients had M3 marrow. 155 patients received phase IA RM2-VHR according to the EORTC 58951 protocol, with HD-MTX (5g/m2) on day 8 and cyclophosphamide (1g/m2) on day 9. The CR was 85, 2%, induction failure and induction death rates were4,3% and10,5% respectively. MRD results available in 128 cases, sensitivity of 0,01%, between 0,01% and 0,1%, between 0,1% and >1% was achieved respectively in 23,4%, 43%, 25,8% and 7,8%. The 3-year RFS and OS were 85,7% and 73,6% respectively. In Univariate analyses, female, WBC<50 G/l, no M3 bone marrow on day 19 and MRD at the end of induction <10-3 have a significantly better RFS. Patients with ≥25% leukemic cells on day 19 had a significantly lower relapse-free survival (RFS) compared to those with rapid early response <25% (64.3% *vs* 87,5% P=0.003). Stratification into 3 MRD groups (≥1%, 0.1-1%, and <0.1%) also showed a statistically significant difference in RFS (33% *vs.* 78.6% [8.7%] *vs.* 83,4%, P=0.003). Multivariate analysis demonstrated that the early marrow response on day 19 and MRD level at the end of induction were an independent prognostic factor.
Summary and Conclusions: Early treatment response as assessed by morphological examination or minimal residual leukemia determination by flow cytometry has important prognostic significance, and can be performed in a resource-poor patient population.

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THE EXPRESSION OF THE EGF-TM7 MOLECULES CD97, EMR2 AND EMR3 IS ASSOCIATED WITH MUTATED NPM1 AND FLT3 IN AML

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Background: Acute myeloid leukemia (AML) is a disease characterized by an increase of immature myeloid blasts in the bone marrow. As for normal hematopoietic stem cells (HSCs), the interaction with the surrounding bone marrow microenvironment mediated by cell surface proteins is important for leukemic (stem) cell fate and characteristics. Therefore, the definition of new marker molecules is fundamental to further unravel the biology of leukemogenesis and to discover new therapeutic targets.
Aims: CD97, EMR2 and EMR3 are EGF-TM7 molecules, a subgroup of adhesion GPCRs, expressed in lymphoid and myeloid cells. However, almost nothing is known about their expression and regulation in normal and malignant hematopoietic stem cells.
Methods: We studied 292 samples from patients with *de novo* acute leukemia, comprising AML M0-2 (n=195), AML M3 (n=16), AML M4/5 (n=63), AML M6/7 (n=4) and c-ALL (n=14). A 4-color immunophenotypic measurement was performed on a FACS Canto II using the following antibodies: CD34 PerCp5.5, CD117 FITC, CD97 APC, CD45 V500. FLT3-ITD mutations and NPM1-mutations were detected as reported in detail previously (Thiede C *et al.* Blood 2002; Thiede C *et al.* Blood 2006). Moreover, EMR2 PE and EMR3 APC were measured in 37 patient samples. In three human AML cell lines, MV4-11, EOL-1 and OCI-AML3, which display a specific FLT3 and NMP1 mutation status, we investigated the expression levels of CD97, EMR2 and EMR3 and a possible influence of the protein kinase inhibitors PKC412 (Midostaurin) and SU5614.
Results: Compared to BM blasts of healthy donors (n=10),we detected significantly higher CD97 expression levels (mean fluorescence intensity, MFI) in 144 AML samples (49%). In detail, the CD97 expression could be observed in 40% out of cases with M0-2, 100% of cases with M3, 59% of patients with M4/5, 75% of M6/7 and 71% of the c-ALL cases, respectively. Of note, higher CD97 expression was accompanied by a significantly higher BM blast count (75% *vs.* 53%, P<0.001) and a lower Hb (5.9 *vs.* 6.5, P=0.02). Interestingly, elevated CD97 expression was associated with mutations in NPM1 (44% *vs.* 19%, P=0.002) and FLT3 genes (43% *vs.* 10%, P<0.0001) as well as lower CD34 (52% *vs.* 83%, P<0.0001) and HLA-DR expression (79% *vs.* 96%, P<0.0001). EMR2 was detected in 16 out of 37 and EMR3 in 24 out of 37 cases. In-vitro, we detected the highest CD97 expression levels (MFI 306.3) in MV4-11 AML cells which carry a FLT3-ITD mutation. Incubation with the multikinase inhibitor PKC412 or SU5614 caused a significant decrease of CD97 expression (MFI 167.6 and 124.1, respectively). Whereas EOL-1 cells expressing wildtype FLT3 displayed low CD97 levels (MFI 16.9) without an influence of the protein tyrosine kinase inhibitors, OCI-AML3 cells carrying wildtype FLT3 but mutated NPM1 expressed moderate CD97 levels (MFI 166.3) which were significantly increased by PKC412 (MFI 266). In EOL-1 cells also EMR2 and EMR3 were expressed at very low levels. The high expression levels of both molecules were decreased by PKC412 in MV4-11 cells but increased in OCI-AML3, whereas SU5614 decreased their expression in MV4-11 cells only.
Summary and Conclusions: For the first time, we present an association between the expression of the EGF-TM7 receptors CD97, EMR2 and EMR3 and AML pathophysiology. The exact mechanism of regulation will be further determined.

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IDENTIFICATION OF NOVEL GENETIC ALTERATIONS BY WHOLE-TRANSCRIPTOME SEQUENCING IN INFANTS WITH CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA

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Background: Pediatric cytogenetically normal acute myeloid leukemia (CN-AML) is a heterogeneous subgroup of myeloid clonal disorders not harboring known mutations. Genome-wide analyses have been used with the aim of determining the full array of genetic lesions of CN-AML and recent studies have provided new insights into the molecular genetics/biology of AML. Nevertheless, there is a considerable proportion of children with CN-AML in whom no genetic abnormality has been detected. Therefore, the identification of the different genetic profiles characterizing this subgroup represents a primary objective to be pursued.
Aims: To identify novel recurrent somatic mutations or genomic rearrangements which could be relevant for prognosis and therapy, we performed whole-transcriptome massively parallel sequencing of 8 cases of infant CN-AML.
Methods: Patients analyzed were children within the first year of life (infant) with newly diagnosed *de novo* CN-AML. All patients had AML other than acute promyelocytic leukemia, were negative for chromosomal rearrangements and for known recurrent genetic abnormalities involving *MLL*, *CBFB*, *NPM1* and *FLT3* genes. RNA-seq was performed at 75×2 in paired-end mode on the Illumina HiScanSQ. Reads were aligned with TopHat2/BowTie2 to the reference human genome. SNVMix2 was used to detect single nucleotide variants (SNVs); deFuse and ChimeraScan packages were used to detect chimeric transcripts. SNP&GO and Provean software were used to identify the SNVs potentially associated with the disease.
Results: Whole-transcriptome massively parallel sequencing in the 8 children with CN-AML, yielded an average of 59.1 million mapped reads/patient, thus reaching an average coverage of 34X. In three out of eight patients we identified a recurrent cryptic inversion of chromosome16, encoding a *CBFA2T3-GLIS2* fusion transcript. Recently, we also published data indicating that this fusion transcript is a novel common feature of pediatric CN-AML, not restricted only to the FAB M7 subtype, predicting poorer outcome, the 5-year event-free survival of patients *CBFA2T3-GLIS2* positive being of 27.4% (SE 10.5) *versus* 59.6% (SE 3.6) of patients *CBFA2T3-GLIS2*-negative (*P*=0.01). Interestingly, in all the three patients in which we identified the CBFA2T3-GLIS2 fusion gene, we have also detected a novel read-through transcript that lead to the fusion of exon 2 of DHH gene, a member of the Hedgehog family, with the exon 2 of RHEBL1 gene which encodes for a small GTPase of Ras family that regulates a wide variety of cellular functions including cell growth, differentiation, and transformation. Further studies will assess the recurrence of DHH-RHEBL1 read-through transcript and its possible prognostic and therapeutic relevance. RNA-seq allowed us also to identified 60 SNVs potentially associated with the disease. Recurrent mutations were detected in genes that regulates apoptosis (TRAP1 [37.5%], BCL3 [12.5%], MCL1 [25%]), in genes implicated in the control of cell proliferation and differentiation (RUNX3 [25%], APBB1[25%], EP300 [25%]) and also in JAK/STAT signaling pathway (Figure 1).

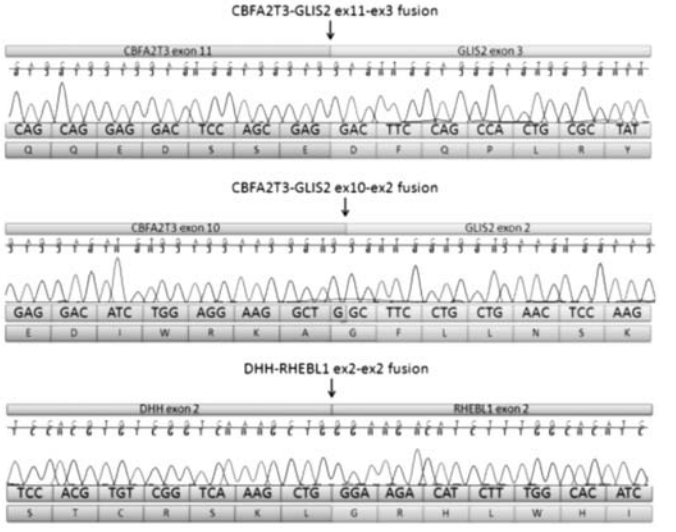

Figure 1. Novel fusion transcripts identified by whole-transcriptome sequencing in infants with cytogenetically normal acute myeloid leukemia. Schematic representation of the fusion breakpoints identified, electropherograms and predicted sequences of the fusion proteins found in infant CN-AML. Black arrow indicates the fusion breakpoints.

Summary and Conclusions: We demonstrated that infant CN-AML CBFA2T3-GLIS2-positive patients also harbor a novel read-through fusion transcript occurring between DHH and RHEBL1. Additionally, we also identified several SNVs affecting key genes of important cellular processes. Future studies will assess whether these genetic alterations could be novel independent prognostic markers and potential therapeutic targets.

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2HG IS A STRONG SERUM BIOMARKER FOR IDH1/2 MUTATIONS IN *DE NOVO* AML

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Background: Mutations in the genes encoding IDH1 and IDH2 produce high levels of 2-hydroxyglutarate (2HG).
Aims: We asked whether, in acute myeloid leukemia (AML), serum 2HG can predict the presence of *IDH* 1/2 mutations at diagnosis, and serve as a marker of minimal residual disease (MRD).
Methods: Serum samples of 82 patients at diagnosis of *de novo* AML (*IDH* mutated n=53; *IDH* wildtype n=29) and 68 non-AML controls were analyzed for total 2HG and its L (S) and D (R) stereoisomers by mass spectrometry. We investigated the relationships between 2HG levels and molecular markers of minimal residual disease (MDR; *WT1* expression and *NPM1* mutation) in serial serum and cellular samples of 36 *IDH* mutated patients after induction therapy.
Results: Total 2HG serum levels were significantly higher in AML patients with *IDH1/2* mutations than IDH wild type AML patients (P=<0.0001). The optimum diagnostic cutoff between IDH mutated and wild type was 2 μM (area under curve [AUC] >99%, sensitivity 100%, and specificity 89%. Quantification of the D/L stereoisomers provided higher specificity (98%) at 100% sensitivity, (P=.002). *IDH2 R172* mutants had 2HG levels higher than other mutants (P=.01). After induction therapy, total 2-HG serum levels <2 μM were associated with better overall survival (P=.007) and better disease-free survival (P=.005). During the follow-up, serum 2HG levels showed strong positive correlation with molecular markers of MRD (P<.001).
Summary and Conclusions: 2HG is a strong serum biomarker for *IDH* mutations and a significant predictor for outcome in IDH 1/2 AML patients. 2 HG measurements serve as a marker of minimal residual disease.

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ALTERED GENES DOWNSTREAM OF MYCN IN ACUTE MYELOID LEUKEMIA IN ZEBRAFISH BY RETINOIC ACID AND VALPROIC ACID

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Background: Amplification of *MYCN* (*N-Myc*) oncogene has been reported as a frequent event and a poor prognostic marker in human acute myeloid leukemia (AML) and neuroblastoma. 13-Cis retinoic acid (AC) and valproic acid (VC) are currently used in the treatment of *MYCN* amplified neuroblastoma patients with minimal residual disease. In early work, we have induced AML in zebrafish using murine *MYCN* (*mMYCN*) transgene and EGFP under control of the heat shock elements (HSEs).
Aims: To identify that AC and VC are appropriated for treating *MYCN* amplified AML.
Methods: The WT and *Tg*(*MYCN*:HSE:EGFP) F1 generation (Tg) embryos were heated shocked at 38° for 1 hour at 16 hours post fertilization (hpf) and hematopoietic cells were collected at 3 dpf by flow cytometry. Total RNA from 5×10^4cells was isolated with Trizol (Invitrogen). The samples were processed and subsequently analyzed in triplicate on Zebrafish Oligo Microarrays (Agilent Technologies Italia, Italy) which contain 43,554 sets of probes. The microarrays were scanned in an Agilent DNA Microarray Scanner and the images were processed using Feature Extraction software. Functional annotation analysis was performed using NIH-DAVID software (version 6.7). Next, the WT and Tg embryos were treated with DMSO (0.1%) or various concentrations of AC (Sigma 4759-48-220:1, 1.5 μM) and VC (Sigma 1069-66-5: 40, 60μM) incubated in 12-well plates (30 embryos/well) at 28.5 °Cfrom 14 hpf to 72 hpf. Then the differentially expressed genes involved in microarray-analysiswere measuredby qRT-PCR. The assay was repeated three times independently.
Results: UsingAgilentmicroarray analysis, we have demonstrated that signaling pathways involved in cell cycle progression, glycolysis/gluconeogenesis,fatty acid metabolism, MAPK/Ras, tyrosine metabolismand p53-mediated apoptosiswere enhanced, while those of mismatch repair, homologous recombination, transforming growth factor β (TGFβ) and base excisionrepair were inhibited in the blood cells of Tg *versus* WT embryos at 3dpf. We observed that both AC and VC counteracted over-expression of *MYCN* induced cell cycle uP-regulation and p53-mediated apoptosis through the expression of vital genes belonging to the cell cycle [S-phase associated kinase 2 (*Skp2*), p27 and p21], MAPK (Ras),p53 andTGFβ pathway(*Smad 4/7*). (Shown in Figure 1. WT: wild type; MYCN: *Tg*(*MYCN*:HSE:EGFP) F1 generation; 1: low dose of AC or VC; 2: high dose of AC or VC. Compared with Tg: *, P<0.05; **, P<0.01.).
Summary and Conclusions: Our results show that AC and VC are potent therapeutics to *MYCN* amplified AML by inhibiting AML cell growth.

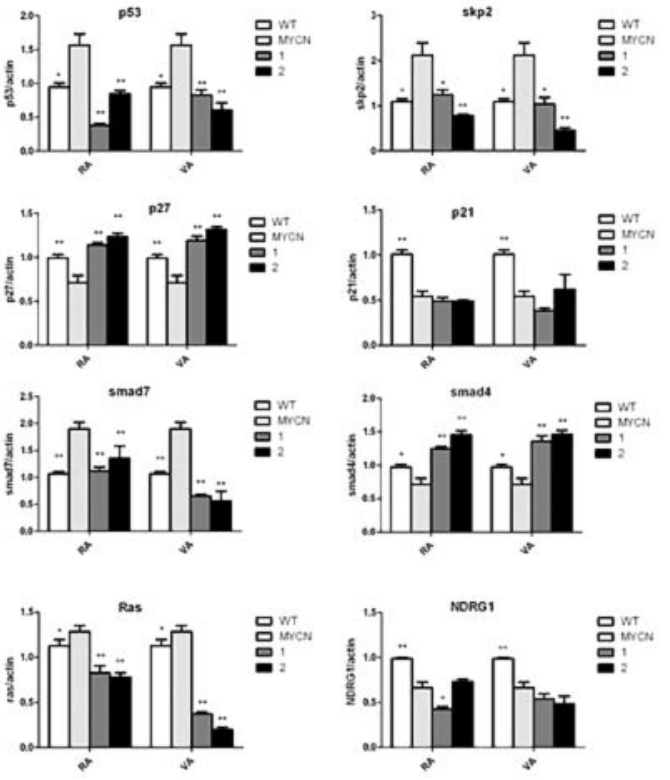

Figure 1.

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THE AAA+ ATPASE RUVBL2 IS CRITICAL FOR THE ONCOGENIC ACTIVITY OF MLL-FUSIONS

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Background: Chromosome translocations that disrupt the Mixed Lineage Leukaemia (*MLL*) gene are associated with a unique subset of Acute Myelogenous and Lymphoblastic Leukaemias. *MLL* translocations are most prevalent in infant leukaemia, where they comprise 80% of cases of Acute Lymphoblastic Leukaemia and 60% of cases in Acute Myeloid Leukaemia. Expression of MLL-fusion proteins is known to induce malignant transformation of normal haematopoietic progenitor cells. To identify transcriptional target genes required for the immortalisation, previous work in our laboratory involved generating constitutively and conditionally immortalised primary mouse haematopoietic progenitor cells. Global gene expression analysis, upon loss of MLL-fusion protein, identified a number of genes that were differentially expressed.
Aims: This study investigated the function of one of these differentially expressed genes, *Ruvbl2*, in MLL-fusion mediated leukaemogenesis.
Results: RUVBL2 is a member of the AAA+ family of DNA helicases and plays an important role in diverse cellular processes. To examine the efficacy of targeting RUVBL2 in leukaemia elimination *in vivo*, inducible models were generated for both the human leukemic cell line, THP1, and mouse MLL-ENL leukemic cells. Upon doxycycline treatment, RUVBL2 is inhibited either via shRNA-mediated knockdown or through the expression of a dominant-negative mutant form of RUVBL2. In concurrence with previous data from the laboratory, RUVBL2 inhibition resulted in a marked increase in apoptosis, reduction in colony formation and a block in cell cycle. In addition, transplantation experiments demonstrated that inhibition of RUVBL2 function significantly delayed leukemic onset *in vivo*. In parallel, while expression of dominant negative RUVBL2 induced apoptosis in mouse MLL-ENL leukemic cells, it had a relatively mild impact on normal haematopoietic progenitor colony formation *in vitro*. Further experiments indicated that RUVBL2 functioned mainly through influencing the transcriptional activity of MYC.
Summary and Conclusions: Taken together, our data suggest that a potential therapeutic window exists for targeting RUVBL2 function in MLL rearranged leukaemia.

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EXPRESSION, REGULATION AND FUNCTIONAL ROLES OF THE CHEMOKINE RECEPTOR CXCR7 IN ACUTE MYELOID LEUKAEMIA CELLS

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Background: Stromal cell-derived factor (SDF)-1 is constitutively expressed and produced by stromal cells in bone marrow (BM). The protein exerts its functions by binding to its receptor, CXCR4, thereby guiding the homing of haematopoietic stem/progenitor cells and leukaemia cells to the BM. Recently, CXCR7 was identified as an alternative receptor for SDF-1, and it has been shown to be involved in adhesion to the endothelium and proliferation of several normal and malignant cell types, rather than cell migration. CXCR7 was shown to be a decoy receptor for CXCR4 in certain cell types. Its expression status and its roles in acute myeloid leukaemia (AML) cells are largely unknown.
Aims: We investigated the expression, regulation and functional roles of CXCR7 in AML cells *in vitro.*
Methods: The expression of CXCR7 in AML cells was studied by reverse transcriptase- polymerase chain reaction (RT-PCR), Western blotting and flow cytometry. The effects of various cytokines and hypoxia on the expression of CXCR7 in AML cells were examined. To determine the functional role of CXCR7, CXCR7 mRNA was knocked down in AML cells using siRNA technology, and subsequent biological alterations in the cells were evaluated *in vitro*.
Results: All AML cell lines examined in this study (U937, K562, KG1a, HL-60 and MO7e), as well as primary CD34$^+$ cells obtained from the BM of patients with AML, expressed CXCR7 mRNA at various levels, as shown by semi-quantitative and real-time RT-PCR analysis. Western blotting showed that all AML cells produce CXCR7. In parallel, all AML cells expressed CXCR7 both in the cytoplasm and on the cell surface at various levels, as revealed by flow cytometric analysis. SDF-1 induced the internalisation of cell surface CXCR7. However, neither hypoxia nor the haematopoietic growth factors (IL-β, IL-3, IL-6, G-CSF, GM-CSF and SCF) and proinflammatory cytokines (IFN-γ, TGF-β and TNF-α) examined altered cell surface CXCR7 expression. The transfection of AML cells with CXCR4 siRNA, but not CXCR7 siRNA, significantly impaired SDF-1-induced transmigration of AML cells. The transfection of AML cells with CXCR7 siRNA did not affect the survival or proliferation of AML cells. Interestingly, knock-down of CXCR7, but not CXCR4, induced the upregulation of SDF-1 mRNA expression and SDF-1 production in AML cells.
Summary and Conclusions: AML cells express CXCR7, which is involved in the regulation of SDF-1 in these cells.

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INTERLEUKIN-3 RECEPTOR ALPHA CHAIN (CD123) AS A MARKER FOR LEUKEMIC STEM CELLS IN ACUTE MYELOID LEUKEMIA AND ITS RELATION TO DISEASE PROGRESSION

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Background: Leukemic stem cells (LSCs) initiate and sustain the clonal growth in acute myeloid leukemia (AML).The poor response to therapy in AML patients raises the expectations that the presence of LSCs in quiescent state, resembling the normal stem cells, may add to the resistance to conventional chemotherapy. An enormous effort has been taken to identify specific surface markers in order to differentiate between normal and leukemic stem cells.
Aims: The study aimed to prove that CD123^{high} is a reliable marker in differentiating the leukemic stem cell in AML from normal stem cells within the CD34$^+$CD38$^-$ cell population. An additional goal is to find a relation between LSC population and disease progression.
Methods: Using multi-color flowcytometry, we analyzed the proportions of CD34+CD38− cells expressing high CD123 as a LSC marker in bone marrow samples of 42 AML patients at presentation and at follow up on day 14 post induction chemotherapy. In addition, 14 nonmalignant bone marrow samples were examined as a control group.
Results: Expression (mean fluorescent intensity) of CD123 was significantly higher in AML cases (median 4.22, range: 2.95-7.60) as compared to control group (median 3.55, range 2.88-5.60) (P value 0.016). In AML cases median percent of CD34+CD38- cells was 1.22% (0.0–41.8%) at the time of diagnosis compared to 0.21% (0.0–3.74%) at day 14 post induction (p value 0.067). At initial diagnosis 69% of AML cases (29/42 cases) showed CD123^{high} LSCs phenotype while 80% of cases (16/20 cases) showed CD123^{high} LSCs marker expression at day 14 post induction of whom 18.75% (3/16 cases) initially lacked the CD123^{high}leukemic stem cells. The median percent of LSC (0.51%) showed a statistically significant higher difference at day 14 post induction when compared to initial percent (0.09%) (P value 0.003). On comparing cases with positive CD123 expression to those lacking expression as regards to complete remission, disease free survival and overall survival no statistically significant difference was found.
Summary and Conclusions: Interleukin-3 receptor alpha chain (CD123) may be used as marker of leukemic stem cells in AML. Presence of leukemic stem cells at diagnosis had no prognostic impact on treatment outcome. Emergence of leukemic stem cells post induction may reflect an induced stem cell theory contributing further to disease resistance.

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EFFECTS OF B-MYB ABERRANT EXPRESSION ON HEMATOPOIETIC PROGENITOR CELL GROWTH AND DETECTION OF GENETIC VARIATIONS THAT MAY MODIFY ITS PROTEIN FUNCTION

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Background: The B-Myb gene encodes a transcription factor involved in cell proliferation. Several studies have shown that it is overexpressed in different cancers conferring poor prognosis. Our previous studies showed that B-Myb is overexpressed in patients with acute myeloid leukemia (AML) relative to CD34+ cells from healthy donors and this overexpression is associated with an unfavorable prognosis.
Aims: Based on our previous results, we decided to study the consequences of B-Myb aberrant expression in hematopoietic progenitor (CD34+) cells with expression modulators. Furthermore, we pretended to find genetic variations in B-Myb coding sequence that may modify the protein function in AML patients.
Methods: CD34+ cells were isolated from umbilical cord blood of healthy donors with the AutoMACS device (Miltenyi Biotec). Cells were transfected in a 2D Nucleofector (Lonza) with B-Myb-IRES-EGFP overexpression plasmid construct (published by Dr. P.García), IRES-EGFP control plasmid, B-Myb specific siRNAs or control siRNAs. After 18 hours of culture, efficiently transfected CD34+ were selected by sorting in a flow cytometer and subsequently plated in triplicate in Methocult GF H84435 (Stem Cell Technologies) medium to perform colony forming cell assays. Colonies were counted after 14 days of culture in a contrast microscope. B-Myb coding sequence was screened in 180 AML patients in a LightCycler®480 (Roche) with the High Resolution Melting method by designing 16 pairs of specific primers. The detected variations were sequenced and contrasted with the accession number NT_011362.10 Gene Bank.
Results: *In vitro* studies demonstrated that total colony count from cells transfected with B-Myb specific siRNAs was significantly lower than those transfected with control siRNAs (median, 41 *vs* 79; range, 36-42 *vs* 63-86; P=0.027). CFU-M count was also significantly lower in cells transfected with B-Myb specific siRNAs (P=0.049). Total colony count of cells transfected with B-Myb-IRES-EGFP plasmid was significantly higher than those transfected with control plasmid (median, 104 *vs* 35.5; range, 78-115 *vs* 29-53; P=0.016), being also significantly higher the number of CFU-G, CFU-M and CFU-E (P=0.031, P=0.031 and P=0.032 respectively) colonies.
Rs2070235, rs11556379 and rs7660 nonsynonymous polymorphisms were detected in the AML patients with incidences similar to those registered in caucasian healthy populations (19.5%, 5.68% and 0.57% respectively). Rs73116571 polymorphism was found in an intronic splicing region with an incidence higher than that reported in a registered healthy population (2.4% *vs* 0.1%) and was associated significantly (P=0.046) with lower B-Myb expression in AML patients. In addition, three new nonsynonymous genetic variations were detected in three different patients: Q67X, E132D (both located in conserved DNA binding domains) and P274L (Table 1).

Table 1. Detected genetic variations in B-Myb coding and adjacent intronic sequences in AML patients.

Sequence	Detected genetic variation	Patients (%)	Identifier
Exon 4	NP_002457.1:p.**Q67X**	1/169 (0.6)	NR
Exon 5	NP_002457.1:p.**E132D**	1/175 (0.6)	NR
Exon 7	NP_002457.1:p.**P274L**	1/177 (0.6)	NR
Exon 8	NP_002457.1:p.**S427G**	33/169 (19.5)	rs2070235
Intron 8	NM_002466.2:c.1365+3G>T	4/169 (2.4)	rs73116571
Exon 12	NP_002457.1:p.**V595M**	1/175 (0.6)	rs7660
Exon 13	NP_002457.1:p.**I624M**	10/176 (5.7)	rs11556379

NR: Not registered variation in the SNP databases

Summary and Conclusions: *In vitro* colony-forming assays suggested that B-Myb overexpression induces CD34+ cell growth. The polymorphisms detected in our series had similar incidences than the reported in healthy population studies. The only exception was rs73116571 (located in a splicing region) for

which we found a higher incidence in our patients and in addition, a significant association (P=0.046) with lower B-Myb expression levels. Therefore, we consider that this association as both its incidence in AML patients should continue being investigated. Finally, new genetic variations (Q67X, E132D and P274L) were detected and its involvement in the function of B-Myb protein should be clarified in future studies.

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MUTATIONS IN RUNX1 AND NPM1 COMBINED WITH IMBALANCES OF CHROMOSOME 21 ARE PREDICTIVE FOR PROGRESSION OF AML

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Background: Extra chromosome 21 is the second most common acquired trisomy after (+)8 in adult myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). It is rarely observed as the sole abnormality but seen as part of complex karyotype in some 3-7% of the AML (*Atlas of Genetics and Cytogenetics in Oncology and Haematology, http:// atlasgeneticsoncology.org*). Although the gene(s) in trisomy 21 associated with leukemia are unknown, the 21q22 region appears to be critical since it houses the RUNX1 gene. Mutation of this gene, along with others such as NPM1, FLT3-ITD, CEBPA, ASXL1, MLL-PTD, IDH1, IDH2, DNMT3A, TET2 have been identified as prognostic factors in AML/MDS patients with a normal karyotype. Recently we reported on 16 MDS/AML patients from a cohort of 83 cases (ASH abstract No2561) carrying imbalances of chromosome 21 identified by high resolution genome array scanning (aCGH). Here we present results from a next generation sequencing (NGS) study of these samples.
Aims: The purpose of this study is to assess the mutation status in the RUNX1 gene and evaluate a link between mutations in NPM1 and RUNX1, in samples characterised by hitherto unreported partial deletions and absence of amplifications.
Methods: 16 MDS/AML patients with acquired copy number changes in chromosome 21 were investigated for mutations in the RUNX1 and NPM1 genes by running a custom library on the Ion Torrent Personal Genome Machine (PGM).
Results: Our previous studies using aCGH and FISH mapping identified chromosome 21 aberrations in 16 patients with MDS/AML. They fall in three groups characterized by: (i) trisomy 21 and intact RUNX1 gene (5/21); (ii) amplification of the 21q22 region including the ERG and ETS2 sequences, in spite of the loss of the whole or part of the RUNX1 gene (5/21); (iii) normal chromosome 21 with partially or whole loss of RUNX1 gene (6/16). The number of total mutations seen in both RUNX1 and the NPM1 genes (average of 6.1 and 4.4 resp.) were significantly higher in patients with RUNX1 deletions and/or amplifications of ETS2 and ERG (groups 2 and 3) as compared (average of 3.5 and 1 resp.) with the first group where no additional changes in the these genes were observed. In the second group, NGS identified mutations in NPM1 exons in all samples where the main feature is amplification of the ERG and ETS2 regions with or without a deletion in RUNX1. In 3 out of the 5 patients mutations of RUNX1 exon 8 and/or in exon 4 (COSM24728) were found to co-exist along with the NPM1 mutations. The same missense mutation of RUNX1 exon 4 (p.F70L) was observed in a further 6 cases, thus making it the most common mutation seen in 8/14 patients (57%) with imbalances in chromosome 21.
Summary and Conclusions: Our study, albeit small, demonstrates the presence of mutations in RUNX1 and NPM1 in 57% of the AML/MDS patients with RUNX1 deletions and/or amplifications of the ETS2/ERG region. These findings suggest that mutations in RUNX1 and/or NPM1 coupled with gains or losses in regions of RUNX1 could provide a useful indicator of progressive disease and also bring a novel insight into the pathogenesis of MDS/AML.

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PROGNOSTIC VALUE OF B-MYB AND ITS REGULATION BY MIRNAS IN ACUTE MYELOID LEUKEMIA

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Background: B-Myb is a transcription factor involved in cell cycle, apoptosis and senescence regulation. B-Myb silencing inhibits cell proliferation, and high expression levels have been found in several kinds of cancer. miRNAs modulate gene expression by inducing mRNA degradation or by blocking translation of their target genes. Thus, we propose that appropriate B-Myb expression levels are necessary for a normal development and function of the hematopoietic cells. We also hypothesize that B-Myb could be modulated by miRNAs and changes in their expression could participate in leukemic development.
Aims: The aims of this project are to analyze the expression of B-Myb and its potential miRNA regulators in Acute Myeloid leukemia (AML), to find association between B-Myb and candidate miRNAs, to perform functional studies in myeloid cell lines in order to test if they modulate B-Myb expression and to evaluate the prognostic value of B-Myb and its regulator miRNAs in AML.
Methods: The study population included 272 adult patients diagnosed with AML (excluding Acute Promielocytic Leukemia), the myeloid cell line KG1 and CD34+ cells selected from umbilical cord blood from 11 healthy donors.
Analyzed miRNAs were miR-30a/b/c/d/e and miR29a/b/c. Criteria for miRNA selection was base complementarity predicted by TargetScanHuman database. Expression levels were analyzed by Real Time PCR with the Assay on demand HS00231158-m1 (Applied Biosystems) for B-Myb and miScript probes (Qiagen) for the miRNAs in an ABI prism 7500. GUS gene and miR-U6b were used as internal controls and CD34+ cells were used as calibrators. Relative expression was calculated with the ΔΔCt method. Median expression was the cut-off value used to categorize patients for the statistical analysis. Functional studies were carried out by nucleofecting the KG1 cell line with mimics (molecules that reproduce miRNAs effects). Changes in gene expression were measured by Real time PCR and Western Blot.
Results: B-Myb median expression in LMAs was 0,22 (0,001-5,56) and in CD34+ cells was 0,14 (0,03-0,17). Median expression was significantly higher in LMAs than in CD34+ cells (P<0,001). Relative expression (ΔΔCt) was1,61. miR-30a/b/c/d/e and miR-29a/b median expression was lower in AML than in CD34+ cells (ΔΔCt=0,69; 0,60; 0,35; 0,26; 0,27; 0,19; 0,25; 0,41 and 0,83 respectively). ΔΔCt for miR-29b was 1,2. We found a significant association between B-Myb and miR-30a/b/c expression: patients who overexpressed B-Myb had lower expression of these miRNAs (P=0,049/0,011/0,002 respectively). The KG1 cell line showed a similar expression pattern: maximal cell proliferation (exponential growth phase of a cell culture) correlated with higher levels of B-Myb mRNA and protein, and lower levels of miR-30a/b/c. We tested if miR-30a and miR-30c modulate B-Myb expression by nucleofecting the KG1 cell line with mimics of these miRNAs. We found that miR-30a blocks B-Myb translation and subsequently cell proliferation decreases approximately 25%. Finally, B-Myb expression showed prognostic value for intermediate cytogenetic risk patients: patients with B-Myb overexpression showed a significantly shorter disease-free survival (DFS) (HR:1,4; IC95%1,02-1,94; P=0,039) and Relapse-free survival (RFS) (HR:1,49; IC1,01-2,20; P=0,047).
Summary and Conclusions: Our results suggest that B-Myb expression is higher in LMA than in CD34+ cells. B-Myb expression is modulated by miR-30a and they could influence leukemogenic development. B-Myb expression could be useful to stratify patients with intermediate cytogenetic risk.

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VLA-4 EXPRESSION OF LEUKEMIC MYELOBLASTS MIGHT BE A GOOD PROGNOSTIC MARKER IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Adhesion and migratory properties of leukemic myeloblasts appear to affect their survival, proliferation and retention in the bone marrow and influence the chemosensitivity. Very late antigen-4 (VLA-4) and CXC chemokine receptor 4 (CXCR4) are representing adhesion molecules expressed on acute myeloid leukemia (AML) blasts in variable ranges. Because VLA-4 may mediate anti-apoptotic signals and CXCR4 is associated with development of AML, therapeutic agents targeting VLA-4 and CXCR4 are investigated. Thus it becomes important to evaluate their expression for clinical use to characterize leukemic myeloblasts and to determine prognosis.
Aims: We prospectively investigated the impact of expressions of VLA-4 and CXCR4 of leukemic myeloblasts on treatment outcomes and leukemogenesis.
Methods: We quantified VLA-4 and CXCR4 expressions of leukemic myeloblasts in consecutive 104 AML patients. We measured the positive fractions and the mean fluorescence intensity (MFI) of VLA-4 and CXCR4 by multi-color flow cytometry using bone marrow aspirates, and correlated expression levels with disease characteristics and clinical outcomes. A combination of cytogenetic and molecular abnormalities was used to stratify the patients into risk groups based on national comprehensive cancer network (NCCN) 2013 guideline. We evaluated prognostic impact in 72 patients who received a cytarabine/anthracycline-based induction therapy by dividing into 2 groups (complete remission [CR] group who achieved CR after 1 cycle of chemotherapy, and induction failure group). We also separated the patients into 2 groups (high and low expression) according to the median positive fraction of VLA-4 or CXCR4.
Results: Good risk group showed higher positive fraction and MFI of VLA-4 than poor risk group (P=.007,.04), but positive proportion and MFI of CXCR4 showed no significant difference between risk groups (P=.352,.831). Positive fraction of CXCR4 increased along with age (r=.215). Proportion and MFI of VLA-4 were significantly higher in CR group than in induction failure group

(P=.01,.013). Those of CXCR4 showed no differences between 2 groups. The high fraction of VLA-4 expession group showed better overall survival, relapse free survival and event free survival than the low VLA-4 expression group (P=.024,.027, 016), but there was no difference of survival between the high and low CXCR-4 expression groups. VLA-4 positive fraction correlated with CD117, CD13 or myeloperoxidase positive fractions (P=.005,.036,.025), and inversely correlated with CXCR4 positive fraction (P=.039).
Summary and Conclusions: We found that high proportion and MFI of VLA-4 led to high complete remission rate, it corresponds with the results from better prognostic group determined by NCCN guidelines, and the high VLA-4 expression group showed better survival. Thus VLA-4 expression of leukemic myeloblasts might be a good prognostic marker in acute myeloid leukemia patients.

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CLINICAL AND HEMATOLOGICAL FEATURES OF ACUTE MYELOID LEUKEMIA WITH REARRANGEMENTS OF EVI1 OR PRDM16

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Background: Acute myeloid leukemia (AML) with EVI1 or PRDM16 rearrangement, which represent 1% to 2% of all AML, are aggressive diseases with poor prognosis and should be diagnosed early. Hint toward the possibility of such rearrangements could be given if these cells present specific features.
Aims: In a cohort of 21 AML with EVI1 or PRDM16 rearrangements a retrospective study of morphological, cytochemical, clinical features and survival was performed and compared to a reference cohort of 1844 AML diagnosed during the same period.
Methods: Rearrangements of EVI1 were observed in 17 AML patients and of PRDM16 in 4. Bone marrow (BM) and blood smears (May Grunwald Giemsa and myeloperoxidase (MPO)) were re-examined by two different cytologists. Information retrieved for both cohorts comprised blood counts, hemoglobin level, platelet counts, bone marrow dysplasia and overall survival.
Results: EVI1 patients had *de novo* AML in 10 cases. For the other7, antecedents of myeloproliferative neoplasm (MPN) were retrieved in4, of myeodysplastic syndrome in 1 and of lymphoproliferative disorder in 2. Karyotypic examination found 9 patients with inv(3)(q21q26.2), 7 with t(3;?)(q26.2;?), 1 with normal karyotype and cryptic inv(3). Monosomy 7 was observed in 8 cases and del(7q) in 1. Fifty-three% of the patients had platelet counts over 100G/L at diagnosis, without the classical thrombopenia observed in AML. This difference was statistically significant (P=0.019) by comparison to the reference cohort. These subnormal counts were associated with platelets dysplasia (giant and hypogranular). Morphological examination of BM smears for FAB classification showed a significant increase of minimal differentiation (M0, n=5, 31% *vs* 7.5%, P=0.002). Megakaryocytes were small, with monolobated or bilobated nuclei and clustered. Multilineage dysplasia of non-blast cells was present in 75% of the cases (*vs.* 17.6%, P<0.001). Cytochemistry for MPO activity was negative in 57% of the patients *vs.* 23.4% in the reference cohort (P=0.008). Of note, 78.5% of EVI1 patients had less than 10% MPO positive blasts, and MPO was also poorly expressed by mature neutrophils. The 4 patients with PRDM16 presented with a history of therapy-related MPN. Cytogenetics showed for all a t(1;3)(p32q21), with an additional del(5q) for 2. As for EVI1 patients, paradoxically normal platelet counts were observed at diagnosis (mean 259G/L). BM smears were characteristically rich in small, monolobated and clustered megakaryocytes (more than 50/smear). All showed multilineage dysplasia and, again as for EVI1 patients, MPO was characteristically low, completely absent in 3 cases. In both groups, prognosis was dismal with 9 months overall survival. The 14 patients who could not receive allogeneic transplantation died within 12 months.
Summary and Conclusions: EVI1 or PRDM16 rearrangement can be suspected in AML patients with subnormal platelet counts, high numbers of bone marrow clusters of monolobate megakaryocytes and multilineage dysplasia. Moreover, these cases are also significantly characterized by a very low or absent expression of myeloperoxidase even in cases not classified as M0. These two very similar entities should prompt for the investigation of EVI1 rearrangement, followed by that of PRDM16 if EVI1 is normal. The poor prognosis associated with these rare diseases should lead to rapid investigation for a donor, in view of allogeneic transplantation of hematopoietic stem cells.

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EFFECT OF MLN4924 ON TRAIL-INDUCED APOPTOSIS IN NON-CLINICAL MODELS OF HAEMATOLOGICAL MALIGNANCIES

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Background: TNF-related apoptosis ligand (TRAIL) is a tumour selective cytokine with potential anti-cancer activity which is currently in clinical trials. Haematological malignancies along with several other cancers have showed varied sensitivity to TRAIL treatment. *In vitro* and *in vivo* studies have shown that TRAIL-resistant tumour cells can be sensitised to TRAIL by various chemotherapeutic agents. This has opened up new possibilities for combination therapies. Proteasome inhibitors have previously been used in this way. MLN4924 is an investigational inhibitor of NEDD8 activating enzyme (NAE), which is part of the ubiquitin proteasome pathway. MLN4924 is currently in Phase 1 trials (Millennium Pharmaceuticals, Inc.). MLN4924 binds to NAE and forms an MLN4924-NEDD8 adduct in place of an NEDD8 adenylate thus locking the enzyme in an inactive state. As a consequence, MLN4924 inhibits the NEDD8-dependent activation of a subset of ubiquitin E3 ligases known as cullin ring ligases (CRLs). When CRL neddylation is disrupted, so is the ubiquitin-dependent turnover of CRL substrates, many of which have important roles in cellular processes associated with cancer cell growth and survival pathways including DNA replication and NFkB activity. Targeting the activity of a specific subset of E3 ligases is particularly attractive because there is the potential to selectively block the degradation of certain cellular proteins and possibly avoid unwanted effects on other proteins.
Aims: We sought to investigate the apoptotic effect MLN4924 in combination with TRAIL may have in multiple myeloma, acute myeloid leukaemia (AML), and diffuse large B cell lymphoma cell lines.
Results: It emerged that MLN4924 and TRAIL have a synergistic effect in a number of AML and MM cell lines. Examples of combination index values obtained for these cell lines include AML2-0.07; MOLM13-0.69; ML1-0.55. In an attempt to identify the mechanism of action we found that MLN4924 induces the expression of Bim and Noxa, pro-apoptotic, BH3-only members of the Bcl-2 protein family. MLN4924 did not significantly affect the expression of apoptotic regulators acting at the TRAIL death receptors, including c-FLIP, pro-caspase-8, cIAP1/2, DR4, DR5, DcR1 or DcR2. Through study of the known transcription factors regulated after MLN4924 treatment and transcription factors regulating Noxa expression, it was found that treatment with MLN4924 led to the induction of p53 and C/EBPa, but not that of Foxo3A or Nrf2 in OCI AML2 cells. Inhibition of p53 action with Nutlin3 reduced MLN4924-mediated Noxa induction and could also sensitise the AML cells to TRAIL-induced apoptosis. Pharmacokinetic studies on OCI-M2 xenografts confirmed that inhibition of cullin ring ligases triggers Noxa accumulation and enhanced TRAIL-induced apoptosis indicated by higher level of pro-casapse-3 processing and cleavage of the caspase-3 target protein, PARP. Finally, OCI-M2 xenografts treated with a biweekly dose of MLN4924 (on days 1 and4, each week for 3 weeks) combined with daily dosage of recombinant human TRAIL (days 1-5 each week for 3 weeks) resulted in very significant reduction in tumour volume (Figure 1).

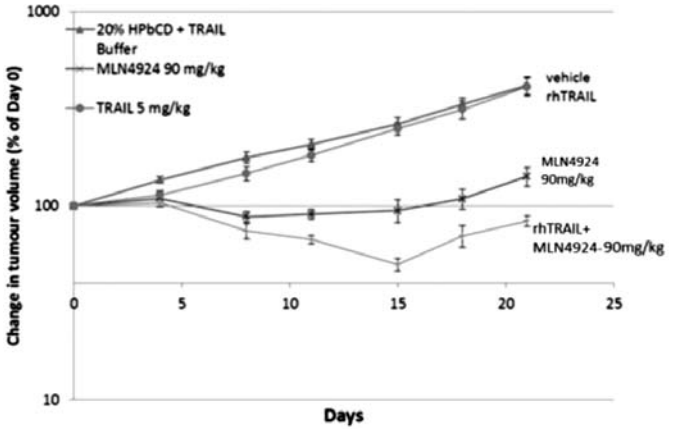

Figure 1.

Summary and Conclusions: In conclusion, the current study shows that MLN4924 can sensitise AML and potentially MM cells to TRAIL induced apoptosis to trigger very robust anti-tumour effects by increasing BH3-only protein expression through an at least partially p53-dependent manner.

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IMPACT OF THE E3 UBIQUITIN LIGASE TRAF2 ON TNF-A-INDUCED APOPTOSIS IN A CELL MODEL OF FLT3-ITD-POSITIVE AML

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Background: Acute myeloid leukemia (AML) is a heterogeneous disease in which multiple signaling pathways can contribute to pathogenesis of leukemia.

The cytokine TNF-a (tumor necrosis factor alpha) has pleiotropic effects on growth, differentiation and apoptosis. One of the key molecules mediating TNF-a signaling is the E3 ubiquitin ligase TRAF2 (tumor necrosis factor receptor-associated factor 2). So far, functional characterization of TRAF2 in AML cells has not been reported.
Aims: The objective of this study was to investigate whether TNF-a can induce apoptosis in cells of FLT3-ITD-positive AML and to elucidate the influence of the E3 ubiquitin ligase TRAF2 on the survival of these AML cells.
Methods: The expression of TRAF2 was suppressed in the FLT3-ITD-dependent human AML cell line MOLM13 by stable lentiviral shRNA expression. TNF-a-mediated signal transduction of MOLM13 shTRAF2 cells was monitored by analysis of AKT, GSK3ß, ERK and p706S. Apoptosis and cell proliferation were detected by flow cytometry and MTS assay, respectively. Furthermore, chemosensitivity assays were performed to investigate a potential functional role after incubation with cytarabine or doxorubicin.
Results: The functional activity of TNF-a was demonstrated by a strong increase of p38 phosphorylation that was detected by Western blotting. In MOLM13 cells, shRNA-mediated knockdown of TRAF2 resulted in a significant suppression of phosphorylation of the anti-apoptotic proteins AKT and GSK-3ß after 24h treatment with TNF-a, which was in contrast to the control cell line. Importantly, the knockdown of TRAF2 caused significantly more apoptosis (33% ±4.2% *vs.* 13%±1.7%) and impaired proliferation after TNF-a exposure (0.58±0.02 fold of control). Furthermore, the TRAF2 knockdown resulted in a different susceptibility to both investigated cytotoxic drugs.
Summary and Conclusions: We revealed the previously unknown functional role of TRAF2 in TNF-a-induced apoptosis in a FLT3-ITD-driven cell model. TRAF2 might elicit cytoprotective effects following activation of the TNF-a receptor. We hypothesize that TRAF2 plays an important role in signal transduction and survival of AML cells as we can demonstrate TRAF2 to be a critical effector of TNF-a-induced apoptosis in a FLT3-ITD-positive AML cell line.

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PLUMBAGIN ENHANCED TRAIL-INDUCED APOPTOSIS OF KASUMI-1 CELLS IN NOD/SCID MICE

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Background: The translocation (8;21)(q22;q22) is one of the most common chromosomal abnormality in acute myeloid leukemia(AML). The incidence of AML with t(8;21) is 6%>10% in primary AML and 18%>40% in AML-M2.AML with t(8;21) is generally considered as a good prognostic type according to WHO classification. However, the recurrence and drug resistance of AML with t(8;21) still threat the life of patients. Therefore, it's important for finding new drugs to overcome the drug resistance of leukemic cells, elevate the rate of complete remission and improve the long-term survival of patients in clinical practice. Our previous research has confirmed that plumbagin can enhance TRAIL-induced apoptosis of Kasumi-1 leukemic cell lines *in vitro*. Therefore, it's necessary to verify the finding in animal experimental leukemic mode *in vivo* before clinical usage.
Aims: To investigate the effect of plumbagin alone, rsTRAIL alone and their combination on leukemic Kasumi-1 cells in the NOD/SCID mice *in vivo* and their possible mechanisims.
Methods: NOD/SCID mouse bearing xenograft tumor of Kasumi-1 cells were randomly divided into normal saline group, Ara-C group, rsTRAIL alone group, plumbagin alone group and rsTRAIL combined plumbagin group. The tumor volume and weight changes of mouse were observed.The pathological slices of tumor and organs of mouse were stained with HE method and observed under microscope.The morphology of tumor cells was observed by microscope. The apoptotic rate of single cell suspension of xenograft tumor was calculated by flow cytometry. The expression of DR4 and DR5 at protein level on the surface of cells was detected by flow cytometry.
Results: (1) Compared with the control group, both rsTRAIL and plumbagin could inhibit the growth of the xenograft tumor (P<0.01).They had a synergistic effect (P<0.05).The inhibitory rates in rsTRAIL, plumbagin and rsTRAIL combined plumbagin were 56.74%,65.17% and 90.07% respectively.(2)The NOD/SCID mouse mode of AML with t(8;21) were successfully established, and the main marker is the formation of subcutaneous mass. (3)The main toxicity of plumbagin 6mg/kg was diarrhea in tumor-bearing mouse. (4)T he ratios of annexin V positive cells were (47.39±9.99)%, (51.35±3.58)% and (68.23±6.74)% in rsTRAIL alone groups, plumbagin alone and the combination of the both group respectively, and was significantly higher in the combination group than in the group of rsTRAIL alone or plumbagin alone.(5)Plumbagin could increase the expression of DR4 and DR5 in cells of xenograft tumor which were demonstrated by flow cytometry (Fiugre 1).
Summary and Conclusions: Plumbagin could enhance TRAIL induced apoptosis of Kasumi-1 cells *in vivo* without obvious damaging of the vital organs, and the major mechanism was related to the upregulation of DR5.

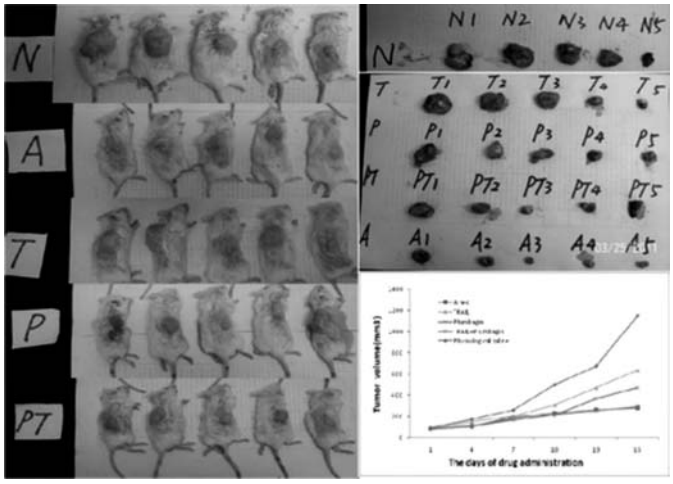

Figure 1.

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PROGNOSTIC RELEVANCE OF ACQUIRED STRUCTURAL GENETIC ABNORMALITIES IN ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is a heterogeneous malignancy, with prognosis remaining unfavorable. In addition to age and leukocytosis, cytogenetic abnormalities are key factors to assess prognosis. However, variability in treatment response remains and is inadequately explained. Furthermore, cytogenetic analysis fails in approximately 10% of patients, thus a correct diagnosis is not possible in these cases.
Aims: In this study, we assessed prognostic relevance of high-throughput single nucleotide polymorphism arrays (SNP-A) analysis in AML.
Methods: We analysed paired (*i.e.*, diagnostic and remission) samples of 119 adult AML patients and in parallel we compared results with conventional cytogenetics and molecular diagnostics. Findings were validated on an additional independent cohort of adult AML (n=127).
Results: We identified SNP-A abnormalities in 57% of the patients. More importantly, we defined unfavorable SNP-A abnormalities based on chromosomal location. These unfavorable SNP-A abnormalities were associated with worse overall treatment response (OS, *P*<0.0001, HR=1.64, 95%CI=1.33-2.02) and with worse relapse-free survival (RFS, *P*=0.0022, HR=1.39, 95%CI=1.13-1.72). Multivariate analysis confirmed independent prognostic impact of unfavorable SNP-A abnormalities with prognostic cytogenetics (*P*=0.013, HR=1.43, 95%CI=1.08-1.90) or with the European Leukemia Net (ELN) classification (*P*=0.013, HR=1.45, 95%CI=1.08-194). The number of unfavorable SNP-A abnormalities was significantly associated with OS and RFS in the validation cohort (*P*<0.0001, HR=1.38, 95%CI=1.20-1.59 and *P*=0.002, HR=1.71, 95%CI=1.23-2.46). Multivariate analysis was validated similarly with prognostic cytogenetics (*P*=0.0082, HR=1.26, 95%CI=1.06-1.50) or with ELN classification (*P*=0.0051, HR=1.28, 95%CI=1.08-1.51) in the independent cohort.
Summary and Conclusions: We have shown independent relevance of unfavorable SNP-A abnormalities compared to conventional cytogenetics and molecular diagnosis. These findings suggest concurrent SNP-A analysis may improve current prognostic risk stratification of AML, especially in cases where conventional cytogenetics is unsuccessful.

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HIGHER PROPORTIONS OF HELPER/INDUCER T CELLS AND CD4+CD31+ NAÏVE T CELLS AT DIAGNOSIS PREDICT BETTER COMPLETE REMISSION RATE IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Physiologic function of the immunocytes in the tumor is to rec-

ognize and destroy clonally transformed cells before they grow into tumors and to kill tumors after they are formed. It is now clear that the immune system does react against many tumors including acute myeloid leukemia. Lymphocyte subpopulation is known to be associated with prognosis with certain malignancies, however, the role of immunocytes in patients with hematologic malignancy is not well studied.
Aims: We prospectively investigated whether the differences in the proportion and absolute number of lymphocyte subsets affect the leukemogenesis and treatment outcomes in patients with acute myeloid leukemia.
Methods: Consecutive 91 AML patients who were newly diagnosed by bone marrow study, immunophenotyping, cytogenetic and molecular analysis, were included and classified into 3 risk groups (good, intermediate, poor) based on national comprehensive cancer network (NCCN) 2013 guideline. We measured lymphocyte subsets (T cells, helper/inducer T cells, suppressor/cytotoxic T cells, naïve T cells, memory T cell subsets, regulatory T cells, natural killer (NK) cell subsets, NK-T cells and B cells) by multi-color flow cytometry with peripheral blood obtained from patients at the time of diagnosis and after chemotherapies (induction, first and second consolidations) and from 10 healthy controls.
Results: At diagnosis, AML patients had significantly lower numbers and proportions of $CD56^{dim}CD16^{+}$ NK cells, central memory T cells and regulatory T cells than healthy controls (P=.005,.007,.019). Higher proportions of helper/inducer T cells and $CD4^{+}CD31^{+}$ naïve T cells and lower proportion of NK cells increased complete remission (CR) rate in 65 non-promyelocytic leukemia patients who received cytarabine+anthracycline-based induction therapy (P=.034,.027,.019), and it was also significant in multivariable analysis with age and risk status (P=.014,.016,.045). In univariate analysis of relapse-free survival (RFS), NK cells <4.8% of lymphocytes demonstrated shorter RFS (P=.006) and it was also significant in multivariable analysis with risk status (P=.037). During chemotherapy, NK-T cell proportion after 1st consolidation therapy, compared with NK-T cell proportion at diagnosis, increased prominently in patients who relapsed later than patients with continuous CR (P=.031).
Summary and Conclusions: Lower numbers of $CD56^{dim}CD16^{+}$ NK cells, central memory T cells and regulatory T cells might contribute the leukemogenesis of AML, and the chemotherapy induced different lymphocyte subset findings. Higher proportions of helper/inducer T cells and $CD4^{+}CD31^{+}$ naïve T cells and lower proportion of NK cells at diagnosis independently predict better CR rate, but the proportion of NK cells <4.8% at diagnosis had adverse prognostic impact in RFS.

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HIGHER EXPRESSION OF ROUNDABOUT 4 (ROBO4) IN THE BONE MARROW IS AN INDEPENDENT POOR PROGNOSTIC FACTOR IN ADULT PATIENTS AFFLICTED WITH *DE NOVO* ACUTE MYELOID LEUKEMIA

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Background: Bone marrow (BM) microenvironment provides support for self-renewal, quiescence, homing, engraftment and proliferative potential for hematopoietic stem cells (HSCs). Roundabout 4 (Robo4) is a transmembrane protein expressed specifically in endothelial cells and HSCs. Recently, *Robo4* expression was shown to be associated with HSC homeostasis.
Aims: Till now, there have been few studies concerning the prognostic implication of *Robo4* expression in *de novo* AML.
Methods: We investigated the mRNA expression of Robo4 by real-time quantitative polymerase chain reaction in the BM cells from a cohort of 201 newly diagnosed *de novo* AML patients, including 148 original and 53 validation cohort, and 20 healthy BM donors. The expression of Robo4 was normalized to that of the housekeeping gene *RPLP0*. The result was correlated with clinical features, cytogenetics, other genetic alterations and treatment outcomes.
Results: Median levels of Robo4 expression were significantly higher in AML patients than in normal BM donors. The patients were then divided into one with low expression of Robo4 (n=87) and the other with high expression (n=61), by using a cut-off point of 0.010 (*Robo4/RPLP0)*. Patients with high Robo4 expression had higher incidence of HLA-DR and CD56 expression on the leukemia cells (85.0% *versus.* 62.4%, P=0.003 and 30.0% *versus.* 8.4%, P=0.001, respectively). Further, high Robo4 expression was closely association with chromosomal abnormalities t(8;21), but inversely correlated with t(15;17) (20% *versus.* 2.3%, P=0.0009 and 1.6% *versus.* 14.9%, P=0.0081, respectively). We also found that Robo4 expression was significantly lower in AML patients with $CEBPA^{doublemutation}$ (P=0.0240). One hundred seven (72.3%) of 148 patients achieved a complete remission (CR). High Robo4 expression was associated with a trend of inferior response to induction chemotherapy (CR rate, 63.9% *versus.* 78.2%, P=0.0643). With a median follow-up of 31 months (ranges, 1.0-160), patients with high Robo4 expression had significantly poorer overall survival (OS) and disease-free survival (DFS) than those with low Robo4 expression (median, 17.0 months *versus.* 95.0 months, P=0.023, and medium, 5.0 months *versus.* 15.0 months, P=0.024, respectively). In the subgroup of 99 patients with intermediate-risk karyotype, the differences of OS (median, 13.5 months *versus.* 95.0 months, P=0.007) and DFS (median, 4.0 months *versus.* 10.0 months, P=0.025) between patients with high and low Robo4 expression were still significant. In multivariate analysis, the independent poor risk factors for OS and DFS were older age, high WBC count, unfavorable karyotype, and high Robo4 expression (Hazard ratio OS, 1.779, 95% CI 1.005-3.149, P=0.048 and DFS, 1.600, 95% CI 1.026-2.495, P=0.038). On the other hand, $CEBPA^{doublemutation}$and $NPM1^{+}$/*FLT3*-ITD^{-}were independent favorable prognostic factors. This result was also validated in an independent validation cohort (n=53).
Summary and Conclusions: Our results demonstrated that Robo4 expression was correlated with surface antigen expression of leukemic cells, cytogenetics, and molecular mutation. Further, high Robo4 expression indicates an unfavorable prognosis in *de novo* AML patients. It may be used as a biomarker for risk-stratification of AML patients.

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IMPLICATIONS OF MIR-10 IN CHEMOTHERAPY RESPONSE OF NPM1 MUTATED AML

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Background: Nucleophosmim (*NPM1*) mutated AML (*NPM1*mut-AML) patients are associated with a high rate of complete response (CR) to induction chemotherapy. However, the mechanisms responsible for such effects are unknown. It has been shown that a unique miRNA signature is associated with cytogenetically normal AML (CN-AML) patients harboring *NPM1* mutations, and includes the strong uP-regulation of miR-10a and miR-10b (miR-10a/b). However, the functional role of these two miRs in *NPM1* mutated AML (*NPM1*mut-AML) is unclear. We hypothesize that chemotherapy sensitivity and the high CR rates observed in *NPM1*mut-AML patients could be mediated through miR-10a/b.
Aims: To investigate the association of miR-10 expression levels with chemotherapy response and sensitivity in *NPM1*mut-AML patients.
Methods: We analyzed miR-10a/b expression levels in pretreatment bone marrow samples obtained from 54 newly diagnosed AML patients treated with idarubicine and cytarabine by using the OSU-CCC miRNA microarray platform. After background subtraction and normalization using quantiles, miRNA data was analyzed using BRB tools (Class comparison). Patients characteristics include: median age (n=61); CN-AML(n=33), FL3-ITD(n=10),*NPM1*mut(n=25), other karytyope (n=21). MiR-10a gain and loss of function studies were performed in AML cell lines and primary AML samples in baseline conditions and after chemotherapy.
Results: To identify whether miR-10a/b are associated with CR, we compared pretreatment miR-10a/b levels of patients who achieved CR (n=28) *versus* no responders (R) (n=26). The median miR-10a levels were 12.1 (4.2-14.2) in CR patients and 9.1 (range 5.1-13.5) in no-CR patients (P=0.002), while the median miR-10b levels were 12.2 (4.2–13.7) in CR patients and 8.1 (4.2–13.1) in no-CR patients (P=0.001). Univariate analysis including cytogenetics, age, *NPM1* status and miR-10a/b revealed that both miRNAs were significantly associated with CR (OR 1.33 and 1.32, respectively). Multivariate analysis including age, *NPM1* status, unfavorable cytogenetic prognostic group, CN-AML and miR-10a/b, revealed that *NPM1* and miR-10a remain significant after adjusting for the other variables. However, there was an interaction effect in the multivariate logistic model with *NPM1* mutation status and miR-10a, where *NPM1* mutation status modifies the effect of miR-10a on CR incidence (P=0.033). To assess the functional role of miR-10a in chemotherapy sensitivity, we over-expressed synthetic miR-10a in AML cell lines with low endogenous miR-10a expression (KG-1a/Kasumi-1) showed no changes in cell proliferation or apoptosis between miR-10a and the controls in baseline conditions or after cytarabine treatment. Knock down of miR-10a in two AML cells lines with high levels of miR-10a (K562/*NPM1*mut OCI-AML3),using antimiR-10a or scramble control showed no differences in cell proliferation and apoptosis in baseline condition or after cytarabine treatment. Identical results were observed in 4 primary AML samples; 2 with *NPM1* wild-type and low level of miR-10a that were transfected with nanoparticle conjugated synthetic miR-10a and 2 with *NPM1* mutated and high level of miR-10a that were transfected with nanoparticle-anti-10a.
Summary and Conclusions: High miR-10a/b expression levels in AML patients are associated with achieving CR. However this association is dependent on *NPM1* mutation status. miR-10a gain and loss of function experiments in cell lines and in primary AML samples did not demonstrate any effect in apoptosis and cell proliferation in baseline conditions nor after chemotherapy treatment.

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INHIBITION OF AUTOPHAGY SENSITIZES LEUKEMIC CELLS TO CYTARABINE

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Background: Autophagy is a process in which a cell degrades its own components in organelles called autophagolysosomes. Cytarabine is a chemotherapeutic drug used to treat different forms of leukemia, including acute and chronic myelogenous (AML and CML), acute lymphocytic leukemia (ALL) and lymphomas.
Aims: In this study we investigated the ability of cytarabine to induce autophagy in different leukemic cells.
Methods: Human leukemia cell lines: promyelocytic (HL-60), lymphocytic (REH) and acute lymphoblastic (MOLT-4), as well as peripheral blood mononuclear cells (PBMCs) from CML patients or healthy controls were treated with cytarabine. Cell viability was determined by measuring the activity of lysosomal acid phosphatase. Intracellular acidification was demonstrated by the acridine orange staining. Autophagosomes and autophagolysosomes were detected by transmission electron microscopy. Expression of autophagy-related proteins was shown by Western blot analysis. Pharamacological inhibitors and RNA interference were used for down-regulation of autophagy.
Results: Cytarabine stimulated cytoplasmatic acidification and appearance of double-membraned autophagosome-like and single-membraned autophagolysosome-like vacuoles with partly digested intracellular content in REH cells. Cytarabine induced degradation of the selective autophagic target p62 and conversion of LC3-I to autophagosome-associated LC3-II in the presence and absence of proteolysis inhibitors in all tested leukemic cells, but not PBMC from healthy controls. In contrast to MOLT-4 cells, pro-autophagic protein beclin-1 was not upregulated in REH, HL-60 and PBMC from leukemic patients. Cytarabine reduced activity of mTOR, major negative regulator of autophagy, and its downstream target p70S6K. Autophagy downregulation markedly increased cell death in all cytarabine treated leukemic cells, but not PBMC from healthy controls.
Summary and Conclusions: Our results demonstrate that the inhibition of autophagy sensitizes leukemic cells to the cytarabine-induced cell death and suggest a promising approach to improve the efficiency of cytarabine in the treatment of leukemia.

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SYNERGISTIC EFFECTS OF HSP70 AND HSP90 INHIBITORS IN PRIMARY HUMAN ACUTE MYELOID LEUKEMIA CELLS

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Background: Acute myeloid leukemia (AML) is a heterogeneous group of malignant hematopoietic disorders characterized by bone marrow accumulation of immature leukemic cells. Heat shock proteins (HSPs) are molecular chaperones that assist proteins in their folding to native structures. HSP90 has emerged as therapeutic targets in human malignancies; including AML there agents have entered clinical trials. However, the results from early clinical trials have not been too promising, and suggested explanations for this have been (i) activation of compensatory mechanisms including increased HSP70, (ii) difficulties in obtaining effective *in vivo* concentration due to dose-limiting side effects especially for the geldanamycin derivatives or (iii) limited effects of HSP90 inhibitor monotherapy. Therapeutic targeting of HSP70 has recently emerged as a new therapeutic approach, and the specific inhibitor VER-155008 has been developed through structure-based X-ray crystallographic design.
Aims: The antileukemic potential of HSP70 inhibition still remains elusive; therefore the aims of the present study were to investigate the *in vitro* antileukemic effect in AML of HSP70 inhibitor both alone and in combination with HSP90 inhibitor.
Methods: We evaluated the effect of the HSP70 inhibitor VER-155008 regarding proliferation, viability, colony forming unit (CFU), cytokine release and levels of intracellular HSPs (HSP27, HSP40, HSP60, HSP70 and HSP90) on highly standardized *in vitro* cultured primary human AML cells derived from 20 consecutive AML patients. In addition we investigated potential synergistic effects between VER-155008 and the HSP90 inhibitor 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG).
Results: VER-155008 caused a dose-dependent inhibition of cytokine-dependent AML cell proliferation both in suspension cultures and in a CFU assay, and also had a significant proapoptotic effect. VER-155008 caused a strong inhibition of the constitutive AML cell release of several growth factors/regulators of hematopoiesis (*i.e.* tumor necrosis factor α, vascular endothelial growth factor, interleukin3, interleukin 1β, interleukin 1 receptor antagonist), although had relatively weak effects on the constitutive chemokine release. When combining VER-155008 with 17-DMAG, we explored an increased antiproliferative and proapoptotic effect compared to both drugs alone. A highly significant decrease in both cytokine and chemokine release were observed of combination treatment, compared to both drugs alone. HSP90 inhibition led to compensatory increase in HSP70 levels, while HSP70 inhibition decreased the levels of both HSP90 and HSP70. Combining VER-155008 and 17-DMAG also increase the HSP70 levels, however the effect was not stronger then use of 17-DMAG alone.
Summary and Conclusions: HSP70 inhibition has potential antileukemic effects in human AML. Furthermore, we provide results supporting stronger effect of HSP70 and HSP90 inhibition in combination, which can increase antileukemic activity in human AML, with the potential to overcome the obstacles associated with HSP90 inhibition alone. These approaches should be future explored in preclinical and clinical settings, to further identify the potential benefits of such a combination in leukemia treatment.

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ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE: IS THE NUMBER OF CONSOLIDATION CYCLES IMPORTANT?

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Background: To this date, the standard treatment of acute myeloid leukemia (AML) in young patients has been the use of increasingly myelotoxic induction followed by consolidation treatment, in which appropriate doses remains uncertain. Furthermore, treatment response is heterogeneous, being that the risk of relapse remains a constant threat. Defining the best consolidation treatment is still a major concern in the treatment of young adults with AML, particularly in patients with a normal or unfavorable karyotype without a HLA-compatible donor. A relevant issue is the ideal number of consolidation cycles with high dose cytarabine (HDAC) that would have an impact on prognosis.
Aims: Determination of the prognostic impact of the number of consolidation cycles in younger patients with non-promyelocytic *de novo* AML and normal karyotype.
Methods: In a total of 336 patients with AML, treated in our center from 1998 to 2010, a retrospective analysis was conducted in the subgroup of younger patients (age ≤65 years old according to our protocol) with non-promyelocytic *de novo* AML, normal karyotype who were undergoing the induction regimen "7+3" (Idarubicin 12mg/m^2 ev, days 1,2,3; Cytarabine 200mg/m^2 ev, days 1,2,3,4,5,6,7) and didn't have an HLA compatible donor. The patients' characteristics were compared with a chi-square test for binary variables and a Mann-Whitney test for continuous variables. The survival curves were estimated based on Kaplan-Meier curves and data for the various groups were compared with a log-rank test. The multivariate analysis was carried out using a Cox model, after the proportional hazard assumption was checked. A p value below 0,05 was considered as being statistically significant.
Results: The median follow-up was 28 months ([1-134]). Of the 47 patients, 48,9% (n=23) were male - with an average age of 52 years old ([20-65]). All patients (n=47) presented intermediate risk SWOG. In this analysis, of the 78,7% (n=37) patients who reached complete response (CR) after one cycle of induction, 76,6% (n=36) underwent consolidation treatment with Cytarabine 3mg/m^2 (CALGB). The median number of consolidation cycles was 3 [(1-4)]. This difference was conditioned by several factors (intention to treat, toxicity, relapse during consolidation, death). 59,6% of the patients relapsed after 13 months (median) [0-92]. It was observed that the disease-free survival (DFS) was better in the group of patients who complete 3 or 4 consolidation cycles, in contrast with the group who complete 1 or 2 cycles (P>0,05). If we done a stratification according to the number of consolidation cycles they carried out, 66.7% (n=24) of them underwent more than 2 consolidation cycles. This group of patients presented higher DFS in comparison to patients who underwent ≤2 cycles (median of 41 *vs.* 9 months, P=0,046), with an impact on overall survival (OS: P=0,021). Given the Cox regression analysis, the completion of more than 2 cycles of consolidation is an independent predictive factor of OS (HR=2,72; 95% CI1,12-6,58; P<0,05), comparatively with the group that performs ≤2 cycles of consolidation, age, number of leukocytes (at diagnosis) and type of response to induction. There was no noted difference between the high/intermediate risk group (n=11) and the low/intermediate risk one (n=4).
Summary and Conclusions: This study demonstrates that the number of cycles performed by a particular patient has prognostic value. For the heterogeneous intermediate risk group with normal karyotype, more cycles of consolidation with CALGB could be considered an important component to the AML treatment and thereby it could be an alternative to allogeneic transplantation in patients without an HLA-matched donor. This analysis needs to be enhanced with randomized studies in order to evaluate and validate this approach.

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IMPROVEMENT, BUT STILL POOR PROGNOSIS FOR ESTONIAN PATIENTS COMPARED TO A WELL-DEFINED REGION OF WESTERN SWEDEN, A POPULATION BASED STUDY OF ACUTE *DE NOVO* LEUKEMIA PATIENTS AGED 16-64 YEARS

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Background: The present population-based survey was carried out over five consecutive 5-year study periods (1982-2006) on the incidence and survival of *de novo* acute leukemia (AL) patients with acute myeloid or lymphoblastic leukemia (AML/ALL) aged 16-64 years at diagnosis in Estonia (n=407) and in a well-defined region in western Sweden (n=517). Estonia regained independence in 1991 after 5 decades of occupation by the Soviet Union and entered a transition from planned to market economy.
Aims: The current population-based study was designed and aimed to investigate how changes as regards political and socio-economic circumstances are likely to affect hematologic health care in a society, especially incidence and survival.
Methods: The present population-based study comprises all *de novo* AL patients aged 16-64 years in Estonia and western Sweden diagnosed 1982-2006. Estonia covers an area of 45,000 km^2 and the Western Swedish Health Care Region covers an area of 27,000 km^2. During the years of the population-based retrospective work (1982-1996) the average population in Estonia and western Sweden were 1.5 and 1.6 million inhabitants, respectively. The average number of inhabitants in Estonia and the western Swedish region during the two prospective population-based studies (1997-2006) were 1.4 and 1.7 million, respectively. *Identification of* de novo *AL.* Since the aim of the present work was to compare the incidence and survival of *de novo* AL in the two countries, the delineation of the diagnosis was critical. Therefore, patients with a history of pre-existing myelodysplasia, polcythemia vera, essential thrombocythemia, idiopathic myelofibrosis, chronic myeloid leukemia or leukemia secondary to chemo-/radiotherapy were excluded from the study. *Statistical methods.* Individual data for the two cohorts of acute *de novo* leukemias from western Sweden and Estonia were put together in a database for the analyses. The incidence in the population was compared by use of age standardized incidence rates. Survival analyses were carried out by estimating relative survival. The relative survival is the ratio between the observed survival of the patients and the expected survival of a comparable group from the general population. Mortality data of the general population in Sweden and Estonia were used to estimate expected survival rates for the study populations. Internal age standardizing of the relative survival rates was done by use of the age distribution of all individuals in the two cohorts.
Results: With regard to age adjusted incidence rates for AL in our current work there is no statistically significant difference between Estonia (1.8/100000) and Western Sweden (1.8/100000)during the period 2002-2006. Corresponding figures for AML were 1.4/100000 and 1.2/100000 respectively and for ALL 0.4/100000 and 0.6 /100000, respectively. Similar incidence rates are noted since the start of our study 1982. Since the first five year cohort 1982-1986, five year relative survival for all AL patients in Western Sweden has increased from 20% to 56%, as compared to 2002-2006 (Figure 1). Corresponding figures from Estonia is an increase in survival from 3% to 22%. The pattern is similar for AML, Estonia (n=280) 2% to 22% and Western Sweden (n=370) 19% to 58%.There is also a brisk improvement in survival for Swedish AML patients during 1997-2001 (45% *vs.* 58%) as compared to Estonia (15% to 22%).

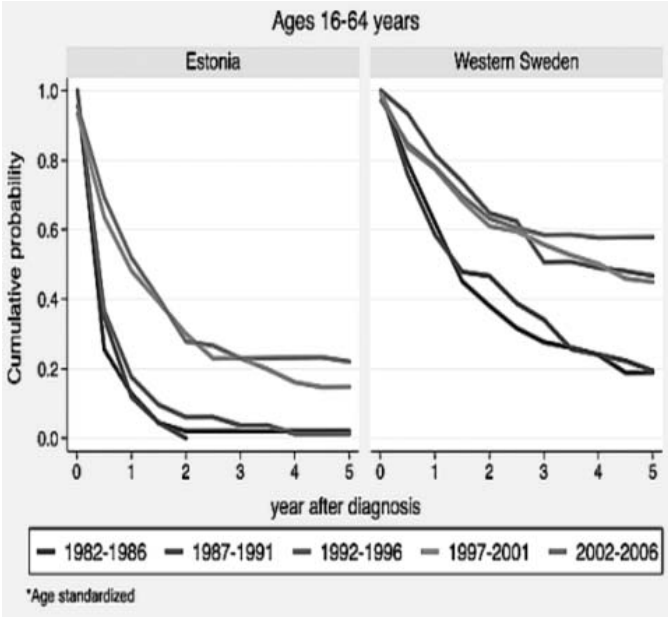

Figure 1. Relative survival* for AML *de novo*.

Summary and Conclusions: Over the years1982-1992 Estonia was still under the mentorship of the Soviet Union. Estonian hematologists did not have access to therapeutic measures readily available to Swedish hematologists, and the results for survival for western Swedish patients with AL far exceeded those for their Estonian counterparts. Of course it is encouraging that survival appears to have increased in Estonia as well as in Sweden. However, despite progress, the results in Estonia are still burdened by very poor survival in acute leukemia.

It is highly unsatisfactory that the improvements in relative survival have not been faster. Differences in survival between the two countries are mainly due to higher intensity chemotherapy regimens and a higher rate of hematopetic stem cell transplantation in Sweden. However, other still unknown factors may play a substantial role. Therefore, it is of particular interest and great importance that we are in the process of collecting specified data also regarding supportive care for *de novo* ALs for the next prospective period from 2007-2011.

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PROGNOSTIC IMPACT OF NPM1, IDH1/2 AND DNAH11 GENE MUTATIONS ON NORMAL KARYOTYPE ACUTE MYELOID LEUKEMIA PATIENTS NOT HARBORING FLT3/ITD MUTATION

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Background: Acute myeloid leukemia with normal karyotype (AML-NK) is known to be heterogeneous in the molecular level. Accordingly, it has become more critical to dissect this group of patients according to their prognosis using a molecular genetic technology.
Aims: We attempted to analyze the incidence and prognostic implication of genetic abnormalities on survival in 426 adult patients with AML-NK.
Methods: A total of 67 AML-NK patients achieved complete remission (CR), candidate mutations in 21 genes were identified by whole exome sequencing which has 41-89X coverage and by single-nucleotide polymorphism array analysis using marrow mononuclear cells at diagnosis of AML-NK. Subsequently, mutation analysis of 11 genes (*i.e. FLT3/ITD, NPM1, DNMT3a, IDH1, IDH2, TET2, NRAS, WT1, DNAH11, SF3B1, and PHF6*) which are known to be involved in the pathogenesis of hematologic diseases, were performed using Sanger sequencing in another subset of 359 AML-NK patients as a validation cohort.
Results: Of 426 patients in total (median age: 51, ranges: 15-85), FLT3/ITD, NPM1, and DNMT3a mutations were associated with higher leukocytes counts at presentation of AML-NK. In 284 patients who received standard remission induction (RI) chemotherapy (excluding 119 patients with conservative treatment and 22 early death/1 follow-up loss after RI chemotherapy), those with FLT3/ITD mutation were significantly associated with a higher risk of relapse (P=0.02), a shorter leukemic-free survival duration (LFS)(P<0.01) or overall survival (OS) (P=0.01). Accordingly, we divided the patients into FLT3/ITD$^+$ and FLT3/ITD$^-$ population, and analyzed their treatment outcomes according to the other mutations. In the FLT3/ITD$^-$ group (n=200), those with NPM1 mutation showed a higher CR rates after one or two cycles of RI chemotherapy (P<0.01) and a longer OS duration (P<0.01), hazard ratio (HR) 0.43, 95% confidence interval (CI) 0.25-0.73, adjusted by other clinical variables including age, leukocyte counts at diagnosis, and transplantation. In the FLT3/ITD$^+$ patients (n=84), NPM1 mutation was found to be a favorable prognostic factor showing a lower relapse rate (P=0.00), a longer LFS duration (P<0.01, HR 0.35, 95% CI 0.18-0.70), and OS duration (P=0.04, HR 0.55, 95% CI 0.31-0.98) in NPM1 mutated patients. In addition, OS was significantly different in favor of those with IDH2, especially R140Q IDH2 mutation, (P=0.04, HR 0.30, 95% CI 0.09-0.99), whereas DNAH11 mutation was associated with inferior OS (P<0.01, HR 5.78, 95% CI 1.65-20.25). Accordingly, we stratified the FLT3/ITD$^+$ patients into three subgroups according to the NPM1, IDH1/2 and DNAH11 mutation status, Group 1: NPM1 mutation and IDH1 or 2 mutations (n=16), Group 2: isolated DNAH11 mutation (n=4) and Group 3: all mutations were negative (n=64). The group 1 showed significantly better OS than group 2 (P<0.01, HR 16.90, 95% CI 3.48-82.15) orgroup 3 (P<0.01, HR 3.40, 95% CI 1.20-9.55).In a subgroup analysis of younger patients less than 60 years of age, similar outcomes were also observed in favor of group 1 in terms of OS.
Summary and Conclusions: Our study confirmed that NPM1 mutation is an independent prognostic factor in adult patients with AML-NK not harboring FLT3/ITD mutation. In addition, several other genetic markers were identified as prognostic including IDH1/2 or DNAH11 mutations as well as NPM1 mutation in a subgroup of AML-NK patients with FLT3/ITD mutation.

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ROLE OF ALLOGRAFTING IN HIGH RISK ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is the most frequent acute leukemia in adults. Prognosis of patients with high risk AML improves with allogeneic stem cell transplantation.
Aims: To evaluate outcomes in newly diagnosed patients, younger than 66, who achieved complete remission (CR) after induction/consolidation therapy at the Divisions of Hematology at Città della Salute e della Scienza, Università di Torino, Torino, Italy, between 2000-2011.
Methods: Three-hundred and two AML patients (except FAB-M3) were consecutively diagnosed and stratified by risk as follows: low risk included presence of t(8;21), inv(16)/t(16;16); high risk features included WBC>50.000/uL at diagnosis, secondary leukemia, presence of extramedullary AML, complex karyotype, chromosomal monosomy, no remission after induction, and FLT3/MLL mutations (since 2004). Intermediate risk included patients who did not meet either low or high risk criteria. Moreover, the standard risk group included low+intermediate risk patients. Patients were treated according to Center guidelines or on clinical trials active at the time of diagnosis. All high risk patients were considered for an allograft since diagnosis.
Results: After induction/consolidation, 229/302 patients (76%) achieved complete remission: 16/229 (7%) were at low, 54/229 (24%) at intermediate, and 159/229 (69%) at high risk respectively. Eighty/159 (50%) high risk patients received an allograft as 1st line treatment; 56% from a HLA-matched sibling, 42% from an unrelated donor and 2% received a haplo-identical transplant. Seventy-nine/159 (50%) did not receive an allograft primarily because of failure to find a suitable donor either sibling or unrelated. At median follow-up of 53 months from induction therapy and 49 months from achieving CR, 5-year overall survival (OS) and 5-year event free survival (EFS) of the entire patient cohort were 45% and 35% respectively. By risk category, standard risk patients showed a 5-year OS of 56% and high risk patients of 40% (P=0.008). Five-year EFS was 37 and 34% in standard and high risk patients respectively (P=0.194). High risk patients who underwent an allograft uP-front showed a 5-year OS of 53% and showed a statistically significant advantage as compared with those who did not receive a transplant (P=0.018).
Summary and Conclusions: Allografting plays a pivotal role in OS and EFS for high risk acute myeloid leukemia. The lack of a donor is associated with bad clinical outcomes. Prospective clinical trials designed to evaluate the use of more readily available donors such as haploidentical siblings or parents are needed.

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PROGNOSTIC IMPACT OF A MONOSOMAL KARYOTYPE ON OUTCOME IN ACUTE MYELOID LEUKEMIA

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Background: Recently, a new cytogenetic category of acute myeloid leukemias (AML), monosomal karyotype AML (AML MK+), was reported to be associated with a poor prognosis and to add prognostic information even in patients with a complex karyotype.
Aims: The aims of this study were to determine the incidence and characteristics of AML MK+, and to estimate the impact of MK+ on overall survival (OS), complete remission rate (CR) and disease-free survival (DFS) in patients with AML.
Methods: This single-center study involved 298 patients with nonpromyelocytic AML. The following parameters were recorded: age, hemoglobin level (Hb), white blood cell count (WBC), platelet count (Plt) and performance status (PS) evaluated by the Eastern Cooperative Oncology Group (ECOG), range 0-4 (<1 *vs* ≥2). The clinical characteristics at presentation and outcome of two subgroups of patients: AML MK+ and AML without MK were compared. MK+ is defined by the presence of one single autosomal monosomy (AM; excluding isolated loss of X or Y) in association with at least one additional AM or one structural chromosomal abnormality (in the absence of core-binding factor AML). Patients were treated according to the Medical Research Council (MRC) 12 protocol. Statistical analysis included: the Fisher exact test, chi-square test and Kaplan-Meier method.
Results: The mean age of the patients was 57 years (range 19-79 years). The incidence of AML MK+ was 32.8% (98 pts). AML MK+ patients were significantly older than those from the control group (AML without MK; P=0.048). The presence of severe anemia at presentation with Hb <80 g/L was more frequent in AML MK+ patients than in those with AML without MK (P=0.046). Likewise, the mean WBC was lower in AML MK+ patients than in participants with AML without MK (P=0.008). AML MK+ patients had a higher rate of poor ECOG PS (≥2) than AML patients without MK (P=0.013). AML MK+ patients had shorter OS (6 *vs* 18 months; P=0.002) and DFS (5 *vs* 12 months; P=0.013) in comparison with AML patients without MK. Similarly, AML MK+ patients had a lower CR rate compared to AML patients without MK (27.3% *vs* 59.5%; P<0.001).
Summary and Conclusions: This research identified MK+ in approximately one-third of AML patients. The characteristics of these patients are older age,

poorer ECOG PS, lower Hb level and WBC at presentation of disease. The study also confirmed a poor prognosis for these patients, with shorter OS and DFS and lower CR rate. These data indicate that this subset of AML patients should be recognized as an adverse risk group and according to this studied in the new context of molecular markers.

P666

RISK FACTORS FOR CENTRAL NERVOUS SYSTEM INVOLVEMENT AT DIAGNOSIS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Central nervous system (CNS) involvement in acute myeloid leukemia (AML) is observed in 2-4% of patients at diagnosis. Hematological parameters associated with an increased risk of CNS leukemia include a high presenting white blood count (WBC), elevated lactate dehydrogenase (LDH), French-American-British (FAB) subgroups M4 and M5 and age less than 50 years. Recently, it was shown that the immunophenotypic pattern of AML–the expression of CD56 antigen (CD56+) on leukemic cells, was associated with a higher rate of CNS involvement in AML at presentation.
Aims: The aims of this investigation were to estimate the incidence of CNS involvement at diagnosis of AML and to determine the risk factors for its occurrence.
Methods: This single-center study involved 191 patients with nonpromyelocytic AML. The following parameters were estimated as risk factors for CNS involvement at diagnosis of AML: age, WBC ($<30\times10^9$/L *vs* $\geq30\times10^9$/L), serum lactate dehydrogenase (LDH) concentration >1.5× the upper limit of normal, expression of CD56 antigen on leukemic blasts (<20% *vs* ≥20%) and cytogenetic risk group (assessed according to European LeukemiaNet recommendations). The presence of leukemic cells in cerebrospinal fluid (CSF) detected by cytomorphological and/or flow cytometric analysis of CSF is considered as CNS involvement. Patients were treated by the Medical Research Council (MRC) 12 scheme. Risk factors were identified using univariate and multivariate analysis.
Results: The mean age of the patients was 55 years (range 21-79). CNS involvement was recorded in 5.8% (11) of them. Univariate analysis detected the following significant risk factors for CNS involvement: age <55 years (P=0.009), WBC $\geq30\times10^9$/L (P=0.001), elevated LDH (P=0.036), FAB M4/M5 (P=0.05) and CD56+ (P=0.001). Multivariate analysis identified CD56+ as the most important risk factor for CNS involvement at diagnosis in AML patients (P=0.001; relative risk (RR)=18.241; 95% confidental interval (CI)=3.305-100.675. The cytogenetic risk group had no influence on CNS involvement in this study.
Summary and Conclusions: According to the result**s** obtained, AML patients with CD56+ on leukemic cells are at high risk for CNS involvement at presentation. Cytomorphological and/or flow cytometric analysis of CSF should be recommended as a routine procedure in these patients for more precise diagnosis of CNS involvement. CD56 antigen is an isoform of the neural cell adhesion molecule, constitutively expressed in normal monocytes and monocyte-derived cells, which could be an explanation for the high risk of CNS involvement in CD56+ patients with AML, particularly FAB subtypes M4/M5.

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SYSTEMIC VERSUS LOCALIZED THERAPEUTIC APPROACH FOR ISOLATED MYELOID SARCOMA: META-ANALYSIS OF INDIVIDUAL PATIENT DATA

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Background: Myeloid sarcoma (MS), also known as granulocytic/monoblastic sarcoma, extramedullary myeloid tumors, or chloroma, is a tumor mass comprising blasts or immature cells of the myeloid series, occurring at an anatomical site other than the bone marrow. MS can occur in patients with active acute myeloid leukemia (AML), in patients with chronic myeloproliferative disease, as the first manifestation of relapse in previously treated patients and as well as isolated MS in patients without bone marrow infiltration. The optimal timing and treatment of isolated MS are not clear, but delayed or inadequately treated isolated MS will almost always progress to AML. Also, in patients treated with chemotherapy postremission therapy has not been adequately studied; therefore, one of the most important questions in the treatment of MS is the role of hematopoietic stem cell transplantation (HSCT).
Aims: The objective of this review was to assess the effects of systemic therapy approach in adults with isolated MS rather than local treatment (surgery or radiotherapy).
Methods: We searched the Cochrane Controlled Trials Register, UKCCCR Register of Cancer Trials, Physicians Data Query, EMBASE, MEDLINE and CancerLit and after that studies in adults with isolated MS treated with chemotherapy and/or HSCT compared with local treatment (surgery or radiotherapy) were included.
Results: Four studies involving 44 patients were included. Individual patient data were obtained. The Kaplan-Meier estimate of median overall survival was 13 months (95%CI 5-21). The median overall survival for patients undergoing chemotherapy and HSCT was 28 months (95% CI, 7 to 48) while the median overall survival for patients locally treated was 9 months (95% CI, 4 to 13). The difference in overall survival was not significant but potentially represents an absolute benefit of 19 months (95% CI 11 to 28). The difference was found in OS between patients treated with HSCT and patients who did not underwent to HSCT in population below 40 years old (P=0,031). The patients undergoing HSCT had a significantly longer overall survival time compared to those who did not [33 months (95%CI 22-44) *vs* 9 months (95%CI 5-13)].There was no consistent evidence of a difference in effect according to age, sex, stage, site, grade, histology, extent of resection, tumour size or exposure to radiotherapy.
Summary and Conclusions: While more individual patient data are needed to collect, chemotherapy with HSCT could be considered as the optimal therapy for patients with isolated MS.

P668

EVALUATION OF BONE MARROW ON DAY 5 OF INDUCTION THERAPY FOR ACUTE MYELOID LEUKEMIA FOR IDENTIFICATION OF CHEMO-RESISTANT BLAST SUBPOPULATIONS

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Background: In most patients with acute myeloid leukemia (AML), different leukemia-associated immunophenotypes (LAIP) co-exist. At diagnosis, rapidly proliferating clones determine the dominant LAIP. However, at relapse, a different LAIP may become dominant. This phenomenon may reflect either a new clone or differentiation change within the original clone evolved over time. Alternatively, a small resistant sub-clone masked at diagnosis, may become evident following eradication of other, chemo-sensitive clones.
Aims: The present study investigated differences in response kinetics among immunophenotypically distinguished cell subpopulations at day 5 of induction in addition to routine assessment of day 14.
Methods: Fifteen consecutive adult AML patients receiving induction therapy with daunorubicin 60mg/m^2 for three days and cytarabine 100mg/m^2 for seven days (3+7) were evaluated. Immunophenotypes of bone marrow samples at diagnosis (day 1), after five and fourteen days of therapy were compared. Samples of relapsed patients were also analyzed. LAIPs were quantitatively evaluated by 6-color FACS analysis (Cytec; FlowJosoftware) and their relative fraction of total blast population was recorded at each time point.
Results: During therapy, the number of detectable LAIPs per patient was found to decrease. The median number of detectable LAIPs reduced from 5 through 3 to 2 at diagnosis, day 5 and day14, respectively. However, in the majority of patients, only scanty leukemic cells were observed in the day 14 marrow and not a single LAIP was detected in 2/12 (16%) patients whose samples were evaluable. In most patients, the cell number in the blast window decreased by day 5. However, eradication rate varied among different leukemic subpopulations. Heterogeneity of leukemic cells decreased with therapy, especially in patients who achieved CR in response to treatment, reflecting different sensitivity of various blast subpopulations to chemotherapy. LAIPs were quantitatively evaluated as percentage of cells in the blast window. Evaluation of each patient was particularly focused on leukemic subpopulations with the slowest eradication rate. A specific LAIP, which doubled its size in the blast window, from diagnosis to day 5 was defined as a personalized LAIP of concern. Personalized LAIPs of concern were identified in 7/15 (46%) patients and their prevalence was as high as 71.5% (5/7) among the individuals who achieved complete remission (CR) following a single induction course. Two of responding patients experienced early relapses. Interestingly, the dominant LAIP at relapse differed from that recorded at diagnosis in both patients. Meticulous analysis of samples obtained at diagnosis revealed that the dominant LAIPs at relapse were identified at day 5 samples in both patients as the LAIP of concern.
Summary and Conclusions: In clinical practice, there is an urgent need for an approach allowing recognition and characterization of leukemia relapse at the earliest possible stage. Herein, a novel approach to early, real-time identification of patient-specific resistant blast subpopulation is suggested. Detection of a personalized unique “LAIP of concern” as early as day 5 of induction would allow sorting these subpopulations out and characterizing their metabolic and genetic factors which could be attributable to later appearing clinically significant chemo-resistance.

P669

LOW MDR1 AND BAALC EXPRESSION IDENTIFIES A NEW SUBGROUP OF ADULT CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA WITH A FAVORABLE OUTCOME

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Background: Cytogenetically normal acute myeloid leukemia (AML) is a heterogeneous disease, in terms of genetic/molecular abnormalities resulting into marked differences in outcome.
Aims: We demonstrated that multidrug resistance 1(*MDR1*), brain and acute leukemia, cytoplasmic (*BAALC*) gene expression was of prognostic significance and high *MDR1* expression correlated with a high *BAALC* expression (*r=0.487,P*<0.001) in cytogenetically normal AML in the prophase study then we hypothesized that *MDR1* and *BAALC* expression together would better identify the patient's risk profile.
Methods: Pretreatment bone marrow samples from 92 cytogenetically normal AML patients were analyzed for *MDR1* and *BAALC* mRNA expression by real-time reverse transcriptase polymerase chain reaction. Patients were divided into low *MDR1* and*BAALC* expression group and combined group(high *MDR1* and/or high *BAALC* expression) according to *MDR1* and *BAALC* levels and were compared for clinical outcome.
Results: 73 cases of 92 cytogenetically normal AML patients got CR after the first block with a CR rate being 79.3%. However, 29 cases of the CR patients relapsed with the relapsed rate being 39.7%. In contrast, Patients with low expression of both *MDR1* and *BAALC* had a higher CR rate (*93.3%vs72.6%*, *P*=0.021), lower relapse rate(*7.1% vs. 42.5%, P*=0.000), longer OS (*50.3% vs 17.8%,P=0.00*1) than high *MDR1*and/or high *BAALC* expression (combined group) in cytogenecally normal AML. Results showed no statistical difference in CR rate (*93.3%vs85.7%*, *P*=0.341),relapse rate (*7.1% vs. 8.8%, P*=0.000) and OS (*50.3% vs 63.1%,P=0.43*1) for cytogenetically normal patients with both *MDR1* and *BAALC* low expression comparing to those with low-risk cytogenetically abnormal.
Summary and Conclusions: The combined assessment of *BAALC* and *MDR1* expression can improve treatment stratification in adult cytogenetically normal AML. Low expression of both *MDR1* and *BAALC* identifies cytogenetically normal AML patients with a favorable long-term outcome.

P670

THE SURFACE MOLECULAR SIGNATURE OF LEUKEMIC CELLS WAS ASSOCIATED WITH NPM1 AND FLT3-ITD MUTATIONS IN ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is a genetically heterogeneous clonal disorder characterized by the accumulation of somatically acquired genetic alterations in hematopoietic progenitor cells. Certain molecular mutations are associated with signs of cell morphology and differentiation in AML. However, only limited data is available to analyze such correlations in detail. Here we report the analysis results of 259 consecutive Chinese AML patients by using J-Express 2009 analysis suite.
Aims: To observe the associations between surface molecular signature of leukemic cells and *NPM1* and *FLT3-ITD* mutations in patients with *de novo* acute myeloid leukemia.
Methods: In present study 259 patients with *de novo* AML were enrolled. The morphological differentiation status of the leukemia cells was evaluated by FAB classification. The mutational status of molecular markers *NPM1* and *FLT3-ITD* were analyzed by polymerase chain reactions (PCR). Surface differentiation markers (CD11c, CD13, CD14, CD15, CD33, CD34 and HLA-DR) of the leukemic cells were analyzed by flow cytometry. Patients gave informed consent prior to enrolment in the study. The obtained data were analyzed using J-Express 2009 analysis suite and SPSS 17.0.
Results: The frequencies of *NPM1* and *FLT3-ITD* mutations were 15.06% (39/259) and 14.51% (37/255) in this study, respectively. Based on the surface differentiation markers of leukemic cells, the patients were classified into 2 distinct subsets: Cluster I ($CD34^{low}$) and Cluster II ($CD34^{high}$). Patients in Cluster I could be further subdivided into Cluster Ia ($CD34^{low}HLA\text{-}DR^{low}$) and Cluster Ib ($CD34^{low}HLA\text{-}DR^{high}$). Similarly, Cluster II patients could be classified into two subsets: Cluster IIa ($CD34^{high}CD11c^{high}$) and Cluster IIb ($CD34^{high}CD11c^{low}$). The frequencies of *NPM1* and *FLT3-ITD* mutations were significantly higher in Cluster I patients [33.33% (35/105) and 31.43% (33/105)] than those in Cluster II patients [2.01% (3/149) and 2.01% (3/149)] (χ^2=47.491, *P*<0.001 and χ^2=45.642, *P*<0.001, respectively). Neither mutation was detected in the Cluster IIb patients. No associations were observed among *NPM1* (*P*=0.531) and *FLT3-ITD* (*P*=0.563) mutations and monocytic morphology in patients without CD34 expression.
Summary and Conclusions: *NPM1* and *FLT3-ITD* mutations are associated with signs of cell morphology and differentiation in AML. The absence of *NPM1* mutations in the immature subset Cluster IIb ($CD34^{+}CD11c^{-}$) patients in this study supports the hypothesis that the cell origin of the *NPM1* mutations is a common myeloid progenitor. Further studies are required to identify similar possible correlations for *FLT3-ITD* mutations.

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IMPACT OF DAY 14 BONE MARROW EXAMINATION ON RE-INDUCTION DECISION AND PREDICTION OF COMPLETE RESPONSE IN ACUTE MYELOGENOUS LEUKEMIA (AML)

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Background: The decision to re-induce patients with acute myeloid leukemia (AML) based on results of the day 14 bone marrow (BM) examination is variable and has limited evidence based data. In adult AML a variety of clinical and biological parameters have been examined for their potential value in predicting treatment response. Early response to induction therapy could be an important prognostic factor in this disease.
Aims: The aim of this study was to evaluate the accuracy of a day 14 BM examination in determining the need for re-induction chemotherapy and prediction of complete response.
Methods: Eighty-four patients with newly diagnosed AML treated with standard induction chemotherapy were retrospectively reviewed for the purpose of evaluating treatment decisions based on their day 14 BM biopsy from 2000-2012. Response to therapy in this analysis was based on morphology alone.
Results: Of the 84 patients undergoing standard induction, 65 patients (77%) had hypocellular bone marrow with less than 5% blast on their day 14 examination. Thirteen patients (16%) had definitive residual disease (RD), and 6 patient's (7%) were classified as indeterminate response (IR). Sixty three out of 65 patients with hypocellular bone marrow on day14 had complete response on day 28 bone marrow examination and 2 patients had residual disease on day 28 bone marrow and these 2 patients received re-induction on day 14. Patients with suboptimal response (SOR) (SOR=IR+RD), 2/13 patients with RD underwent re-induction chemotherapy on day 14. Seventeen patients with SOR (6 with IR and 11 with RD) were observed until count recovery without any re-induction therapy. All 6 patients with IR and 8 out of 11 patients with residual disease who were observed without re-induction eventually attained a morphologic complete remission (CR) on day 28.
Summary and Conclusions: Day 14 BM examination may have suboptimal sensitivity for the detection of residual leukemia in AML patients. Some patients with an indeterminate response and residual disease on day 14 may not require re-induction chemotherapy, but instead, may benefit from careful observation until count recovery to avoid the morbidity and mortality associated with prolonged bone marrow suppression related to early re-induction chemotherapy.

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ADVERSE PROGNOSTIC SIGNIFICANCE OF FLT3 MUTATIONS IN ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Internal tandem duplication (ITD) of *Fms-like tyrosine kinase-3* (*FLT3*) gene and point mutation in its activation loop (*FLT3*-D835) are frequent aberrations in acute promyelocytic leukemia (APL), but their prognostic value is not well established.
Aims: To evaluate prognostic value of *FLT3* gene mutations in *de novo* APL patients.
Methods: *FLT3*-ITD and *FLT3*- D835 mutations were assessed by PCR and PCR-RFLP method respectively in 45/61 newly diagnosed APL patients, on DNA isolated fromdiagnostic bone marrow aspirates or smears. The presence of t(15;17)(q22;q12) was assested using cytogenetics or RT-PCR method. All of the patients (median age 44 years, range 19-78; female/male 32/29; median follow-up 32 months) were managed at the Clinic of Hematology from 2004 to 2012 with all-trans retinoic acid and anthracycline-based chemotherapy.
Results: Material for *FLT3 gene* mutations analysis was available in 45/61 (75%) patients. *FLT3*-ITD was detected in 11/45 (24.4%) and *FLT3*- D835 in 4/45 (8.9%) patients. *FLT3*-ITD was associated with significantly higher WBC count (median 28.4×10^9/L *vs.* 1.4×10^9/L, P=0.0022), higer percentage of peripheral blood blasts (median 19% *vs.* 4%, P=0.042), lower platelet count (median 21×10^9/L *vs.* 32×10^9/L, P=0.029) higher relapse-risk score (low risk 1/11 (9%), intermediate 3/11 (27%), high 7/11 (64%) *vs.* 12/34 (35%), 10/32 (30%), 12/34 (35%) respectively, P=0.034), more frequent microgranular subtype (3/11 (27%) *vs.* 2/34 (5.9%) P=0.0001), CD2 expression (5/9 (55.5%) *vs.* 5/30 (16.7%) P=0.0007), CD56 expression (3/9 (33%) *vs.* 1/12 (8%), P=0.047) and thrombotic events (4/11, (36%) *vs.* 5/34, (15%), P=0.025). No correlation was found with age, fibrinogen, prothrombin time, activated prothrombin time, D-dimer, International Society of Thrombosis and Haemostasis Scoring System for diseminated intravascular coagulation (ISTH DIC score), expression of the other surface markers, additional cytogenetic abnormalities and incidence of differentiation syndrome. In contrast, *FLT3*-D835 was not significantly associated with any hematologic characteristic. Early death

(ED) rate in our series was 8/45 (17.78%). ED rate was significantly higher (P=0.002) in patients with *FLT3*-ITD (4/11, 36%) than in patients without mutation (4/34, 11.7%). Furthermore, multivariate analysis identified WBC count >20×10^9/L (*P*=0.002337), ISTH score ≥6 (P=0.008) and *FLT3-ITD* (*P*=0.02) as independent prognostic factors for ED. Seven relapses (7/37, 18.9%, 5 overt and 2 molecular) occured, three (3/7, 42%) in *FLT3*-ITD positive cohort and four (4/30, 13%) in *FLT3*-ITD negative cohort. Median time from the achievement of complete remission to relaps was 30 months (range: 4–44). *FLT3*-ITD positive patients had significantly lower 4-year RFS than *FLT3*-ITD negative ones (56% *vs.* 77%; *P*=0.002). However, multivariate analysis identified only WBC count >20×10^9/L as an independent predictive factor for relapse (P=0.05). Besides, 4-year overall survival (OS) was significantly lower in *FLT3-ITD* positive patients (62% *vs.* 87%, P=0.016) too. In contrast FLT3- D835 mutation had no impact on both 4-year RFS and OS.
Summary and Conclusions: Our study speaks in favor of negative prognostic value of *FLT3*-ITD mutation in APL. Further prospective trials should 1) investigate independence of FLT3-ITD predictive value and 2) evaluate whether this parameter might be included in risk stratification.

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MINIMAL RESIDUAL DISEASE BY WT1 GENE EXPRESSION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AFTER INDUCTION AND CONSOLIDATION CHEMOTHERAPY: CORRELATION WITH MULTIPARAMETER FLOW CYTOMETRY

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Background: Evaluation of minimal residual disease (MRD) in early stages of treatment can refine the risk stratification of patients with acute myeloid leukemia (AML).
Aims: The objective of this study was to analyze the MRD detection by of the Wilms tumor (WT1) gene expression after induction and consolidation chemotherapy in patients with AML and to correlate the results with those obtained by multiparametric flow cytometry (MFC).
Methods: Between 2007 and 2012, 65 patients were diagnosis of AML in our institution. Twenty patients were excluded due to earlydeath, lack of intensive treatment, promyelocyticleukemiadiagnosis or absence of available data. Therefore, 45 AML patients were included in the analysis (Table 1a). WT1 expression was quantified in bone marrow (BM) samples at diagnosis, after induction and after consolidation therapy, using real-time quantitative PCR (relative quantification with K562 cell line as calibrator and GUS as reference gene). The cutoff value for overexpression was set at 0.55% in BM. The presence of subpopulations associated with leukemia aberrant immunophenotype (LAIP) was analyzed by MFC with 4 colors (Beckman Coulter FC500). Residual disease was reported as positive above 0.1%.

Table 1.

Tabla 1a Caracteristics of Patients

Patients (N)	45
Age, Median (range)	50 (18-75)
Gender M/F	29/16
Classification (FAB)	M1=12; M2=7; M4=11; M5=6; Displasia=9
Cytogenetic Risk	Favorable=2; Intermediate=31; Unfavorable=6
WT1 overexpression at diagnosis	39 (86%)
CMF (LAIP) at diagnosis	36 %= Optimal LAIP 8 = Suboptimal LAIP 1 = Whithout LAIP
Induction Treatment*	IA 3x7 = 40 (88%) Reduced IA = 5 (11%)
Consolidation Treatment	IA 3x7 = 36 (80%) Reduced IA = 3 (6%) I-FLAG = 3 (6%) HDAC = 3 (6%)

* Reinduction treatment 6 patients

Table 1b. Correlation between WT1 overexpression and MFC for MRD detection after induction (A) and consolidation (B) therapy

(A)		MFC*		
		POS	NEG	
WT1*	POS	3	0	3
	NEG	9	12	21
		12	12	24

(B)		CMF**		
		POS	NEG	
WT1**	POS	4	0	4
	NEG	4	15	19
		8	15	23

*Not available MFC 2 patients / WT1 13 patients
**Not available MFC 5 patients / WT1 11 patients

Results: Out of the 45 patients, 39 (86%) showed WT1 overexpression at diagnosis. No correlation was observed between WT1 overexpression and clinical characteristics such as age, gender, FAB classification and cytogenetic risk at diagnosis. In 1 patient no LAIP could be identified and 8 more patients showed suboptimal LAIPs for follow-up. The post-induction and post-consolidation analysis showed MRD by both WT1 and MFC in 3 and 4 patients, respectively (Table 1b). No patients were positive by WT1 and negative by MFC either post-induction or post-consolidation. Finally, 9 patients post-induction and 4 post-consolidation showed positive MRD by MFC without WT1 overexpression. Among these patients 2 showed suboptimal LAIPs for follow-up. The limited number of patients in the present series as well as the heterogeneity of post-consolidation subsequent treatments does not allow testing the clinical utility of MRD results.
Summary and Conclusions: Overexpression of WT1 in the present series of AML patients is similar to that reported in the literature. In most cases, MRD positivity by WT1 overexpression correlates with that of MFC. Although clinical value of these results could not be tested in the present study, WT1 expression would be especially useful in patients without other molecular markers and those with suboptimal or without LAIP. Further analysis with a larger cohort of patients is needed to determine the true prognostic value of this MRD approach.

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SERUM FERRITIN AS A PROGNOSTIC MARKER IN YOUNG ADULT ACUTE MYELOID LEUKEMIA

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Background: Different studies have demonstrated an iron overload contribution to post-transplantation liver toxicity, infectious events and poor survival in patients undergoing hematopoietic stem cell transplantation for haematological malignancies. So far, in the clinical setting of adult acute myeloid leukaemia (AML) there is no evidence of the possible role of iron in response and survival rates.
Aims: Our aim was to study the role as a prognostic factor of pre-treatment serum ferritin in young adult AML.
Methods: The study sample included 77 consecutive adult *de novo* AML patients (38 males and 39 females, median age 55 years, range 16 to 60 yrs). The serum ferritin level was determined at onset of the disease in each case. According to the FAB criteria the subtypes were: 7 M0, 43 M2, 16 M4, 6 M5, 5 M6. M3 subtypes were excluded from the analysis. NPM, FLT3 and cytogenetic evaluation was performed for all cases. The NPM mutation was present in 35 patients (45%) and 34 (44%) harboured the FLT3 alteration. Twenty-five (32%) patients were in the adverse, ten (13%) in the favorable and forty-two (55%) in the intermediate molecular-cytogenetic risk group. The patients were subdivided into two groups according to serum ferritin values (less than 800 *versus* greater than 800 ng/mL). Student t-test or the Mann-Whitney test was performed for comparisons of means. Two-tailed Fisher exact test exact test was used to compare categories. Overall survival (OS) was measured from the time of diagnosis to death or last follow-up visit and was calculated using the Kaplan-Meier method; the log-rank test was used to compare survival curves. Logistic regression was performed for multivariate analysis. Only p values less than 0.05 were considered statistically significant.
Results: Thirty (39%) patients showed a ferritin serum value greater than 800 ng/mL. Compared with the less than 800 ng/mL group, patients with serum ferritin grater than 800 ng/mL were more frequently non responders to chemotherapy (29 *vs* 58%, P=0.03) and they had a shorter median OS (250 *vs* 665 days, P=0.04). Moreover, patients with serum ferritin greater than 800 ng/mL showed a higher frequency of documented infections during induction treatment (48% *vs* 16%, P=0.004). At multivariate analysis, NK-FLT3ITD+,NK-NPM, Cytogenetic and Ferritin value (less than or greater than 800 ng/mL) all showed a statistical correlation with the response rate (P=0.04; P=0.05; P=0.04; P=0.05, respectively).
Summary and Conclusions: The results of our study suggest a link between serum ferritin and AML prognosis. Further studies are needed to confirm the utility of serum ferritin as a prognostic marker in the adult acute myeloid leukaemia setting.

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PERIPHERAL BLOOD BLASTS ON DAY 7 OF INITIAL INDUCTION THERAPY PREDICT RESPONSE TO CHEMOTHERAPY AND SURVIVAL IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Cytogenetics and molecular markers are powerful prognostic indicators for outcome of patients with acute myeloid leukemia (AML). However, predictors of response after initiation of chemotherapy are not available. A rapid decrease in blood blasts is often considered a favorable indicator of response in patients with AML who receive induction chemotherapy. Time of peripheral blood

blast (PBB) clearance has been reported to be a predictor for complete remission (CR) and survival of AML patients, but litter attention was paid to the correlation between response, outcome of AML and PBB percentage.
Aims: To explore the associations between chemotherapy response, outcome of AML and PBB percentage on day 7 of the initial induction therapy.
Methods: In this study, 46 consecutive *de novo* AML (excluding acute promyelocytic leukemia) patients treated with standard '3+7' induction course were evaluable for bone marrow (BM) response on day 14 and day 28. Flow cytometry was carried out to identify the percentage of cells with leukemia-associated aberrant immuno-phenotype (LAIP) in each patient from the initial BM aspirate. LAIP-positive blast percentage was determined on peripheral blood on day 7 of the induction therapy. Patients gave informed consent prior to enrolment in the study. Statistical analysis was performed with the software SPSS 17.0.
Results: PBB percentage on day 7 (PBBPD7) was 0.35% (0.0%, 80.74%) in all patients, and in those achieved CR, the percentage was 0.03% (0.0%, 0.45%), which was significantly lower than those did not achieve CR (10.85% [1.13%, 19.38%]) (u=- 3.92, P<0.001). PBBPD7 was significantly correlated with CR rate of the first course (r=0.584, P<0.001). The cutoff value was 0.945% (area under curve [AUC]=0.84, sensitivity [Se]=78.9%, specificity [Sp]=81.5%, P<0.001). The CR rate of patients with PBBPD7 ≤0.945% (84.62%, 22/26) was significantly higher than those with PBBPD7 >0.945% (25.0%, 5/20) (χ^2=16.571, P<0.001). PBBPD7 was significantly correlated with overall survival (OS) (r=- 0.437, P=0.003) and relapse free survival (RFS) (r=- 0.388, P=0.007). The cutoff value was 0.43% for both OS (AUC=0.723, Se=73.4%, SP=71.4%, P=0.009) and RFS (AUC=0.723, Se=66.7%, SP=69.2%, P=0.009). The OS and RFS were significantly higher in patients with PBBPD7 ≤0.43% compared with those with PBBPD7 >0.43% (P=0.002 and P=0.005, respectively). PBBPD7 was proved to be an independent prognostic parameter in multivariate analyses for both OS (P=0.036) and RFS (P=0.035).
Summary and Conclusions: Peripheral blood blast percentage on day 7 of the induction therapy may be an important predictor of early response to initial chemotherapy and long-term survival for AML.

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PROGNOSTIC VALUE OF TH17 CELLS IN ACUTE LEUKEMIA
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Background: The net outcome of the battle between the anti-tumor and the tumor promoting immune cells and their associated cytokines within the tumor environment will determine the ultimate fate of the affected tumors. Th17 cells and their effector cytokines have emerged as important mediators in inflammatory and autoimmune diseases and serve as an ambitious field in current immunology research. Recent studies suggest a potential impact of Th17 cells on solid tumors but relatively little is known about their contribution in hematological malignancies.
Aims: The current study was designed to investigate the possible involvement and clinical significance of circulating Th17 cells in acute leukemia
Methods: Flow cytometry was used to analyze percentages of Th17 cells in peripheral blood from 93 acute leukemia patients and 40 controls
Results: Compared with healthy controls; acute leukemia patients had a higher proportion of circulating Th17 cells. Furthermore, increased serum concentrations of IL-17 and IL-21 accompanied the increased Th17 cell levels in these patients. Circulating Th17 cells were reduced when patients achieved complete remission (CR) after chemotherapy, suggesting that circulating Th17 cells can be used to evaluate response to therapy in acute leukemia patients. Notably, the increased prevalence ofinitial Th17 cells was significantly associated with probability of achieving CR as well as with longer overall survival of acute leukemia.
Summary and Conclusions: These results strongly suggest that Th17 cells may be a powerful new prognostic determinant which could serve as a potential therapeutic target to modulate anti-tumor response in acute leukemia. Mechanisms by which Th17 cells influence acute leukmia, and whether these mechanisms mediate direct or indirect effects need to be determined.

Chronic lymphocytic leukemia - Immune triggering and microenvironmental interactions

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BCR AND TLR ACTIVATION OF AGGRESSIVE CHRONIC LYMPHOCYTIC LEUKEMIA: THE CASE OF STEREOTYPED SUBSET 1
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Background: Chronic lymphocytic leukemia (CLL) B cells express auto/xeno-antigen-reactive immunoglobulin receptors that bind to self-epitopes, such as oxidation-modified low-density lipoprotein (oxLDL), and certain microbial structures. Chronic infections are hence possible triggering events in the pathogenesis of CLL. In addition to B cell receptor (BCR) crosslinking, interaction with the microenvironment through other receptors such as innate Toll-like receptors (TLRs) could be important to promote CLL cell survival and proliferation.
Aims: In this study we ought to get insight in oxLDL binding, and to study the outcome of BCR and TLR co-stimulation on activation and/or proliferation of subset 1 CLL cells.
Methods: We used primary CLL cells from eight patients belonging to the poor-prognostic stereotyped subset 1 (*Clan I IGHV genes-IGKV1(D)-39),* and seven non-subset 1 patients. The specificities of IgM Abs from six different subset 1 patients were evaluated in competition chemiluminescent ELISA. Furthermore, binding of oxLDL to cells from five subset 1 and four non-subset 1 CLL patients were studied in flow cytometry. To investigate the effect of BCR and TLR co-stimulation on activation and/or proliferation, cells from 8 subset 1 CLL patients and 2 non-subset 1 CLL patients were either unstimulated or stimulated with oxLDL (BCR) alone or in combination with the TLR9 ligand CpG-oligodeoxynuclotide (ODN). Activation of CLL cells was evaluated using kinetic measurements, during 5 min, of intracellular Ca^{2+} release and surface expression of the activation markers CD25 and CD86 after 24h. Proliferation was measured with BrdU-incorporation after 72h stimulation.
Results: All six CLL Ab clones showed specificity for oxLDL. Interestingly oxLDL not only bound to subset 1 CLL cells but also to non-subset 1 cells indicating that binding to other oxLDL (scavenger) receptors may also occur. In none of the subset 1 cases was antigen alone sufficient to induce Ca^{2+}mobilization, proliferation, or CD25 expression. Interestingly however, uP-regulation of the co-stimulatory ligand CD86 could be seen in 2/2 patients studied. CpG-ODN induced proliferation in all but 1 subset 1 CLL cases, dual stimulation with oxLDL and CpG-ODN however, gave a heterogeneous outcome. 4/7 responding patients showed an increase in proliferation compared to single stimulated cells, and 2/7 showed suppression of proliferation. Addition of the TLR inhibitor chloroquine or a Syk-inhibitor totally abrogated the oxLDL-CpG induced proliferation, indicating BCR/TLR collaboration.
Summary and Conclusions: Taken together, we document that BCR-to-self-antigen trigger may act in synergy with TLR signaling for activation and proliferation of clonal CLL cells in some patients.

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THE PHOSPHO-PROTEOMIC PROFILE OF CD49D-EXPRESSING CIRCULATING CHRONIC LYMPHOCYTIC LEUKEMIA CELLS IS CONSISTENT WITH CONSTITUTIVE RECEPTORIAL ENGAGEMENT BY BLOOD-BORNE LIGANDS
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Background: CD49d is a negative prognosticator in chronic lymphocytic leukemia (CLL) marking a subset of ~40% of CLL cases. CD49d, the a4 integrin subunit, is a key molecule for CLL cell microenvironmental interactions, through the binding to fibronectin and VCAM-1. Despite the great deal of studies investigating CLL cell microenvironmental interactions in tissue sites, little is known regarding the constitutive engagement of adhesion receptors in circulating CLL cells.

Aims: Defining the role of plasma and plasma components in the activation of circulating CLL cells.
Methods: The proteomic profiles of circulating CLL cells was explored using a reverse phase protein microarray (RPMA) approach. Phospho-proteins expression was validated by western blot assay. F-actin polymerization and apoptosis were evaluated by flow cytometry through phalloidin and AnnexinV/7-AAD staining, respectively.
Results: Comparison of the phospho-proteomic profiles between 40 CD49d+ and 40 CD49d- CLL cases highlighted the over-expression in the CD49d+ group of proteins involved in the regulation of integrin-mediated cytoskeletal dynamics, such as phospho-p21-activated kinase (pPAK; P=0.0005), phospho-LIM kinase (pLIMK; P=0.00001) and phospho-CrkII Tyr221 (P=0.039). The constitutive overexpression of these phospho-proteins in the CD49d+ CLL group, confirmed by western blot analysis, suggest that integrin signalling is active in CD49d-expressing circulating CLL cells, pointing to a constitutive receptor engagement in peripheral blood. To test whether plasma constituents could modulate integrin-signaling proteins, CD49d- and CD49d+ CLL cells, were challenged with autologous plasma. The presence of plasma induced a stronger increase of pPAK and pLIMK in CD49d+ CLL cells, compared to CD49d- cases (P=0.018 and P=0.017, respectively). Of note, pretreatment of CD49d-expressing CLL cells with the anti-CD49d HP1/2 blocking antibody, resulted in a lower uP-regulation of pPAK and pLIMK (P<0.01). Since PAK and LIMK are key players in the regulation of actin reassembly, we examined the effect of plasma stimulation in the actin polymerization. Addition of plasma induced actin polymerization in CD49d expressing CLL cells (P=0.004) but not in CD49d- CLL cells, this effect being impaired by the pretreatment with the anti-CD49d HP1/2 blocking antibody. ELISA assay on plasma samples from 24 CLL revealed the presence of high fibronectin level (mean concentration=336 ug/mL) and VCAM-1 (mean concentration=3.7 ug/mL) without substantial difference between CD49d- and CD49d+ cases. Given the high molecular weight of both fibronectin and VCAM-1 (>100 kDa) fractionation through filter devices was allowed, yielding plasma fractions enriched or not CD49d ligands. These plasma fractions, upon verification by western blotting for the presence/absence of fibronectin and VCAM-1, were used to culture CLL cells from 6 CD49d- and 6 CD49d+ cases. Only plasma containing CD49d ligands protected CLL cells from apoptosis and this effect was evident in CD49d+ group (P=0.03) and partially reverted by blocking CD49d activity.
Summary and Conclusions: Altogether these results sustain the hypothesis of an active role of plasma components in the activation of CD49d-mediated integrin pathway, thus favoring the delivery of pro-survival signals even in the context of circulating CLL cells. Our results may be of interest in the perspective of novel therapies, *e.g.* those targeting the B-cell receptor signalling, known to provoke a massive egress of neoplastic cells from tissue sites into the blood stream.

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IDENTIFICATION OF CIRCULATING CHRONIC LYMPHOCYTIC LEUKEMIA INTRACLONAL SUBGROUPS WITH VARYING B CELL RECEPTOR FUNCTION

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Background: Chronic lymphocytic leukemia (CLL) is a tumor of circulating B cells, with the clonal B-cell receptor (BCR) variably stimulated and downmodulated (anergized) following exposure to antigen in lymphoid tissues. However surface expression and signal capacity of the tumor BCR IgM is dynamic and can recover *in vitro*, and apparently *in vivo* during recirculation in the blood. Levels of surface IgM (sIgM) may be critical to tumor cell behavior and sensitivity to clinically relevant BCR-signalosome inhibitors.
Aims: We aimed to dissect individual tumor CLL clones by differential levels of sIgM. We then determined i) if IgM function and sensitivity to BCR-signalosome inhibitor ibrutinib varied and ii) if phenotypic characteristics changed depending on those levels.
Methods: A bead-bound anti-IgM assay was developed to discriminate and investigate subgroups (SGs) of CLL cells, defined by sIgM levels. SGs were determined by number of anti-IgM beads bound to cells. Single cells were visualized by imaging flow cytometry. Expression of Ig light chain, CD19, CD5, CD38, CD25 and CXCR4 and intracellular Ki67 were determined in SGs in flow cytometry. BCR signaling analysis of phosphorylated PLCgamma2 or ERK1/2 was determined by Phosflow in the presence or absence of BTK inhibitor ibrutinib.
Results: Four clear subgroups (SG1-4) with increasing sIgM were identified in 37/37 cases (Figure 1). Engagement of sIgM induced phosphorylation of PLCgamma2 and ERK1/2 at levels ranging from very low in SG1 to high in SG4. Remarkably, phosphorylation was suppressed by Ibrutinib. Expression of CXCR4 also increased from SG1 to SG4 but markers of activation (CD25 and CD38) and proliferation (Ki67) were dominant in SG1. Incubation of whole CLL populations *in vitro* led to striking increases in CXCR4 expression, as well as recovery of sIgM.

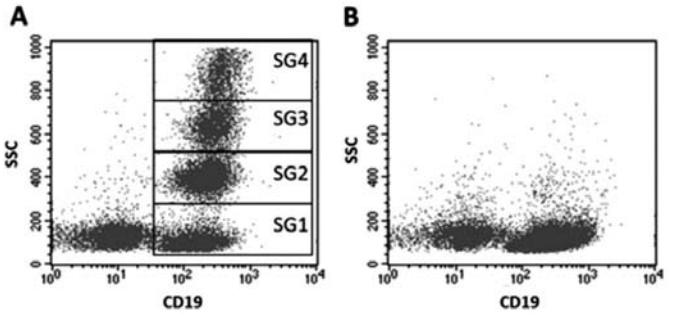

Figure 1. CLL clonal subgroups by anti-sigM coated beads separation. PBMCs were incubated either with goat F(ab')$_2$ anti-human IgM coated beads (panel A) or with isotype control coated beads (panel B). CLL subgroups (SGs) were identified within the CD19+ tumor population according to different side scatter (SSC).

Summary and Conclusions: Clonal analysis reveals dynamic SGs following presumed antigen stimulation in tissues. SG4 represents a fully recovered, potentially dangerous population equipped to migrate to tissue and to receive a proliferative stimulus. SG1 likely represents a post-mitotic unresponsive "resting" population. The suppressive effect of ibrutinib on the small SG4 population may be the critical factor in therapeutic success.

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AN UNBIASED SCREEN OF FUNGAL EXTRACTS IDENTIFIED CHAETOGLOBOSIN A AS A METABOLITE THAT PREFERENTIALLY INDUCES APOPTOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS BY TARGETING THE CYTOSKELETON

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Background: Chronic lymphocytic leukemia (CLL) is a malignancy of mature B cells that is very common in the western world and not curable with currently available treatment options. Even though the bulk of leukemic cells in the peripheral blood is eradicated upon treatment in most patients, relapse frequently occurs from cells that are located in secondary lymphoid tissues, often leading to chemoresistant disease.
Aims: In search for novel therapeutics that target CLL cells also in protective microenvironments, we performed a fungal extract screen using primary CLL cells in coculture with the bone marrow-derived stromal cell line HS-5, which is known to support long-term survival of CLL cells.
Results: One of the major hits of our screen was a metabolite of *Penicillium aquamarinium*, that was subsequently identified as Chaetoglobosin A, a member of the cytochalasin family. Extensive testing of the pure compound with primary blood cells of CLL patients or normal donors (Figure 1), leukemic cell lines, as well as a variety of different tumor and fibroblast lines revealed a preferential activity of Chaetoglobosin A for CLL cells (LD50 value of 2.8 µM compared to 9.5 µM for normal PBMC), which was also observed in culture systems that mimic the protective microenvironments of bone marrow and lymph nodes. The inclusion of 74 CLL cases with various genetic aberrations (del11q: n=14; del13q: n=44; del17p: n=12; trisomy12: n=11; mutBRAF: n=4; mutMYD88: n=2; mutNOTCH1: n=4; mutSF3B1: n=11; mutTP53: n=13) in our study revealed effective targeting of CLL cells by Chaetoglobosin A, also in cases harboring bad prognosis characteristics. To provide insight into its mechanism of action, we further showed that Chaetoglobosin A induces apoptosis in CLL cells and confirmed filamentous actin as its target. As the cytoskeleton is known to regulate B cell receptor (BCR) signalling, we hypothesized that apoptosis induction by Chaetoglobosin A is due to impairment of signalling cascades that deliver pro-survival signals in CLL cells. In support of that, our data show that Chaetoglobosin A alters activation-relevant molecules in CLL cells and sensitizes them for treatment with BTK or PI3K inhibitors, which are known to interfere with BCR signalling. The cytoskeleton changes induced by Chaetoglobosin A further impaired migration of CLL cells in transwell assays. In mice and rats, no severe toxicity was observed upon administration of relevant doses of Chaetoglobosin A. In ongoing experiments using CLL xenotransplantation as

well as Eμ-TCL1 mice, a genetic animal model for CLL, we are currently evaluating the efficacy of Chaetoglobosin A for CLL *in vivo*.

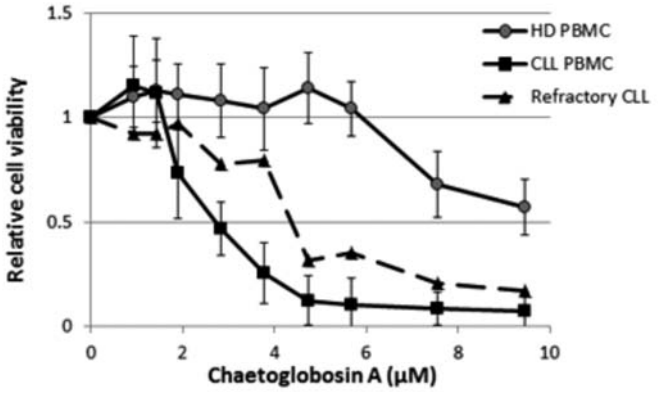

Figure 1. Viability of peripheral blood mononuclear cells (PBMC) isolated from blood samples of CLL patients (n=8) or healthy donors (HD; n=4) was measured by Cell Titer Glo assay after 24 hours of treatment with Chaetoglobosin A. Median values normalized to control samples and standard deviations are depicted.

Summary and Conclusions: In summary, our findings suggest Chaetoglobosin A as a novel potential drug for CLL that prevents pro-survival signalling and migration of leukemic cells.

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THE ELASTIN MICROFIBRIL INTERFACER1 (EMILIN1), IS HIGHLY EXPRESSED IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)-INVOLVED TISSUES, AND PROMOTES CLL CELL ADHESION VIA CD49D

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Background: CD49d is a negative prognosticator in chronic lymphocytic leukemia (CLL) with a key role in microenvironmental interactions. CD49d triggering by its main ligands VCAM-1 and CS-1 fragment of fibronectin activates pro-survival signaling pathways, and promotes resistance to drug-induced apoptosis in CLL. Recently, the globular (g) C1q-like domain of EMILIN1, an adhesive extracellular matrix constituent, represents a new ligand for CD49d and operates as a negative modulator of proliferation signals in non-hematopoietic cells (Danussi *et al.*, J Cell Biol, 2011).
Aims: Investigating the distribution of EMILIN1 in normal and CLL-involved tissues, and the effects of CD49d/EMILIN1 interaction in CLL in terms of adhesion and survival.
Methods: Immunohistochemical (IHC) detection of EMILIN1 in CLL-involved tissues was done utilizing a non-commercial anti-human EMILIN1 affinity purified rabbit polyclonal antibody (AS556). Adhesion assays were performed on coverslips coated with VCAM-1, the CS-1 fibronectin fragment, the gC1q-like domain of EMILIN1, and gC1q-like domain mutants devoided of segment Leu932–Gly945, that contain the Glu933 responsible for integrin interaction. Immunofluorescence (IF) and biochemical assays were used to study the activation of signaling proteins. Apoptosis was evaluated by annexinV/7-AAD staining.
Results: Exploratory IHC staining in reactive lymphoid tissues indicated an extracellular EMILIN1 specific reactivity in the outer zone of the mantle/marginal areas. IHC analysis in CLL lymph node tissues (n=3) and bone marrow biopsies (n=5) showed a clear EMILIN1 staining intermingled with the neoplastic component, in close proximity of venules, close to marginal sinuses and in CLL proliferation centers. In in-vitro CLL cell adhesion assay, both the CLL-derived CD49+ Mec-1 cell line and primary CD49d+ CLL cells (n=12) were specifically able to adhere onto EMILIN1 substrates (mean values of adhered cells/control 28±3 and 24±3, respectively), the adhesion efficiency being similar to that observed on VCAM-1 and CS-1. Adhesion was specifically blocked by pre-treatment with the anti-CD49d HP1/2 blocking antibody, and absent when the gC1q like domain mutant was employed. After short-term adhesion assay onto EMILIN1 substrates, western blot analysis documented increased phosphorylation of Akt and ERK1/2, mediators of survival signals, similar to that observed upon CD49d engagement by VCAM-1 and CS-1. These results were corroborated by IF analysis showing pAkt and pERK uP-regulation, and the concomitant increase of pVav1 and F-actin reorganization, confirming the activation of the integrin signaling pathway. Finally, we studied the effects of CD49d/EMILIN1 interaction on CLL cells survival. CD49d engagement by both EMILIN1 and VCAM-1, were able to significantly delay the CLL cells spontaneous apoptosis (P=0.002 and P=0.009, respectively), and the viability obtained on EMILIN1 was even significantly higher than that observed on VCAM-1 (P=0.005).
Summary and Conclusions: For the first time we showed that EMILIN1 is present in normal and CLL-involved tissues, and it is able to efficiently bind to CD49d, as expressed by CLL cells. At variance of what demonstrated in non-hematopoietic models, EMILIN1 was able to deliver anti-apoptotic/pro-survival signals to circulating CLL cells. CD49d/EMILIN1 interactions may have a role in the maintenance of the neoplastic clone in CD49d-expressing CLL.

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SINGLE CELL NETWORK PROFILING (SCNP) ANALYSIS REVEALS DYSFUNCTIONAL SIGNALING WITHIN THE TH17 AXIS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: Chronic lymphocytic leukemia (CLL) cells obtain pro-survival signals from non-tumor leukocytes such as T cells and monocyte-derived nurse-like cells. Recent reports demonstrate that increasing numbers of Th17 cells, a subset of $CD4^+$ T cells that can be induced by TLR agonists, IL-1b, and IL-6 and express IL-17A and IL-17F as effector cytokines, correlates with improved prognosis in CLL. The functional basis for this correlation, including how these factors in the Th17 axis affect signaling in CLL, is unknown. SCNP is a multi-parametric flow cytometry based technology that quantifies signaling in multiple intracellular pathways at the single cell level, without prior cell subset isolation, providing a means to interrogate the signaling interplay of Th17 cells with B-CLL cells and the tumor microenvironment.
Aims: Using SCNP, investigate signaling in the Th17 axis in peripheral blood mononuclear cell (PBMC) samples to identify a functional role for Th17 cells in CLL pathophysiology.
Methods: SCNP was performed on PBMCs from 22 CLL patients and 8 healthy donors, examining signaling in $CD4^+$ and $CD4^-$ T cells and $CD14^+$ monocytes in both groups as well as $CD19^+$ B cells in healthy donors and $CD19^+CD5^+$ cells in CLL patients. Induction of intracellular signaling in the NFkB (IkB, P-NFkBp105), PI3K (AKT), MAPK (P-ERK), and STAT (P-STAT1, P-STAT3) pathways was examined in response to TLR agonists and cytokines involved in Th17 differentiation and effector function.
Results: Engagement of TLR7/8 (R848) or of TLR9 (CpG-B) resulted in reduced IkB degradation in CLL B cells and monocytes compared to healthy cells. This reduced responsiveness was associated with lower basal levels of IkB in CLL. The magnitude of TLR9-induced IkB degradation in CLL B cells significantly correlated with Th17 numbers, suggesting that TLR activation induces factors (*e.g.* IL-1b, IL-6) triggering Th17 differentiation. Upon IL-1b modulation, CLL $CD4^+$ and $CD4^-$ T cells displayed greater phosphorylation of NFkBp105 than healthy cells. IL-6 also induced greater phosphorylation of STAT3 relative to STAT1 in $CD4^+$ but not $CD4^-$ CLL T cells. Because STAT3 promotes whereas STAT1 inhibits Th17 differentiation, these data suggest that CLL $CD4^+$T cells have dysregulated signaling pathways that favor Th17 generation. Moreover, analysis of Th17 effector cytokines identified IL-17F but not IL-17A induced NFkBp105 phosphorylation only in CLL B cells whereas no response was detected in healthy B cells. In addition, combination of IL-17F with BAFF, a factor that regulates CLL B cell survival, led to ERK and AKT phosphorylation only in CLL B cells. Although significant differences in IL-17F modulated signaling between CLL and healthy B cells were identified, IL-17RA and IL-17RC receptor expression were comparable between CLL and healthy cells, demonstrating that analysis of modulated signaling reveals functional alterations not revealed by surface phenotyping.
Summary and Conclusions: Collectively, these data identify multiple novel alterations in Th17 axis signaling in CLL, providing a functional basis for the role of Th17 cells and IL-17 cytokines in CLL pathophysiology. The application of SCNP to further explore these signaling differentials, and their potential as novel prognostic factors in CLL, is in progress.

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TOSO-DEFICIENT B CELLS SHOW IMPAIRED B CELL DEVELOPMENT

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Background: In the recent years, TOSO–alias FAS apoptosis-inhibitory molecule 3 - has been controversially discussed whether it exerts anti-apoptotic effect or is the long-sought-after bona fide Fcμ-receptor.
Aims: Since our group previously identified TOSO to be overexpressed in CLL and to be correlated with progressive disease, it is indispensible to clarify the biologic significance of TOSO, particularly in the CLL relevant B cells.
Methods: Therefore, we generated a B cell-specific knockout mouse model and crossbred FAIM3-floxed C57BL/6 mice with CD19-specific Cre recombinase expressing mice. B cells from the *toso*$^{CD19-/-}$ (KO)micewere isolated and gene expression was analyzed via mRNA based Illumina microchip array. Convincing results were verified by flow cytometry and blood count was carried out in addition.
Results: Peripheral blood and spleens of the TOSO deficient mice displayed decreased level of lymphocytes, in which B cells were determined as reduced entity. Other cell types, like NK and T cells, remained thereby unaffected.
Downstream effects of TOSO were validated via microarray-based gene expression analysis. Results displayed a clear clustering of deregulated genes compared to controlmice. Nearly 500 genes showed expression alterations. Genes involved in the NF-κB pathway and migration processes were downregulated in *toso*$^{CD19-/-}$ mice, suggesting that TOSO represents an important factor in these processes. Flow cytometry analysis confirmed these results and additionally referred to an impaired B cell maturation process.
Summary and Conclusions: Thus, our results might reveal a new function of TOSO in migration, pro-survival signaling and B cell homeostasis, supporting the anti-apoptotic feature of TOSO in B cells.

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EXPRESSION OF ZAP-70 IN CLL ACTIVATES NF-KB SIGNALING

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Background: The clinical course of chronic lymphocytic leukemia (CLL) is variable, but can be predicted by prognostic markers. Two frequently used prognostic markers include the mutation status of the immunoglobulin variable heavy chain genes (IgVH) and the expression level of the zeta-associated protein of 70 kDa (ZAP-70). The clonal excess of CD5+, CD19+ CLL cells is thought to be due to a defect in both apoptosis and cell proliferation.
Aims: Although the exact mechanisms responsible for this inappropriate cell growth and survival are poorly understood, one of the key factors that has been related to the biology of CLL cells is the constitutive activation of nuclear factor-KB (NF-KB). Since NF-KB is a downstream target of the B-cell receptor (BCR) signaling in normal B lymphocytes and since ZAP-70 might directly enhance IgM signaling, we wondered if ZAP-70 affects NF-KB target gene expression following IgM stimulation.
Methods: We introduced a functional ZAP-70 protein in CLL cells with undetectable ZAP-70 expression by electroporation of *in vitro* transcribed capped mRNA that is translated in the cell into ZAP-70 protein (Van Bockstaele F. *et al.* Leukemia 2007). After electroporation, cells were stimulated with anti-IgM-polyacrylamid beads (anti-IgM stimulated) to activate the BCR signaling pathway and to activate the introduced ZAP-70 protein. As a negative transfection control we electroporated mRNA coding for ΔNGFR or we used uncapped mRNA that cannot be translated in the cell, anti-IgA-polyacrylamid beads (anti-IgA stimulated) were used as a negative control for stimulation. We determined the mRNA expression profile using a Human Illumina Gene Expression BeadChip and measured interleukin (IL) expression levels in CLL cells differing in ZAP-70 expression.
Results: In samples only differing in ZAP-70 expression after exogenously introducing ZAP-70 in ZAP-70 negative CLL samples, expression of IL-1β, IL-6 and IL-8 after 24 hours of IgM stimulation was increased in the presence of ZAP-70 (Figure 1). The observed increased mRNA levels were translated into increased amounts of secreted proteins. We also observed similar enhanced cytokine expression in cells of ZAP70+ cases compared to of ZAP70- cases, after 24 hours of IgM stimulation. The enhanced cytokine expression in ZAP-70 expressing cells could be a NF-KB mediated downstream event of BCR triggering. To test this hypothesis BMS-345541, a selective inhibitor of the catalytic subunit of IKK, was used. As expected, without inhibitor ZAP-70 expression increased endogenous cytokine expression. This induction of cytokines in ZAP-70 expressing CLL cells was completely blocked upon inhibition of IKK. Furthermore, we analyzed by whole genome expression profiling the genes affected by ZAP-70 in BCR stimulated CLL cells. By functional annotation pathway analysis, we found the gene set most significantly upregulated after 24h to be associated with RelA activation, RelA being one of the five members of NF-KB transcription factors. This suggests that ZAP-70 indeed enhances NF-KB signaling upon BCR signaling. This signature fits in a functional network of genes involved in cell movement and immune cell trafficking.

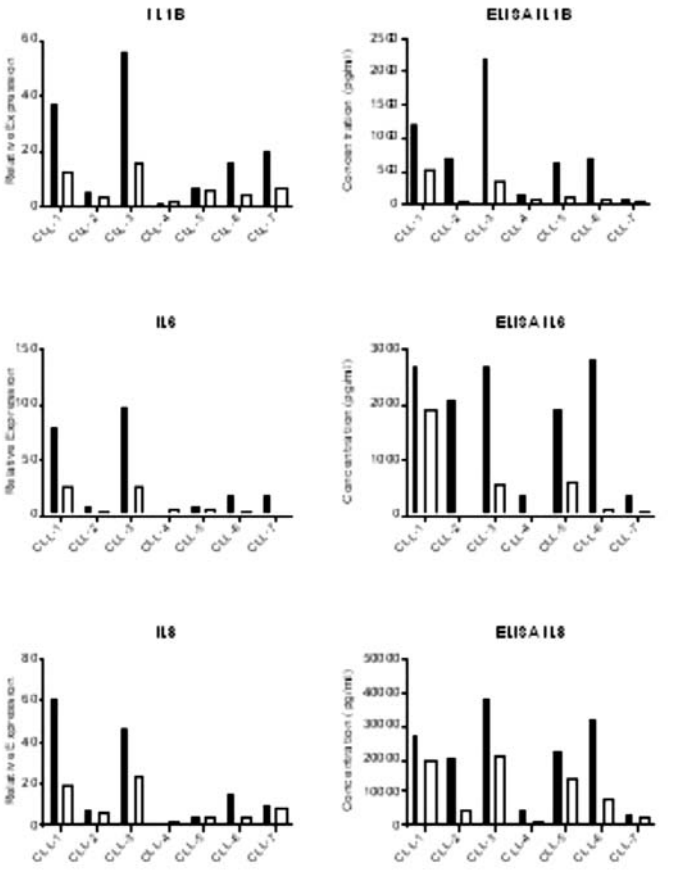

Figure 1. Expression of ZAP-70 enhances cytokine expression up on IgM stimulation, on mRNA level (left) and protein level (right). Black bars: ZAP-70 electroporated; open bars ΔNGFR control electroporated cells.

Summary and Conclusions: By enhancing the NF-KB pathway, ZAP-70 expression can contribute to increased motility, survival and proliferation of CLL cells and thus contributes to the worse prognosis observed in these patients. More than simply a correlate with prognosis of CLL, ZAP-70 therefore potentially has a direct pathognomonic role in the disease. Identification of the molecular pathways involved requires more research, what could lead to the identification of novel therapeutic targets.

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THE PRO-APOPTOTIC MIR-132~212/SIRT1/TP53 AXIS IS ACTIVATED UPON B-CELL RECEPTOR TRIGGERING IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background: Numerous studies indicate a prominent role for the B-cell receptor (BCR) pathway in defining the patho-biological features of chronic lymphocytic leukemia (CLL). MicroRNAs (miR) represent a class of small non-coding RNAs that, by degrading selected mRNAs or inhibiting their translation, modulate key cellular functions. Altered miR expression in CLL by genetic abnormalities or microenvironmental interactions has been reported, but nothing is known regarding their modulation upon BCR triggering.
Aims: To investigate the expression and function of microRNAs modulated by BCR triggering in CLL.
Methods: Negatively selected CLL cells were stimulated in-vitro (20 hours) with immobilized anti-IgM (aIgM) and analysed by miRNA/gene expression profiling (miRome/GEP), quantitative real-time PCR (qRT-PCR) and western blotting. All CLL cases were characterized for IGHV gene mutation status and common genetic lesions, including *TP53* mutations, by FISH and/or sequencing analyses.

Results: Supervised analysis of miRome of 16 aIgM-stimulated or unstimulated CLL samples (9 IGHV unmutated, UM; 7 IGHV mutated, M) showed substantial upregulation of the *miR-132~212* cluster. This phenomenon was validated by qRT-PCR in additional 28 cases in which a mean 19.5-fold increase (range 1.5-50; P<0.0001) was documented upon aIgM exposure. No differences were observed between UM- and M-CLL samples. UP-regulation of *miR-132~212* required sustained BCR signaling, as demonstrated by the following observations: i) it occurred upon exposure of CLL cells to immobilized but not soluble anti-IgM antibodies; ii) it was abrogated by co-treating CLL cells with the SYK inhibitor R406. An in-silico analysis aimed at identifying candidate target genes for *miR-132~212* revealed responsive elements in the 3'UTR of *SIRT1*, a gene encoding for a histone deacetylase that targets several proteins including TP53. This finding was experimentally validated by western blot analysis of 9 CLL samples (5 UM-, 4 M-CLL), where SIRT1 levels were reduced by 13% (range 5-23%) upon aIgM-treatment (P=0.001). A significant increase in TP53 acetylation was also observed in the same cases (mean increase 30%, range 3-82%; P=0.0072). Notably, GEP analysis of the 16 CLL samples investigated in the miRome analysis identified both *TP53* and the TP53 target gene *CDKN1A* among the 917 toP-ranked genes uP-regulated in aIgM-stimulated CLL. We also evaluated the constitutive expression of *miR132* by qRT-PCR in highly purified CLL cells from a cohort of 134 CLL cases. The obtained values were of similar magnitude to those obtained in the first series of anti-IgM-stimulated CLL samples, and spanned from negative values to values comparable to those observed upon aIgM exposure. By using the median value as cut-off to discriminate miR132-high from miR132-low cases, miR-high CLL cases unexpectedly displayed a longer time-to-first-treatment interval than miR132-low cases (P=0.02). By correcting for IGVH mutation status, *miR-132* levels above the established cut-off retained a favorable prognostic impact in M-CLL (P=0.005), but not UM CLL patients (P=0.968).
Summary and Conclusions: We describe an inter-chained cascade of events sequentially characterized by BCR triggering, *miR-132~212* uP-regulation, SIRT1 down-regulation and TP53 uP-regulation/acetylation. In addition, we show that high *miR132* expression is associated with a longer time-to-first treatment in M CLL patients. The activation of this pro-apoptotic axis should be considered in the light of emerging drugs targeting the BCR pathway in CLL.

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HETEROGENEOUS FUNCTIONAL EFFECTS OF CONCOMITANT B CELL RECEPTOR AND TOLL-LIKE RECEPTOR STIMULATION IN CLL WITH MUTATED VERSUS UNMUTATED IMMUNOGLOBULIN GENES

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Background: Antigenic stimulation through the B cell receptor (BcR) is critical for CLL development and evolution. However, significant differences in responsiveness to BcR crosslinking are observed among cases with similar sIgM levels, suggesting that additional factors may be implicated in shaping the functional outcome. Support for this notion emerged from studies showing that CLL cells may also crosstalk with their microenvironment via non-BcR mediated modalities, including the TLRs.
Aims: We recently reported that CLL subgroups with distinct clonotypic BcRs present discrete patterns of TLR expression, function and/or tolerance. Here, to explore whether specific types of BcR/TLR collaboration exist in CLL, we studied the effect of single *vs.* dual BcR and/or TLR stimulation on different CLL subgroups.
Methods: We stimulated negatively isolated CLL cells from 11 mutated (M-CLL) and 10 unmutated CLL (U-CLL) cases with anti-IgM, Imiquimod (IM) and CpG ODN for BcR, TLR7 and TLR9 stimulation, respectively, for15, 50 and 60 minutes and, for apoptosis studies, also for 48 hours. Cultures were established under the following conditions: (1) non-stimulated; (2) single BcR, TLR7 or TLR9 stimulation; (3) dual BcR/TLR7 or BcR/TLR9 stimulation.
Results: After *in vitro* culture in the absence of stimulation at any time point, differences in constitutive phosphorylation of ERK (pERK) were identified, with higher pERK levels in U-CLL *vs.* M-CLL. In single-stimulated cultures, the great majority of cases, both M-CLL and U-CLL, responded to all stimuli with uP-regulation of pERK. However, the magnitude and time-course of pERK induction varied significantly: at each time-point, significantly (P<0.05) higher induction was seen in U-CLL for all types of stimuli. In dually-stimulated cultures, anti-IgM+IM or anti-IgM+CpG significantly (P<0.05) uP-regulated pERK levels in comparison to the unstimulated control, in both M-CLL and U-CLL, with maximum response at 50 min. However, comparison of dually-stimulated cells to single-stimulated cells revealed great heterogeneity, with some cases showing uP-regulation of pERK contrasting others exhibiting pERK suppression. Exclusively among M-CLL, co-stimulation with anti-IgM+CpG significantly increased pERK levels compared to single stimulation with either anti-IgM or CpG. Effects on apoptosis were studied by Western blotting for the active isoform of caspase 8 (aCasp8) and the cleaved fragment of PARP (cf-PARP). In unstimulated cultures, the aCasp8 isoform was detected in almost all cases, while cf-PARP was detected in only one-third of cases, with relatively higher values in U-CLL. In single-stimulated cultures, great heterogeneity was observed regarding apoptosis induction in that anti-IgM alone had no effects, whereas stimulation with IM or CpG increased both aCasp8 and the cf-PARP, mainly in M-CLL. Dual stimulation with anti-IgM+CpG or anti-IgM+IM uP-regulated both aCasp8 and cf-PARP, again mainly in M-CLL, to significantly (P<0.05) higher levels compared to those observed in IM or CpG single stimulation.
Summary and Conclusions: Altogether, these findings suggest that pronounced pERK induction can be seen by single stimulation in U-CLL, whereas BcR/TLR synergism is required in M-CLL. Regarding apoptosis, an opposite pattern was observed, with M-CLL responding even to single stimulation, contrasting U-CLL that showed minimal responses, at least in the present setting. Differential intensity and duration of responses in M-CLL *vs* U-CLL indicates that the differences in signal transduction between the two subgroups may be primarily quantitative rather than qualitative.

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IGG-SWITCHED CLL HAS A DISTINCT IMMUNOGENETIC SIGNATURE FROM THE COMMON MD VARIANT: ONTOGENETIC IMPLICATIONS

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Background: IgG-switched CLL (G-CLL) is a relatively rare variant of CLL whose origin and ontogenetic relationship to the common IgM/IgD (MD-CLL) variant remains undefined. Previous studies have reported that most G-CLL cases carry somatically mutated B-cell receptors (BcR) pointing to possible derivation from post-germinal center B-cells. However, the relevant studies concerned small series, precluding definitive conclusions.
Aims: We sought for evidence about the origin of G-CLL *versus* MD-CLL by comprehensively profiling the respective immunoglobulin (IG) gene repertoires in the largest series thus far studied for this purpose.
Methods: Detailed immunogenetic analyses were conducted in a series of 1256 cases selected based on expression of MD (n=1087) or G isotype (n=169) as determined by peripheral blood flow cytometry (n=1019) or expression of tumor-specific Cμ/Cδ and/or Cγ transcripts as determined by RT-PCR (n=186).
Results: The IGHV gene repertoire of G-CLL differed significantly from that of MD-CLL, with over-representation of the IGHV4-34 (P<0.0001) and IGHV4-39 (P=0.003) genes, and, in contrast, under-representation of the IGHV1-69 (P=0.003), IGHV3-21 (P=0.017), IGHV1-2 (P=0.04) and IGHV3-48 (P=0.05) genes. Regarding somatic hypermutation (SHM), G-CLL included significantly more cases with mutated IGHV genes (following the 98% cut-off value for identity to the germline) compared to MD-CLL (84% *vs* 46%, respectively; P<0.0001). At the other extreme of the SHM spectrum, significantly more MD-CLL cases carried BcR IGs with no SHM (100% identity to the germline) compared to G-CLL (32% *versus* 11%, P<0.0001). The search for CLL-specific stereotyped BcR IGs revealed that the extreme skewing of the G-CLL repertoire was due to the fact that almost one-third of all cases was represented by only three subsets utilizing either the IGHV4-34 gene, namely mutated subsets #4 (relative frequency 18.3% of all G-CLL) and #16 (4%), or the IGHV4-39 gene, namely unmutated subset #8 (7.3%). These subsets (especially, subsets #4 and #8) represent polar opposites in terms of prognosis and outcome, with subset #4 cases experiencing indolent disease unlike subset #8 cases who tend to follow an aggressive disease course often complicated by the development of Richter's syndrome. In keeping with our previous reports, subset #4 and #16 rearrangements identified in the present study exhibited recurrent SHM at certain positions throughout the VH domain. However, despite an overall high SHM load, almost all IGHV4-34 rearrangements assigned to these two G-CLL subsets carried intact FR1 motifs for superantigenic-like interactions with N-acetyllactosamine epitopes present in self and exogenous antigens. In sharp contrast to subsets utilizing the IGHV4-34 and IGHV4-39 genes, all major sterotyped subsets utilizing the IGHV1-69, IGHV3-21, IGHV1-2 and IGHV3-48 genes (defined as reported in Blood 2012;119:4467) concerned exclusively MD-CLL.
Summary and Conclusions: G-CLL exhibits an overall distinct immunogenetic signature from MD-CLL, even when restricting the comparison to cases with mutated BcR IGs, prompting speculations about distinct ontogenetic derivation and/or distinct immune triggering. Nonetheless, questions abound regarding *e.g.* the presence of class switch recombination in the absence of SHM in subset #8 or the over-representation of the IGHV4-34 gene in G-CLL, especially

in light of studies showing that healthy IGHV4-34 cells are physiologically excluded from germinal center reactions in order to maintain peripheral B-cell tolerance.

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MIMICKING LEF1 OVEREXPRESSION OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS IN MICE

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Background: One of the hallmarks of CLL is the abnormal activation of the WNT signaling pathway and the strong overexpression of its down-stream transcription factor LEF1. The overexpression is highly associated with disease progression and induction of leukemia, while overall WNT activation appears to contribute to the maintenance of the apoptotic block. The aim of this study was to overexpress LEF1 in mouse B cells in order to test its leukemogenic potential.
Methods: LEF1 cDNA was introduced into the ROSA26 locus of C57/Bl6 mice. The gene is controlled via a floxed STOP cassette that inhibits gene expression in absence of Cre recombinase activity. LEF1 transgenic mice where crossed with CD19-Cre mice for B cell specific expression. Leukocytes were isolated from the peripheral blood, the spleen and the bone marrow and analyzed via FACS.
Results: In our mouse model LEF1 was overexpressed specifically in B cells and the expression appeared to be stable over 12 months. The oncogenic potential of LEF1 expression in B cells seemed to be low since no B cell leukemia was detected after monitoring more than 15 LEF1 expressing mice over 20 month. Complete blood counts also revealed no significant differences, but LEF1 expressing mice showed a significant reduction of B cells in peripheral blood lymphocytes after 16 (LEF1=38.39% *vs.* Control=50.80%; P=0.002) and 32 weeks (LEF1=43.44% *vs.* Control=52.51%; P=0.047). Percentage of B cells in the spleen was also significantly reduced (LEF1=46.26% *vs.* Control=56.54%; P=0.011), while the bone marrow appeared to be unaffected.
Summary and Conclusions: In our mouse model the LEF1 overexpression was not able to induce B cell dependent leukemia. This might be due to the moderate LEF1 over-expression in this model or insufficient activity of the WNT signaling cascade under physiological conditions, which is needed for LEF1 dependent gene expression. Nevertheless LEF1 expression affected either B cell proliferation or survival, which underlines the importance of WNT signaling for B cell homeostasis. The underlying mechanisms are to be clarified by future experiments.

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ABERRANT EXPRESSION OF PD-1 IN CHRONIC LYMPHOCYTIC LEUKEMIA IS INDEPENDENT FROM BCR SINGLING AND PDCD1 GENE POLYMORPHISM

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Background: The programmed death-1 (PD-1, CD279) is an immunoreceptor responsible for peripheral tolerance. Through inhibition of TCR/BCR signaling PD-1 impairs activation and effectors functions of T and B lymphocytes, where it is expressed upon activation. PD-1 was described on exhausted T cells and it is aberrantly expressed in several malignancies, where PD-1 pathway was related to a potential tumor escape mechanism from the immunosurveillance. Previously, we characterized PD-1 as a novel phenotypic feature of chronic lymphocytic leukemia (CLL) cells. PD-1 was uP-regulated on both transcript and surface protein levels in CLL patients in comparison to healthy volunteers (HVs). Moreover, activated *in vitro* CLL cells in conditions mimicking microenvironment of proliferation centres uP-regulated PD-1 on their surface.
Aims: Despite PD-1 aberrant expression in CLL patients, its mechanism and function is unclear. To investigate PD-1 influence on BCR signaling in CLL cells we analyzed expression and phosphorylation of SYK, LYN, and ZAP70 kinases. To verify relation of PD-1 expression to microenvironment *in vivo*, we analyzed PD-1 expression in 149 peripheral blood (PBMC) and 140 bone marrow (BM) CLL samples, accumulation and proliferation compartment respectively and 30 HVs. Finally, we characterized polymorphism of *pdcd1* gene in 114 CLL patients and 150 HVs.
Methods: Quantitative reverse transcriptase PCR was performed for the PD-1 transcript for 182 CLL patients (148 PBMC, 140 BM). Western blot (SYK and Lyn) and flow cytometric (ZAP70 and PD-1) analysis was performed for CD19+ magnetically separated cells from 12 CLL patients. For each kinase two separate phosphorylation sites were analyzed, responsible for activation or inhibition respectively: Y525/526 and Y323 for SYK, Y507 and Y396 for Lyn, Y315/319 and Y292 for ZAP70. Five single nucleotide polymorphism sites (SNPs) were analyzed by restriction fragments length polymorphism (RFLP-PCR) in group of 114 CLL patients and 150 HVs.
Results: The median level of PD-1 transcripts in CLL patients was higher in comparison with HVs (P<0.0001), but no difference between blood and bone marrow compartments. The expression and phosphorylation sites of LYN and SYK were not dependent on PD-1 expression in 12 CLL patients. Flow cytometric analysis showed that ZAP70 expression is not related to PD-1 level. Of note, median percentages of cells with phosphorylated ZAP70 activation/inhibition sites were low (Y315/319: 0.62%, Y292: 0.5%). Range of PD-1 expression varied from 67.53% to 4.2%. Analysis of *pdcd1* polymorphism showed that neither of SNPs frequency (loci: rs36084323, rs11568821, rs2227981, rs2227982, rs41386349) was different in CLL group compared to HVs. Particular substitutions were not related to the PD-1 expression level. Frequencies of haplotypes in CLL and HVs showed no significant difference. Linkage disequilibrium comparison of CLL and HVs revealed complete dependency of rs2227981/rs2227982 (P=0.03) and rs2227981/rs36084323 (P=0.03) loci in CLL patients. Those SNPs were associated with disorders like breast cancer and ankylosing spondylitis.
Summary and Conclusions: PD-1 expression is strongly uP-regulated in CLL patients regardless microenvironment of leukemic cells. No PD-1 influence on expression and activation of BCR related kinases might suggest impaired or alternative function of PD-1 pathway in CLL cells. Characterization of *pdcd1* polymorphism revealed no association of PD-1 SNPs with CLL, therefore, its upregulation might not be driven by genetic alternations.

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2-PHENYLETHYNESULFONAMIDE INDUCES THE UPR DEPENDENT APOPTOTIC PATHWAY IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: CLL is currently incurable partially due to resistance caused by non-functional p53/ATM. We previously showed that 2-Phenylethynesulfonamide (PES, Pifithrin μ) induced apoptosis of CLL cells independently of p53. However the mechanism of PES induced apoptosis has remained elusive. Recently PES was suggested to inhibit HSP70, a key protein responsible for protein folding. Inhibition of HSP70 function results in accumulation of misfolded proteins within the endoplasmic reticulum (ER) and causes activation of the unfolded protein response (UPR). Under normal conditions the ER chaperone BiP prevents UPR activation by binding and inactivating the ER transmembrane receptors; IRE-1α, PERK and ATF6. If the stress is resolved the UPR is switched off, however if the aggregation of unfolded proteins is persistent then the UPR remains active and switches from an anti-apoptotic to a pro-apoptotic pathway. The pro-apoptotic cascade involves the induction of XPB1/JNK/JUN/p38MAPK signalling, phosphorylation of eif2α (eif2α-P) and the induction of CHOP. We previously showed that PES treatment of CLL cells promoted apoptosis via the UPR, however the precise mechanisms have never been elucidated. Here we show for the first time which pathways are involved in PES induced cell death and suggest novel targets which could be used to treat patients with an ATM or TP53 deletion or mutation.
Aims: To determine how PES activates the UPR in CLL cells and whether the UPR pathway contributes to PES mediated apoptosis.
Methods: Fifty two CLL samples were treated with PES (5-20μM), an activator of the UPR, in the presence and absence of the pan-caspase inhibitor ZVAD. Molecular responses to treatment with PES and/or other agents were investigated by western blotting, flow cytometry and MTT assay.
Results: Here we demonstrate for the first time that treatment of CLL cells with PES generated reactive oxygen species (ROS) resulting in apoptosis since treatment with the antioxidant N-acetyl cysteine (NAC) inhibited PES induced apoptosis. PES treatment lead to increased expression of NRF2, p62 and HSP70 within 24 hours, indicating that the UPR and autophagy pathways had become activated. Immunoblotting identified that key proteins in the UPR pathway (cleaved XBP-1, phosphorylated eiF2α, c-Jun and p38 MAPK) were also induced following PES treatment. However surprisingly AKT was also activated and induced. Pharmacological inhibition of p38 MAPK or PERK provided protection from PES mediated apoptosis, as assessed by PARP cleavage and Propidium iodide/Annexin V staining. This indicates that the UPR is involved in the induction of CLL cell death following PES treatment. To confirm these observations we treated MEFS which are unable to induce eiF2α-P and subsequent downstream signalling. PES treatment could not induce eiF2α-P. In addition Ser51 mutated cells showed reduced autophagy following PES treatment compared to wild-type cells. Following from our observation that PES induced phosphorylated AKT we co-treated CLL cells with PES and an AKT inhibitor (AKTi) to identify whether we could enhance PES induced apoptosis of CLL cells.

AKTi treatment enhanced PES induced apoptosis compared with either agent alone.
Summary and Conclusions: PES represents a novel class of cytotoxic drug that induces UPR mediated apoptosis of CLL cells and may be of value in the treatment of CLL patients who have become refractory to conventional therapy through loss/mutation of TP53.

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ANTI-ROR1 MONOCLONAL ANTIBODY INDUCED APOPTOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA CELLS THROUGH PI3-KINASE/AKT/MTOR SIGNALING PATHWAY

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Background: The PI3-kinase/AKT/mTOR pathway is an intracellular signaling pathway important in cancer as well as apoptosis of cells. Mammalian target of rapamycin (mTOR) is a 289 kDa serine/threonine protein kinase and a member of the PIKK (Phosphatidylinositol 3-Kinase-related Kinase) family. It functions as a central component in the signaling pathway involved in the control of cell growth and proliferation. In CLL cells PI3K pathway is constitutively activated leading to AKT activation. ROR1 is a type I transmembrane receptor tyrosine kinase (RTK) belonging to one of the twenty families of RTKs. ROR1 is overexpressed on CLL cells but not in white blood cells of healthy donors. ROR1 is constitutively phosphorylated in CLL and siRNA transfection induced apoptosis. We have developed a unique anti-ROR1 mAb directed against CRD (cysteine-rich domain) of the extracellular region of ROR1 capable of inducing direct apoptosis of primary CLL cells. Our anti-CRD mAb could also induce dephosphorylation of the ROR1 molecule.
Aims: To study the apoptotic effect of an anti-ROR1 CRD mAb and effects on downstream signaling pathways involved in CLL, specially the PI3K/AKT/mTOR pathway using primary CLL cells.
Methods: Apoptosis was detected by the MTT assay and AnnexinV/PI methods in a 24 h assay. Antibody untreated and treated cell lysates were prepared and subjected to Western blot analysis for identification of the signaling molecules involved in apoptosis induced by the anti-ROR1 CRD mAb. We analysed total and phosphorylated levels of the following signaling proteins: AKT, P-AKT, PI3K, P-PI3K, mTOR, P-mTOR, ERK, P-ERK, PKC and P-PKC. Phosphoproteins were measured before incubation with the mAb and after 20 min-24 h.
Results: ROR1 surface expression was detected on 80-85% of the CLL cells. The frequency of apoptotic cells induced by the anti-CRD mAb was in the range of 45-50%. Time kinetics experiments using anti-ROR1 CRD mAb incubated with primary CLL cells revealed dephosphorylation of ROR1 downstream signaling molecules. After co-culturing CLL cells with the anti-ROR1 CRD mAb, Western blot analysis showed decreased level of phosphorylated AKT in treated compared to untreated samples. No changes in the phosphorylation levels of ERK and PKC proteins were seen. Furthermore, we analysed the PI3K protein which is upstream of AKT, and noticed that in CLL cells treated with the anti-ROR1 CRD mAb, the phosphorylation intensity of PI3K p85 isoform decreased but not p55 isoforrn. Moreover, we also studied the mTOR phosphorylation in treated and untreated CLL samples and detected dephosphorylation of mTOR in treated as compared to untreated samples.
Summary and Conclusions: Incubation of CLL cells with an anti-ROR1 CRD mAb induced apoptosis of primary CLL cells. Apoptosis was preceded by dephosphorylation of PI3K, AKT and mTOR proteins indicating deactivation of these signaling proteins by the anti-ROR1 mAb. In untreated CLL cells no effect on phosphorylation of these proteins was noted. Furthermore our ROR1 mAb did not dephosphorylate PKC or ERK. Our data may suggest that activation of mTOR molecule occur via the PI3K/AKT pathway and may be a survival signal in CLL cells associated with the aberrant expression of ROR1. The constitutive phosphorylation of PKC and ERK seen in CLL might not be related to the overexpression of ROR1. Further studies are warranted to understand better the signaling pathways associated with ROR1 and the downstream signaling effects of ROR1 targeting drugs.

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ROLE OF WNT-5A AND NON-CANONICAL WNT SIGNALING IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Transmembrane receptor tyrosine-protein kinase (ROR1) is almost uniformly uP-regulated in patients with chronic lymphocytic leukemia (CLL). ROR1 is a receptor for ligands of the Wnt family, which activate the non-canonical Wnt pathway, so called planar cell polarity - PCP-pathway. We have shown recently that PCP pathway is required for the migration of CLL cells in the chemokine gradient and the expression of PCP genes corresponds with the clinical course of CLL (Kaucka *et al.*, Cancer Res 2013).
Aims: In the present study we investigated the role of Wnt5a, the putative ligand of ROR1 and the activator of PCP pathway, in CLL.
Methods: B-lymphocytes of CLL patients were negatively separated using RosetteSep (StemCell) and gradient density centrifugation. The expression of Wnt-5a was assessed by quantitative RT-PCR using B2M and actin as endogenous controls. The migration of CLL cells in chemokine gradient of CXCL12 was conducted in HTS Transwell-96 well plates.
Results: Quantitative RT-PCR analysis of 80 CLL samples taken up to two years from diagnosis showed highly variable expression of Wnt-5a. The expression ranged from low levels comparable to healthy controls (peripheral B-cells) to high levels (10^2 fold change) indicating autocrine production of this ligand in a subgroup of patients. Significantly, patients with unmutated IGHV (N=46) showed higher levels of Wnt-5a (P=0.0014) which was also reflected by the worse treatment-free survival in Wnt-5a-high cohort (P<0.05). Furthermore, analysis of follow-up samples from 26 patients have shown that in several cases CLL cells uP-regulated Wnt-5a during disease progression. In patients treated in the interim, this effect was the most prominent. Functional data revealed that Wnt-5a promotes chemotaxis of CLL cells whereas inhibitors of Wnt-5a production (IWP2) block it.
Summary and Conclusions: Based on expression analysis and functional assays we propose that Wnt-5a is a ligand of Ror1/PCP in CLL responsible for the autocrine stimulation of the PCP pathway, chemotaxis and disease progression.
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EXPLORING THE ROLE AND USE OF CANNABINOID RECEPTORS AND CANNABINOIDS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Cannabinoids are the active compounds of the marijuana plant *Cannabis sativa L*. They exert their function by binding to the two cannabinoid receptors CNR1 and CNR2, CNR1 being expressed mostly in the nervous system while CNR2 mainly is found in immune cells. Various studies reported that cannabinoids induce apoptosis and inhibit cell proliferation and invasion in solid tumours. We and others have previously observed an overexpression of both receptors and induction of apoptosis upon incubation with cannabinoids in hematologic malignancies, including chronic lymphocytic leukemia (CLL). Furthermore, cannabinoids interfere with the microenvironment interaction of T cells by inhibiting the CXCL12/CXCR4 axis, which also plays an important role in CLL cell survival and resistance to therapy.
Aims: To evaluate the role of the two receptors and to test whether cannabinoids might serve as novel therapeutic approach in CLL.
Methods: A cohort of 102 well characterized CLL patients was screened by real-time PCR for CNR1 and 2 mRNA expression, normalized to the mean expression ofCD19 sorted healthy donor (HD) cells (N=4). Also, peripheral blood mononuclear cells (PBMC) from CLL patients (N=10-16) and HD (N=2-3) were incubated with different cannabinoids in suspension and co-culture with the mouse fibroblast cell line M2-10B4 and in combination with fludarabin at concentrations ranging from 5 to 100 µM for 48h. IC50 values were calculated based on standard viability assays. Migration inhibition of PBMC from CLL patients toward CXCL12 was evaluated in migration assays.
Results: Although both cannabinoid receptors were overexpressed in CLL, only CNR1 was found to have prognostic value. CNR1 mRNA expression was increased from 0 to 140.4 fold compared to HD. With a cut-off set at median CNR1 expression (1.34), high CNR1 expression was associated with Binet stages B+C (P=0.049), unmutated IGHV (P=0.006), and high CD38 expression (P=0.032). Furthermore, CNR1 high expressing patients had shorter overall (median 154.2 months *vs.* median not reached; P=0.002) and treatment free survival (median 53.6 *vs.* 141.4 months;P=0.000). Based on these data, we evaluated CNR 1 & 2 as potential therapeutic targets. As shown in Table1, in particular the antagonists reduced cell viability considerably in suspension culture. In co-culture, the feeder cells protected tumour cells from compound induced cytotoxicity except for methanandamide (RM), cannabidiol (CBD), and AM630 which retained their toxicity. However, some of the compounds also had significant impact on healthy cells showing in some instances IC50 values similar to that of CLL cells (Table 1). Interestingly, the sensitivity of samples to cannabinoids was not associated with mRNA expression of either of the receptors. In combination treatments with fludarabine, only the CNR1 agonist ACEA exerted a small additive effect albeit not statistically significant. In contrast to observations in T cells, none of the tested cannabinoids could significantly inhibit CLL cell migration toward CXCL12.

Table 1. IC50 values achieved after 48h incubation of primary CLL and HD cells and M2-10B4 mouse fibroblasts with cannabinoids. Concentrations in µM, NR=50% viability reduction not reached.

Compound	Action	Primary CLL suspension	Primary HD suspension	Primary CLL co-culture	M2-10B4 fibroblasts
RM*	CNR1 agonist	33.19	60.13	29.27	34.55
CBD§	CNR1 antagonist, CNR2 inverse agonist	21.74	15.09	16.78	13.52
ACEA	CNR1 agonist	31.78	39.01	NR	NR
AM251	CNR1 antagonist	**9.43**	11.44	NR	NR
JWH133	CNR2 agonist	75.68	78.12	NR	NR
AM630	CNR2 antagonist, CNR2 inverse agonist, weak CNR1 agonist/inverse agonist	**12.08**	28.51	27.64	28.27

*RM = R-(+)-Methanandamide
§CBD = (-)-Cannabidiol

Summary and Conclusions: Although some cannabinoids reduce viability in neoplastic cells considerably independent of CNR expression, also healthy cells are affected. The important CXCL12/CXCR4 axis could not be inhibited by cannabinoids in CLL. These data indicate a poor therapeutic potential for cannabinoids in chronic lymphocytic leukemia, although CNR1 mRNA expression could be determined as novel prognostic marker.

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MICRORNA MIR-150 CONTRIBUTES TO THE DISEASE AGGRESSIVENESS AND REGULATION OF B-CELL RECEPTOR SIGNALLING (BCR) IN CLL

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Background: We and others have shown that expression of certain miRNAs associates with disease activity and pathogenesis of CLL (Calin, 2005; Mraz, 2012; Mraz, 2009). However, it remains largely unclear which from the ~1400 described miRNAs in human genome are relevant for the regulation of malignant B cell functions.
Aims: In this study we first screened (TaqMan miRNA Cards-ABI, 750 miRNAs) for abundantly expressed miRNAs in CLL cells hypothesising that miRNAs with strong expression are more likely significantly involved in the regulation of cell functions.
Results: We identified miR-150 as the most abundant miRNA in CLL cells, however, its lower levels associated with stronger calcium mobilization (a marker for active BCR signalling) after stimulation of BCR on CLL cells with anti-IgM ($P<0.05$, N=36). Additionally, miR-150 levels were directly down-modulated after stimulation of BCR with anti-IgM in CLL cells ($P<0.01$). This miRNA has been previously reported to influence the gene expression of normal B cells (Xiao, 2007) altogether suggesting that it might be involved in the BCR-pathway. Several target genes have been described for miR-150 in studies performed on various cell types. However, it is becoming clear that the preferential target(s) of a certain miRNA depend on the context of all mRNAs with potential binding sites and their expression levels in a specific cell type. More importantly, the identification of mRNAs regulated by a certain miRNA can be performed based on the analysis of mRNA expression since most miRNAs affect not only mRNA translation but predominantly decrease target mRNA levels (Guo, 2010). To describe novel targets regulated by miR-150 specifically in CLL cells we performed array-based transcriptome analyses of 110 CLL samples. This identified differential expression of 2 genes with evolutionary conserved binding sides for miR-150 (GAB1 and FOXP1) between CLL cells expressing low *vs.* high levels of miR-150. The immunoblot analysis of GAB1 and FOXP1 in CLL cells confirmed their higher protein levels in cases with low miR-150 expression ($P<0.005$, fold change >10.0) and the transfection of CLL cells with artificial miR-150 led to GAB1/FOXP1 down-regulation. GAB1 is an adaptor molecule that recruits PI3K to the plasma membrane after BCR stimulation and is required for amplification of PI3K signalling and subsequent AKT activation (Ingham, 2001). FOXP1 is a transcription factor uP-regulated by BCR stimulation and implicated in the progression of several B-cell lymphomas (Hu, 2006). We found that, CLL cells with higher expression of *GAB1* or *FOXP1* were more responsive to BCR stimulation *in vitro* and higher expression of each associates with shorter overall survival (OS) (13.9 *vs.* 22.7 years, 13.9 *vs.* 21.1 years; N=168; $P<0.05$). Most notably, a reverse trend was observed for miR-150, where higher levels (>median) were associated with significantly longer OS and TTFT in a multivariate analysis, which included other 6 other routinely used prognostic markers (OS HR: 3.4 [CI 1.4-8.6], P=0.009; TTFT HR: 2.3 [CI 1.3-4.2], P=0.004). Additionally, we have noted differences in the miR-150 expression and methylation profile of its coding region after disease progression (analysis of serial CLL samples).
Summary and Conclusions: We conclude that miR-150 is a novel regulator of genes that control BCR-signalling, which is a factor that prominently affects the biology of malignant B cells.
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Chronic myeloid leukemia - Biology 2

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A NEW ONCOGENIC ROLE OF BCR/ABL-OOF IN CHRONIC MYELOID LEUKAEMIA PATHOGENESIS

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Background: chronic myelogenous leukaemia is a myeloproliferative disorder characterized by the presence of Philadelphia chromosome, derived by a reciprocal translocation between chromosome 9 and 22, involving respectively the genes c-Abl and Bcr. Next to the main BCR/ABL transcripts we identified previously alternative BCR/ABL splice variants involving BCR exons1, 13 or 14 and ABL exon 4. Their translation products are characterized by the change in the reading frame of ABL exon 4 leading to the formation of a early stop codon within ABL exon 5. Bcr/Abl fusion proteins (Bcr/Abl OOF) resultant have an initial and correct Bcr portion attached to a sequence of aminoacids arising from the out of frame (OOF) reading of the c-Abl exon 4 and 5 gene sequence, which has been studied in the past for its immunogenic rule. It contains the characteristic Bcr domain but its specificity is the absence of COOH-terminal Rac GAP domain.
Aims: To determine the role of the Bcr/Abl OOF we analyzed the effects of the protein in term of cytoskeleton modelling, adhesion and activation of Rho GTPases, given the role of Rho GTPase family proteins in cytoskeletal remodelling.
Methods: Bc/Abl OOF cDNA was cloned in an expression vector in order to investigate new functions of this protein that differ from Bcr for the absence of the GAP domain, essential for the inactivation of Rho GTPases proteins. Immunofluorescence and western blot of different fraction (cytosolic and microsomes) were performed to investigate its localization. Adhesion assay on fibronectin was used to analyse a possible change in cytoskeleton modelling after transfection with Bcr/Abl OOF. Pulldown assay was performed by using Rac GTPase binding domain as GST fusion protein bound to glutathione-sepharose beads (GST PAK-CD). Proliferation assay was performed by evaluation of ^{3}H incorporated in cells during division while apoptosis assay was done by using Annexin V-FITC and Propidium PE.
Results: By immunofluorescence and western blot assay we observed that the localization of Bcr/Abl-OOF is predominantly cytoplasmatic and that colocalizes in endosomal vescicles with Bcr protein. Bcr/Abl-OOF confers an altered adhesion if compared to empty pcDNA, with a morphology quite similar to hela cells transfected with Rac active form and unlike to that of cells transfected with Rho and Cdc42 active form. This is confirmed by analysis of Rac endogenous, which assumes peculiar submembrane localization typical of Rho proteins when present in the activated form. This result suggest that Rac activation could be one of the responsible of Bcr/Abl-OOF cell morphology during cell adhesion on fibronectin. The GST PAK-CD fusion protein was able to trap a significant proportion of GTP-bound form of Rac in Bcr/Abl-OOF cells lyses, supporting the presence of active Rac. We also analysed a possible involvement of Bcr/Abl-OOF in the most important oncogenic pathway. Apoptosis assay showed that transfected cells with Bcr/Abl-OOF presented lower apoptotic rate than control cells, with 67% of reduction (P=0,01). Proliferation assay shown a 2 fold of induction of proliferation rate (P=0,001) in Bcr/Abl-OOF cells, which is restored after Rac inhibitor treatment. We also observed an increase in Ciclin D, pAkt and pp38 proteins, increase that become more significant in presence of growth factor stimuli.
Summary and Conclusions: Summary and Conclusions: all these results therefore suggest a possible oncogenic rule of Bcr/Abl-OOF, which can cooperate with Bcr/Abl in the process of proliferation, survival and apoptosis of chronic myeloid leukaemia. Next step will be the analysis of Bcr/Abl OOF in a big cohort of CML patients in order to understand if different quantity of our gene can be predictive of a non suboptimal answer to Bcr/Abl specific therapy.

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INITIAL OCCUPANCY OF LEUKEMIC STEM CELL CORRELATES WITH PROGNOSTIC FACTOR IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: Chronic myeloid leukemia (CML) stem cells are less sensitive to tyrosine kinase inhibitors (TKIs) compared to downstream progenitors. It might be possible that the initial leukemic stem cell burden affect the prognosis of chronic phase (CML-CP) patients, but this possibility has not been verified.
Aims: The purpose of present study is to explore the involvement of BCR-ABL positive clone in stem cell fraction at diagnosis and to evaluate the prognostic impact of leukemic stem cell occupancy.
Methods: Bone marrow (BM) aspirate samples were obtained from 9 newly diagnosed CML-CP patients CD34+ stem/progenitor cells were first enriched using a magnetic bead selection kit. HSC (hematopoietic stem cell; CD34+CD38), CMP (common myeloid progenitor; CD34+CD38+IL3RlowCD45RA-), GMP (granulocyte/macrophage progenitor; CD34+CD38+IL3R+CD45RA+) and MEP (megakaryocyte/erythrocyte progenitor; CD34+CD38+IL3R-CD45RA-) fractions were isolated by BD FACS Aria and subsequently analyzed the frequency of BCR-ABL positive clone by FISH analysis. The protocol of the study was reviewed and approved by the institutional review board prior to initiation of the study. Written informed consent was obtained from all the patients.
Results: Among myeloid progenitors, CMP and MEP populations robustly expanded and were composed almost entirely of BCR-ABL positive clone in all patients (FISH positive rate, 95.1+/-7.4% and 96.0+/-5.5%, respectively), while surprisingly GMP fraction was less involved in leukemia (56.3+/-37%), suggesting that CML hematopoiesis is able to bypass the GMP phenotype. Importantly, CD34+CD38- HSC fraction was suppressed in CML patients (~1% of CD34+ cells) compared to healthy volunteers (~10% of CD34+ cells), and the frequencies of BCR-ABL positive clone in HSC fraction (46.1+/-40.5%) were always less than those in CMP and MEP, suggesting that CML stem cell is less addictive to growth signaling of BCR-ABL compared to myeloid progenitors. According to the leukemic stem cell occupancy, CML-CP patients were classified into 3 subgroups. In the high involvement group, >90% of HSCs were BCR-ABL positive, while in the low and intermediate involvement groups, <5% and ~50% of HSCs were involved, respectively. We evaluated relationship between BCR-ABL positive rates in HSC fraction and patients' data. The leukemic stem cell occupancy fabulously correlated with platelet count and basophils in peripheral blood, and consequently with prognostic scores such as Sokal, Hasford, and EUTOS.
Summary and Conclusions: The risk of disease progression and/or TKI sensitivity in CML-CP patients might be prospectively classified by the leukemic stem cell occupancy.

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MICROVESICLES TRANSCRIPTOME REFLECTS PHYSIOLOGICAL CONDITIONS OF LEUKEMIC PARENTAL CELLS

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Background: Microvesicles (MVs) are nano-sized lipid bodies (100-1000 nm in diameter) that are released by cells into the extracellular space and as carriers of proteins, mRNA and miRNA shown to represent vectors from donor to target cells[1]. Through fusion with target cells MVs may well modulate the functional state of receiving cells. In leukemia MVs have been suggested to modulate the hematopoietic niche[2,3].
Aims: 1) Transcriptome comparison between MVs and leukemic parental cells. 2) Investigate the effects of a hypoxic environment similar to that at the hematopoietic endosteal niche on transcripts carried by MVs. To this end, we collected MVs released from cell cultures in hypoxic condition and compared the transcriptome with that of MVs isolated from cultures at normoxia.
Methods: MVs released from K562, a BCR-ABL positive human erythromyeloblastoid leukemia cell line, were isolated by culture medium centrifugations (2500g for 15 minutes). The supernatant was filtered by means of a 1.2µm filter to eliminate larger vesicles. The filtered medium was re-centrifuged at 18000g for 1h at 4°C. MVs and parental cells RNA was extracted using Trizol. Cell line RNA quality was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies) and quantified by means of NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc.). To check MVs RNA integrity GAPDH 5':3' amplicon ratios were determined by RealTime-PCR. Afterwards, cDNA was synthesized from 1µg of total RNA by reverse transcription–polymerase chain reaction. Gene expression profiles of K562 cell line and MVs were analyzed using GeneChip Human Genome U133 Plus 2.0 arrays (Affymetrix).
Results: Gene expression data have been compared between cells and MVs. We identified 5294 common probe sets with a Present call (fold-change lower than 1.5). Analysis of these probe sets using DAVID Functional Annotation Bioinformatics Microarray Analysis revealed a conservation of MVs transcripts involved in pathways of cell function such as RNA processing, protein translation, aminoacid metabolism and cell respiration. Remarkably, in MVs we observed a high presence of gene transcripts coding for protein belonging to the Chronic Myeloid Leukemia pathway that are expressed downstream of the BCR-ABL tyrosin kinase fusion protein. Furthermore, different gene expression profiles were identified between cells and MVs by a paired-T test. 290 probe sets resulted uP-regulated in MVs compared to cells lines (fold-change higher than 1.5). On the other hand, 1899 probes were down-regulated in MVs compared to parental cells. In addition, transcriptome comparison of MVs released under hypoxic *versus* normoxic conditions revealed two upregulated transcripts, both involved in HIF1α regulation, in MVs released from hypoxic cell cultures[4,5]
Summary and Conclusions: We isolated MVs released by K562 leukemic

cells and for the first time a whole transcriptome gene expression analysis has been performed comparing K562 parental cells and MVs. Moreover, we identified an enrichment of transcripts coding for proteins involved in several essential pathways in the MVs supporting the hypothesis of a functional selection from the parental cell transcriptome. MVs released from cells under hypoxic conditions carried a higher amount of two transcripts related to HIF1α compared to MVs isolated from cells in normoxia.

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THE REFERENCE STANDARD FOR BCR-ABL TRANSCRIPT MONITORING IS IMPORTANT TO DEFINE CMR IN CML PATIENTS AND TO TEST THE RQ-PCR METHOD PERFORMANCES

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Background: Real-time quantitative polymerase chain reaction (RQ-PCR) of BCR-ABL transcripts is now the standard method needed for monitoring minimal residual disease in patients with chronic myeloid leukemia (CML) treated with tyrosinekinase (TK) inhibitors. RQ-PCR is a molecular method for monitoring response to tretment in clinical trials.
Aims: Internal quality assurance is crucial for high fidelity results. In order to test comparability of standard curve we used a common calibration plasmid–pME2 (Scientific Laboratory, III. Medizinische Klinik, Medical Faculty Mannheim) and universally accepted refference standard - ERM-AD623 (Institute for Reference Materials and Measurements).
Methods: In order to compare results previously obtained using the Abl control gene, we performed RQ-PCR on 20 leukemic samples. We choose samples with different levels of molecular response in international scale (IS). Molecular analysis was performed using a standardized RQ-PCR method with Hybridization Probes detection method on LightCycler instrument. For ABL measure we used five dilution points with pME standard and six dilution poits with ERM standard in duplicate. For BCR-ABL measure we used six dilution points with pME standard and five dilution poits with ERM standard in duplicate. All quantification parameters were set up acording to international guidelines for the measurement of BCR-ABL transcripts in CML.
Results: We found a two fold difference between the ERM absolute numbers and pME2 standard curves. Comparing BCR-ABL/ABL with both plasmids for samples where residual disease is detectable, the ratio is within the limits of normal variation but the differences in absolute control gene numbers impacts on definitions of complete molecular response (CMR) when BCR-ABL is undetectable.
Summary and Conclusions: For reliable results and to minimize variability of the results, a universal, traceable standard/calibrator is needed to be available to all MRD monitoring labs. Definitions of CMR need to take into account variations in the assignment of absolute copy numbers.
We gratefully acknowledge the support of the European LeukemiaNet/EUTOS for the international standardization process.

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THE BCR/ABL TYROSINE KINASE INHIBITOR IMATINIB PRODUCES PROFOUND MAST CELL DEFICIENCY IN CHRONIC MYELOID LEUKEMIA

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Background: Although mast cells (MC) play an important role in allergic reactions, their physiologic role in healthy tissues remains largely unknown. In mice, several MC-deficiency models have been developed. In several of these models, MC deficiency is triggered by a lack of *KIT* or lack of functional KIT ligand stem cell factor (SCF). However, no comparable human model is available.
Aims: We examined the *in vitro* and *in vivo* effects of the ABL/KIT-targeting drug imatinib on growth and development of MC.
Results: Imatinib was found to inhibit SCF-induced differentiation of MC from their cord blood-derived progenitors in a dose-dependent manner (IC50: 0.01 μM). Correspondingly, continuous treatment of chronic myeloid leukemia (CML) patients with imatinib (400 mg daily) resulted in profound MC deficiency. In patients entering a major molecular response during therapy, MC numbers in the bone marrow decreased to less than 5% of pre-treatment values, and serum tryptase concentrations decreased to low or even undetectable levels (tryptase before therapy: 32.0±11.1 ng/mL *versus* after therapy: 3.4±1.8, P<0.01). Moreover, as assessed by qPCR, KIT mRNA levels and tryptase mRNA levels decreased substantially during imatinib-therapy. Other myeloid lineages in the bone marrow, known to develop independently of KIT, were not affected substantially by treatment with imatinib.
Summary and Conclusions: In conclusion, imatinib produces profound MC deficiency *in vitro* and *in vivo*. However, the imatinib-induced MC deficiency is not accompanied by specific symptoms or side effects. Based on these observations, we hypothesize that MC may be less relevant to physiologic homeostasis in healthy tissues than has been assumed so far.

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IDENTIFICATION AND FOLLOW-UP OF TWO NEW RARE E8A2 BCR-ABL FUSION GENES

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Background: Less than 5% of patients with chronic myeloid leukaemia (CML) have breakpoints falling outside of the M-*BCR* in chromosome 22, and as the e8a2 transcript several other fusion transcripts have been identified to date. Although rearrangements have been described, involving a fragment insertion from *ABL* intron Ia or Ib between *BCR* exon 8 and *ABL* exon2, rearrangement with a third partner gene occur even more rarely.
Aims: We investigated two newly diagnosed cases of CML patients with novel fusion transcripts that give rise to a chimeric molecule involving an e8a2 fusion transcript and a third gene thus keeping the reading frame. We report here the precise features of the rearrangements and the follow-up of minimal residual disease (MRD) of these two patients.
Methods: Two cases were diagnosed with CML in chronic phase based on typical peripheral blood findings and standard karyotyping analyses indicating t(9;22)(q34;q11). An unusual band, other than the common transcripts, was observed in multiplex reverse transcription-polymerase chain reaction (RT-PCR) for the *BCR-ABL* gene rearrangement. The PCR products were then sequenced to assess the identification of the transcript. The monitoring of MRD was carried out using RT-PCR with specific primers located either close to the e8-*BCR* or in the inserted fragment. SNP-array was performed to accurately identify additional copy number variations.
Results: In case no.1, the fragment turned out to be an e8a2 fusion transcript with 112-bp inserted fragment corresponding to the exon 4 of the *PRDM12* gene. This gene is located on chromosome 9q34 and encodes the PR domain zinc finger protein 12 involved in transcriptional regulation. Deletion on locations 22q11.23q12.1 was highlighted by SNP-array. In case no.2, we also identified an e8a2 transcript with a 154 base pair (bp) insert that wholly matched the sequence from the exon 4 of the *SPECC1L* gene. The *SPECC1L* gene is located on chromosome 22q11 and encodes the cytospin-A protein implicated in cytokinesis and spindle organization. Besides, SNP-array analysis revealed deletions on locations 9q34 and 22q11 for this patient. Both patients showed favorable response to imatinib therapy, and achieved major molecular responses (MMR) less than 1 year after imatinib introduction. In case no. 1 (rearranged with *PRDM12*), the levels of transcript have remained undetectable for the 4-year follow-up. The patient no. 2 (with *SPECC1L* gene rearrangement), although achieving MMR, never obtained undetectable molecular response, but transcript levels that have been fluctuating below the MMR threshold for 5 years.
Summary and Conclusions: The insertion of 154 bp and 112 bp of a third partner gene, *SPECC1L* and *PRDM12* genes respectively, resulted in a novel in-frame *BCR-ABL* fusion transcript e8-[ins]-a2. Deletions on the derivative chromosome 9 occur in approximately 15% of CML patients and a potential role for *PRDM12*, located on 9q11, has been suggested previously in the pathogenesis of CML. Nevertheless, this is the first report of a novel fusion transcript involving an exon from this gene, as for *SPECC1L* gene. Structure/function studies would bring a more comprehensive survey on the real biological significance of such BCR-ABL chimeric protein.

P701

THE PROSPECTIVE ANALYSIS OF LOW LEVEL BCR-ABL1 T315I MUTATION IN THE CD34+ CELLS OF *DE NOVO* CML PATIENTS

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Background: The BCR-ABL1kinase domain (KD) mutations are associated with resistance to tyrosin kinase inhibitors (TKIs) and thereby with poor prognosis in chronic myeloid leukemia (CML) patients.Thus, an early detection of these mutations is important and could potentially leads to early therapeutic intervention and optimization of ongoing treatment regimen. The particular focus should be given to T315I mutation, which is resistant to all approved TKIs (imatinib, nilotinib and/or dasatinib). Considering the hierarchy of hematopoiesis, it should be expected that BCR-ABL1 KD mutations expand directly from stem cells or early progenitor cells. It has already been reported that these mutations were detected in these cells before their occurrence in bone marrow or peripheral blood.
Aims: The prospective detection of low level T315I mutation in newly diagnosed CML patients, especially in early progenitor CML cells ($CD34^+$; $CD34^-$).
Methods: The cell sorting (using a FACSVantage SE) of bone marrow $CD34^+$ and $CD34^-$cells from 50 *de novo* CML patients wasperformed at the time of diagnosis. Isolated RNA from sorted cells was reversibly transcribed into cDNA, which was subsequently used for amplification of *BCR-ABL1* fusion gene followed by sensitive detection of T315I mutation by quantitative Ligation-PCR ($ligPCR^{T315I}$). The estimated sensitivity of $ligPCR^{T315I}$ was 0.5% $BCR\text{-}ABL1^{T315I}/BCR\text{-}ABL1^{total}$.
Results: Within our cohort of 50 *de novo* CML patients, low level T315I mutation was detected in 7/50 (14%) cases with obtained positivity closed to detection limit of applied method (~0.5%). These suspected samples were tested repeatedly to avoid any false-positive results. Moreover, additional $CD34^+/CD34^-$ samples were tested for the presence of T315I mutation after the 3 months of TKI therapy. From these re-tested patients only 1/7 wasconsidered as positive for the low level T315I mutation. ThisT315I positive CML patient wassubsequently followed-up using $ligPCR^{T315I}$ analysis for the possible T315I mutation evolution. The expansion of T315I mutated clone was observed at the months 3 and 6 of TKI therapy with level of 0.7% and 4.2%, respectively. Other samples for T315I kinetics monitoring were not available since this patient died very quickly due to the progression of CML (8 months after diagnosis).
Summary and Conclusions: Our prospective analysis of low level T315I mutation in early progenitor CML cells ($CD34^+$; $CD34^-$) of *de novo* CML patients showed that this key mutation does not frequently occur in CML patients at the time of diagnosis. We suppose that the presence and evolution of T315I mutation is more likely associated with increased genomic instability, advanced phase of CML or selective pressure of applied TKIs.
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P702

EVALUATION OF TOB1 GENE EXPRESSION IN CML PATIENTS AND BLOOD DONOR AND THE EFFECT OF GENE SILENCING IN BCR-ABL+ CELL LINE

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Background: The TOB1 gene is a transcription factor responsible for the transduction of the gene ERBB2. It is a member of a family of cell suppressor proliferation proteins called TOB/BTG1 family; also, this gene operates on the inhibition of neoplastic transformation. The TOB1 gene presents a low expression in several types of cancer such as lung, breast, thyroid and stomach cancer. However, the function of this gene in chronic myeloid leukemia (CML) remains unknown.
Aims: The purpose of this study is to assess the gene expression in CML and blood donors, to evaluate the inhibition of gene TOB1 in BCR-ABL positive cells and try to elucidate the molecular mechanisms associated with the inhibition of this gene in the CML.
Results: We identified differentially expressed gene among CML patients granulocytes and healthy individuals using the Subtractive Supression Hybridization technique. We used 101 patients and 20 blood donors to proceed the analysis of gene expression characterization, and found that patients presented a lower gene expression. Then, the inhibition of this gene in K562 cells was performed using specific lentivirus. The effect of silencing TOB1 in the proliferation of K562 cells was assessed by the MTT assay after 48 hours of culture; in shTOB1 the proliferation was increased in comparison with shControl cells. To analyze the clonogenicity, we performed a formation of colonies assay, in methylcellulose, to determine whether silencing TOB1 could cause a change in the clonal growth of positive BCR-ABL cells. We found an increase in the number of colonies in shTOB1 cell culture compared to shControl cells. In the assessment of cell cycle, the flow cytometry analysis revealed a significant accumulation of K562 cells in S phase, with consequent reduction of cells in the G2 phase of the cell cycle in shTOB1 cells compared to shControl cells. The TOB1 gene silencing in K562 cells kept the cells in the S phase and prevented the entry of cells in the G2 phase, showing that the inhibition of TOB1 gene induced an increase in proliferation of K562 BCR-ABL cells. The level of apoptosis was assessed by flow cytometry after labeling the cells with anexin-V/PI, and by assays of caspases3, 8 and 9. It was found an increase of the caspase activity of shControl cells in relation of the shTOB1 cells, showing that the inhibition of this gene also changes the level of apoptosis.
Summary and Conclusions: The results corroborate the literature data that report the relationship of this tumour suppressor gene in signalling pathways related to angiogenesis, carcinogenesis, apoptosis and metastasis. When we relate the results obtained with the LMC, we can consider the possibility of the changes in TOB1 regulation be associated to alteration of important signalling pathways such as AKT, PI3K, STAT3 and STAT5, among others. Furthermore, the inhibition of TOB1 may be related with an increase on the number of BCR-ABL positive cells and subsequent disease progression. In conclusion, this study confirmed literature data showing that TOB1 gene works as a tumour suppressor protein in cells of many types of cancer. From this work we can infer that in CML the expression of TOB1 is transformed, resulting in changing of the capacity of induction of apoptosis, decrease tumour necrosis and increase cell proliferation. This work was supported by FAPESP and INCT.

P703

CYP1A1, GST GENE POLYMORPHISMS AND RISK OF CHRONIC MYELOID LEUKEMIA (CML)

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Background: Chronic myelogenous leukemia (CML) is a clonal myeloproliferativedisorder that results from an acquired genetic change in a pluripotential haemopoietic stem cell. The ability of the chemical carcinogens to induce malignancies depends on the body activation/detoxification of these carcinogens.
Aims: of this work was to study the genetic polymorphism of xenobiotic metabolizing enzymes: phase I enzymes; cytochrome P450 (CYP1A1) and phase II enzymes; glutathione S-transferase (GSTM1 and GSTT1).
Methods: 30 chronic myeloid leukemia patients (CML) (17 males, 13 females; age (Mean±SD) 36.87±12.45 years) and 30 age and sex matched healthy controls were analyzed for the frequency of CYP1A1 alleles and of GSTT1 and GSTM1 homozygous deletions by PCR- RFLP and multiplex PCR methods, respectively, using peripheral blood samples. The relationship between these genotypes and risk of CML was assessed by means of Odds Ratio (OR) with 95% confidence limits.
Results: Present study revealed that genotype frequency of (CYP450) 1A1*2C of patients was 66.7% were of the wild-type homozygous Ile/Ile genotype, 30.0% heterozygous Ile/Val genotype and 1(3.3%) homozygous mutant Val/Val genotype. In the control group, 96.7% with Ile/Ile genotype, 3.3%with Ile/Val genotype and 0% Val/Val genotype. CYP1A1 variant genotype was higher in patients group than in controls group, this difference was statistically significant. The frequency of CYP1A1 Val allele was higher CML patients, indicating that persons carrying this allele had an increased risk of CML. Although the frequency of GSTT1 null genotype was higher among CML patients compared to controls, this difference was not statistically significant.GSTM1 null genotype, the frequency was slightly higher in the patient group than that of the controls, however this difference also was not statistically significant. GSTT1 and GSTM1 double null genes carried a 9.0 greater risk for CML.
Summary and Conclusions: In conclusion this data suggests that polymorphic CYP1A1 and GSTT1 genes appear to affect susceptibility to CML.

P704

ENHANCED ADHESION & MIGRATION AND INDUCTION OF PYK2 EXPRESSION IN K562 CELLS FOLLOWING IMATINIB EXPOSURE

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Background: Since the introduction of imatinib treatment for CML there has been increasing concern about extramedullary relapse (EMR) progression despite favorable response in the bone marrow.
Aims: We postulated that imatinib induces molecular changes associated with enhanced migration and adhesion abilities probably enabling CML cells to inhabit extramedullary sites. Pyk2 is a tyrosine kinase localized to focal adhesion sites that has been implicated in mediating cell adhesion and motility. We have recently shown an increase in the migration, adhesion and invasion capabilities of NB4 cells (APL cell model) following treatment with the targeted therapy ATRA routinely used for the treatment of APL patients and identified Pyk2 as one of the major mediators of these processes.
Methods: We studied the effect of imatinib on K562 cells by combining adhesion, migration and invasion assays, real-time PCR, Western blots and siRNA experiments.

Results: In agreement with our previous report, our current studies reveal that CML cell lines and patient cells also demonstrate a 2-3-fold (P<0.006) elevation of Pyk2 mRNA and protein expression when exposed to imatinib. This increase in pyk2 expression subsequently led to enhanced adhesion (5-fold, P<0.0001), migration (7-fold, P<0.0001) and invasion (2-fold, P<0.001) capabilities of K562 cells. Interestingly, the non-adherent population was 50% more sensitive to imatinib treatment, suggesting that the acquired adhesive phenotype of K562 cells following exposure to imatinib may grant the cells with a certain level of resistant. Selective inhibition of Pyk2 expression by shPyk2 resulted in a 2-fold (P<0.02) reduction of K562 cell adhesion and a 5-fold (P<0.002) reduction of K562 cell migration abilities following imatinib treatment. These results correlate with our previously published data regarding NB4 cells exposed to ATRA.
Summary and Conclusions: Relying on these data, we suggest an active role for the novel targeted therapies in the occurrence of EMR in hematological malignancies. Agents such as ATRA and imatinib could induce expression of molecules that promote migration and extravasation of the leukemic cells and eventually stimulate their adhesion to extramedullary sites forming a reservoir of viable cells that could later proliferate and result in an extramedullary recurrence. Since the use of targeted therapies such as ATRA and tyrosine kinase inhibitors (TKIs) is broadening, we should be aware that alongside their therapeutic effect they may potentially obtain a detrimental nature with respect to extramedullary manifestation in hematological malignancies. Other novel agents in clinical practice should also be evaluated for this phenomenon.

P705

PATTERNS OF ABL KD MUTATION IN ASIAN PATIENTS WITH IMATINIB-RESISTANT CHRONIC PHASE CHRONIC MYELOID LEUKEMIA

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Background: Imatinib (IM), the first Bcr-Abl1 tyrosine kinase inhibitor (TKI), is a novel compound for managing chronic myeloid leukemia (CML), however, substantial numbers of patients are presenting with resistance. Among the causes of resistance, the point mutation in the BCR-ABL1 kinase domain (KD) is most common. However, the characteristic of mutations in most of reported data was based on the clinical trials that can be different in clinical practice.
Aims: Therefore we conducted this retrospective study to elucidate BCR-ABL1 mutation pattern detected in real clinical practice.
Methods: A retrospective cross sectional study has been conducted to describe the patterns and rate of BCR-ABL mutations in Asian CML patients. All reported mutation data of chronic phase CML (CP-CML) patients upon development of IM resistance in 2001-2012 were collected from medical charts and transcribed into a database for analysis.
Results: One hundred thirty six patients were enrolled from 9 institutions of 3 Asian countries in this study. Disease phase at diagnosis were CP in 111 (81.6%) patients, accelerated phase (AP) in 15 (11.0%), blast crisis (BC) in 8 (5.9%), ALL in 1 (0.7%) and unknown in 1 (0.7%) patients. Here, we report the result of mutation in 111 CP-CML patients. The median age was 52 (18-85) years and male patients were dominant (n=79, 71.2%). Prior interfereon-alpha therapy ws received in 32 (28.8%) patients. A majority of patients received 400mg/day or more as initial IM dose. Median duration of IM treatment was 17.1 (range, 2.1-96.2) months. Methods for detecting mutation were direct sequencing (80.2%), pyrosequencing (11.7%), ASO-PCR (6.3%) and others (3%). One hundred twenty three individual mutations were detected in 111 CP-CML patients. As shown in Table1, P-loop (43.1%) and T315I (17.1%) were most frequently developed among 123 mutations. There were no differences between patients remaining CP and patients evolved into advanced stage at the time of IM resistance in terms of P-loop mutations (P=0.142), non-P-loop (P=0.224), T315I (P=0.305) and multiple mutations (P=0.103). The most common single mutation was T315I (n=21/123, 17.1%). Subsequently common mutations were G250E (9.8%), Y253H (8.9%), E255K/V (8.9%), F317L/I (7.3%), F359C/V (5.7%), M244V (4.9%), E459K (4.9%), Q252H (2.4%) and H396R (2.4%) among 123 mutations. No mutation was found in E256K, E257K, V299L and E355G. Multiple mutations were detected in 11 (2 mutations in 10 and 3 mutations in 1) patients. T315I mutation was most common compartment comprising of 18.2% patients harboring multiple mutations. Sensitivity to 2nd generation TKIs based on the IC^{50} value of individual mutation estimated that 37.8% were sensitive to dasatinib and 21.0% to nilotinib. Median overall survival after IM resistance was 47.1 (95% CI 26.9-67.4) months with 42.5% at 5 years and 32.1% at 7 years. Patients harboring T315I, P-loop mutations, and multiple mutations had shorter survival than those having other mutations (median survival 32.6 *vs.* 87.6 months; P=0.012).

Table 1. Frequency of 111 CP-CML patients harboring mutations according to location in Bcr-Abl kinase domain.

No. of mutations	n=123
T315I	n=21 (17.1%)
P-loop	n=53 (43.1%); G250E (9.8%), Y253H (8.9%), E255K/V (8.9%), M244V (4.9%), Q252H (2.4%)
Others	n=49 (39.8%); F317L/I (7.3%), F359C/V (5.7%), E459K (4.9%), H396R (2.4%)
Multiple (duplicated)	n=23; T315I (n=2/23), M244V (n=2/23)

Summary and Conclusions: In conclusion, T315I and P-loop requiring more potent TKI therapy were most frequently detected mutations. And the result demonstrated some differences in the frequency and location of BCR-ABL1 KD mutation based on real clinical practice.

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COMPARISON OF NON AUTOMATED BCR-ABL1 RT-QPCR WITH THE XPERT BCR-ABL MONITOR TM ASSAY

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Background: *BCR-ABL1* quantification by reverse transcriptase quantitative PCR (RT-qPCR) is largely used in different centers to monitor chronic myeloid leukemia (CML) patients. Efforts towards standardization of the *BCR-ABL1* quantification have been made in the last years. To address this issue semi or automated assays have emerged. The automated cartridge-based assay (Xpert BCR-ABL Monitor™, Cepheid) showed very good inter-laboratory reproducibility. Nevertheless intra-laboratory reproducibility was not evaluated for blood samples from CML patients.
Aims: This study aimed to compare intra-laboratory reproducibility of automated and non automated *BCR-ABL1* quantification.
Methods: Twelve blood samples from CML patients with *BCR-ABL1* values between 0.001% and 10% were tested in quadruplicate for each method, from RNA extraction to qPCR. The non automated but standardized RT-qPCR followed the EAC protocol with a validated conversion factor according to the EUTOS program. Correlation of mean values between both methods, standard deviations (σ) and coefficients of variation (CV) of replicates for each sample were assessed.
Results: Three samples were found undetectable for at least one replicate on the GeneXpert®. These same samples were found positive with very low level of *BCR-ABL1* ($<RM^{4.5}$) for each replicate with the non automated RT-qPCR except for one. This last one was found undetectable in one out of 4 replicates whereas it was undetectable in all 4 replicates with the GeneXpert®. Regarding the nine other positive samples, a very good correlation (R^2=0.99) was found between both methods and a trend towards a better reproducibility was observed for the automated RT-qPCR as compared to the non automated method for *BCR-ABL1* values over 1% (σ_{mean}: 0.12% *vs* 0.5%; CV_{mean}: 6.68 *vs* 17.15%). Similar reproducibility was observed for *BCR-ABL1* values below 1% (σ_{mean}: 0.03% *vs* 0.04%; CV_{mean}: 25.8% *vs* 25.1%).
Summary and Conclusions: The GeneXpert® shows results highly comparable to the EUTOS validated technique down to $RM^{4.5}$. Past this limit, the 200µl sampling size seems insufficient, a larger volume of sample should resolve this problem. A better reproducibility was observed for values over 1%.

P707

SAFETY AND DURABILITY OF PONATINIB IN PATIENTS WITH PHILADELPHIA CHROMOSOME-POSITIVE (PH+) LEUKEMIA: LONG-TERM FOLLOW-UP OF AN ONGOING PHASE 1 STUDY

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Background: Ponatinib is a potent oral pan–BCR-ABL tyrosine kinase inhibitor (TKI) that is active against native and mutated forms of BCR-ABL.
Aims: The safety and anti-leukemic activity of ponatinib in patients with chronic myeloid leukemia (CML) or Ph+ acute lymphoblastic leukemia (ALL) were evaluated in a phase 1 clinical trial.
Methods: Patients (N=81) with resistant/refractory hematologic malignancies were enrolled in this ongoing, open-label, dose escalation, phase 1 study. Ponatinib was dosed once daily (2–60 mg). Sixty-five patients had Ph+ leukemia and are included in the present analysis (data as of 9 Nov 2012). Median follow-up was 25 (0.5–44) months.
Results: The median age of patients was 55 years; median time since diagnosis was 6.5 years. Patients were heavily pretreated (94% had received ≥2 prior TKIs, 62% had received ≥3). Baseline BCR-ABL mutations were detected in 65% of patients. Forty-six percent (67% chronic phase [CP] CML) of patients remained on study. Progressive disease and adverse events (AEs) were the most common reasons for discontinuation (17% each). The most common treatment-related AEs were rash (42%), thrombocytopenia (34%), arthralgia (20%), and increased lipase (20%). Rash, arthralgia, and increased lipase were generally grade 1 or 2 in severity; thrombocytopenia was generally grade 3 or 4. Significant anti-leukemic activity was observed (Table 1). Responses (major cytogenetic response [MCyR] for CP-CML or major hematologic response [MaHR] for accelerated phase [AP] CML, blast phase [BP] CML, or Ph+ ALL) were observed against 6 of the 7 mutants detected in >1 patient at baseline: 14/19 T315I, 4/7 F317L, 2/4 G250E, 2/2 M244V, 2/2 M351T, 1/2 F359V, and 0/2 H396R.Among CP-CML patients, 81% with complete cytogenetic response (CCyR) and 74% with major molecular response (MMR) are estimated to maintain response at 1 year (Kaplan-Meier); 73% with CCyR and 63% with MMR are estimated to maintain response at 2 years. The duration of CCyR ranged from 1.9–35+ months, and the duration of MMR ranged from 0.02–38+ months (median not yet reached for CCyR or MMR). Of 28 CP-CML patients with CCyR, 25 remained on study (19 with continuous CCyR); of 22 patients with MMR, 21 remained on study (15 with continuous MMR). Updated data will be presented.

Table 1.

	CP-CML N=43	AP-CML N=9	BP-CML N=8	Ph+ ALL N=5
MaHR	n/a	44%	25%	40%
MCyR	72%	22%	38%	40%
CCyR	65%	22%	13%	20%
MMR	51%[a]	11%	n/a	n/a

n/a=not applicable
[a]14 (33%) CP-CML patients achieved MR[4]; 8 (19%) achieved MR[4.5]

Summary and Conclusions: Significant and durable responses were observed in heavily pretreated CP-CML patients, regardless of mutation status, and ponatinib was generally well tolerated. ClinicalTrials.gov ID NCT00660920

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PHARMACOKINETIC AND -DYNAMIC MONITORING DURING FIRST LINE TREATMENT WITH NILOTINIB IN EARLY CHRONIC PHASE CML–FIRST RESULTS FROM THE ENEST1ST SUB STUDY CAMN107EIC01

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Background: Imatinib trough plasma level testing and pharmacodynamic evaluation (*i.e.* quantification of phosphorylated CrkL) may help to tailor patient-specific therapy.For the 2nd generation TKI nilotinib the value of such information is less clear.
Aims: We here present first data from a substudy of the phase IV ENEST1st trial testing uP-front nilotinib therapy in early chronic phase CML. The study addressed the value of plasma-level testing and protein phosphorylation pharmacodynamics for clinical outcome.
Methods: Drug levels were centrally quantified by means of mass spectrometry (HPLC-MS/MS) in 54 patients at 3 hours after first drug intake at day (d) 1 (C_{max}) and at d7, d28 and months (m)3,6, 12 prior to morning drug intake (C_{min}). Single cell quantification of intracellular protein phosphorylation was performed by flow cytometry (phosphoflow) in the myelocyte cell population after full blood fixation/cell permeabilization/erythrocyte lysis. BCR-ABL phosphoprotein signalling was detected by phosphoflow at d1 prior to first drug intake, 3 hours after first drug intake as well as at d7 and d28.
Results: In our first analysis we focussed on correlation with early molecular response, *i.e.* IS 0.1% (MMR) and CMR at months3,6, and 12. Of the 54 patients, 21% achieved MMR at 3m and 55% at 6m of therapy (including 2 CMR). Only 4 patients at 3m and 1 patient at 6m did show less than IS 10% BCR-ABL load. 11% achieved CMR at 12m with remaining patients except one showing MMR. At d1 three hours after first drug intake median peak plasma levels reached 405 (range 0-1188) ng/mL increasing to a median trough level of 820 ng/mL at d7 and 880 ng/mL at d28. Thereafter from d28 to d360 plasma trough levels remained stable with individual values ranging from 347 to 2522 ng/mL. Initial C_{max}-levels at d1 significantly correlated with steady state levels thereafter. In addition, the final steady state levels were also influenced by the slope of the subsequent increase (ranging from -21% to 580%). Higher plasma levels of nilotinib were significantly associated with lower BCR-ABL mRNA burden at 3m with a similar trend observed at 6m and 12m. If median steady-state drug levels were compared in different response categories (CMR *vs.* no CMR, MMR *vs.* no MMR, >IS 10% at 3m *vs.* <IS 10%) we could identify significantly higher levels in MMR at 3m (P=0.0083), as well as increased 3m plasma levels were associated with MMR at 6m (P=0.045) and CMR at 12m (P=0.001). Phosphoflow of myelocytes revealed that compared to baseline, phosphorylation of almost all investigated proteins decreases over time, including significant reduction of phospho-Gab2(Y452), Abl(Y245), STAT5(Y694), Erk1/2(T202/Y204), p38(T108/Y182) at d28. Correlation of plasma levels with changes in phosphorylation status revealed a negative correlation of increasing drug levels with decreasing phospho-p38 and phospho-STAT5 status.
Summary and Conclusions: Inter-individual plasma levels show a relatively wide variability. C_{max} levels upon first drug exposure correlate with later trough levels pointing out to a potential individual pharmacogenetic background regulating systemic drug availability. Higher trough nilotinib plasma levels are associated with improved response rates. Single cell myelocyte phosphoprotein detection allowed direct monitoring of components in the BCR-ABL signalling network. More detailed analyses will be presented at the meeting.

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RESPONSE DYNAMICS AND OUTCOMES IN PATIENTS DEVELOPING MUTATIONS DURING FIRST-LINE TREATMENT WITH DASATINIB OR IMATINIB FOR CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): DASISION 3-YEAR FOLLOW-UP

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Background: BCR-ABL mutations may be responsible for 40–60% of secondary resistance to imatinib. Newer therapies enable many patients (pts) to over-

come imatinib resistance, including pts with mutations. A small number of mutations confer resistance to second-line dasatinib (DAS) or nilotinib, but less is known about their emergence during first-line treatment. Previous analyses identified mutations in CML-CP pts who discontinued treatment in the first-line phase 3 DASISION trial of DAS *vs* imatinib (IM).

Aims: Perform a mutational analysis to include pts with on-treatment events and at discontinuation to potentially identify pts at higher risk of developing clinically relevant mutations. Explore relationships between development of mutations, response dynamics, long-term outcomes, and baseline factors.

Methods: In the DASISION trial, 519 CML-CP pts were randomized to receive DAS 100 mg QD (n=259) or IM 400 mg QD (n=260). Based on 3-year (y) minimum follow-up, an exploratory mutational analysis was conducted evaluating 1) pts who discontinued treatment for any reason and 2) pts on treatment with ≥1 clinically relevant event: no complete cytogenetic response (CCyR) or major molecular response (MMR) within 1 y, loss of CCyR, and 5-fold increase in BCR-ABL with loss of MMR. Pts were screened for mutations at time of discontinuation from treatment or by retrospective analysis for pts on treatment having ≥1 clinically relevant event.

Results: This analysis evaluated pts identified on-treatment with ≥1 clinically relevant event or who discontinued treatment (Table 1). Among all pts analyzed, mutations were identified in a small number of pts in both arms (17 DAS; 15 IM), most in pts screened due to discontinuation, with 6 pts (5 DAS, 1 IM) identified by on-treatment events. While a similar number of pts with mutations were identified in both arms, there were several differences: DAS *vs* IM had (1) a narrower spectrum of mutations, (2) fewer pts with >1 mutation (1 *vs* 6; see footnote [a] in Table), and (3) more pts with T315I. Pt follow-up after 3 y showed that most pts with mutations discontinued treatment, primarily due to disease progression, with death occurring in 10 of these pts (6 DAS, 4 IM). Of the 5 pts with mutations remaining on treatment (4 DAS [1 G250E, 1 V299L, 2 T315I] and 1 IM [F359V]), 3 of the DAS pts remain in CCyR at last follow up, including both pts with T315I, 1 with 40%, and 1 with 80% T315I abundance. Analysis of molecular and cytogenetic response dynamics showed that most pts found to have mutations either (1) had no initial response or (2) experienced a transient modest early response rarely deep enough to achieve an MMR.

Table 1. Summary of mutational analyses.

	DAS 100 mg QD n=259	IM 400 mg QD n=260
Pts identified for mutational analysis, n (%)	169 (65)	194 (75)
Discontinued treatment	75	79
Pts with mutations	12	14
On treatment with ≥1 clinically relevant event	94	115
Pts with mutations	5	1
Pts with mutations (total), n	17	15
Mutations detected[a], n	F317I/L (3) V299L (3) T315I (11) G250E (1)	M244V (1) L248V (1) G250E (1) E255K/V (4) D276G (1) M351T (3) E355G (2) F359C/V (4) L387M (1) H396P (1) E450G (1) Y253H (1)
Current status of pts with mutations[b], n		
On treatment	4	1
Discontinued treatment and alive	7	10
Died	6	4

[a]Includes pts with 2 mutations: 1 DAS (V299L/F317I) and 6 IM (M351T/F359V, E255V/E450G, L248V/E355G, E255K/M351T, E255V/Y253H, D276G/F359C).
[b]Based on most recent follow-up (after 3 y).

Summary and Conclusions: Consistent with previous reports, a narrow spectrum of mutations was found in pts treated with first-line DAS *vs* IM. Most pts who developed mutations failed to achieve or maintain cytogenetic or molecular response. While in both arms few patients with mutations were identified, outcomes were generally poor for pts with mutations regardless of therapy. Notably, more pts with IM *vs* DAS had multiple mutations.

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VALIDATION OF THE EUTOS SCORE FOR PREDICTION OF COMPLETE CYTOGENETIC RESPONSE AND PROGRESSION FREE SURVIVAL: APPLICATION TO INDEPENDENT DATA FROM THE EUTOS REGISTRY AND REVIEW OF PUBLICATIONS

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Background: The EUTOS score is predictive for complete cytogenetic remission (CCyR) at 18 months and subsequent progression free survival (PFS) in patients with chronic phase Chronic Myeloid Leukemia (CML) treated first line with imatinib. The score uses percentage of basophils and spleen size to classify patients into a high and a low risk group (Hasford *et al.*, Blood 2011).

Aims: The EUTOS score was developed and independently validated on patients enrolled in prospective, protocol-based phase III- and IV- studies of frontline therapy which typically recruit highly selected patients only. Now the score is tested in routine CML-patients, registered prospectively by National Study Groups and who were not included in clinical trials. Additionally, publications on validity of the EUTOS score are reviewed.

Methods: A total of 1288 patients were collected from a hospital-based national group located in Madrid (n=193), from the national Polish registry (n=281), from two Russian registries located in Moscow and in St. Petersburg (n=493), from two Czech registries (Camelia and Infinity) (n=309, including 96 patients from Slovakia), and from a Romanian registry (n=12). PFS, time to CCyR and CCyR status 18 months after the start of therapy were calculated and analyzed using Kaplan-Meier and cumulative incidence curves, and respective Gray and Log-Rank tests. For cumulative incidence analyses death was considered a competing risk. PFS was measured from the start of imatinib treatment to date of death or progression to blastic or accelerated phase. PubMed and Abstract Books of relevant conferences were searched for publications on the EUTOS score. Information on sample size, percentage of high risk patients, median age and follow up time was collected.

Results: Of 1288 patients 52% were male. Median age was 49 years (range 18-85), and median observation time was 65 months (range 2-112). There were 161 (12.5%) high risk patients. At 18 months 61.3% of the patients in the high risk and 73.0% of the low risk group were in CCyR (P=.036). In high risk patients, the cumulative probability of ever achieving a CCyR over the whole observation time, was significantly lower than in low risk patients (P<.0001), being 67.6% (95% CI 59.1–74.7) *vs.* 84.4% (95% CI82.1% - 86.7%) at 5 years. This indicates that patients not treated in clinical studies achieve CCyR later than patients enrolled in clinical trials. PFS differs significantly for EUTOS high and low risk group (P=.0395). After 5 years PFS was 89.3% (95% CI 87.1% - 91.1%) in low risk patients *vs.* 82.0% (95% CI 74.7% - 87.3%) in the high risk ones. Thus even in this sample of non-trial patients the EUTOS score identified two distinct prognostic groups. There were eight publications on the validity of the EUTOS score identified. Authors were located in Europe, Asia, North and South America and their reports included 139 to 279 patients. Median age ranged from 43 to 55 years, and median follow up time ranged from 29 to 117 months. Percentage of patients in the EUTOS high risk group started at 5% in Italy and went up to 31% in Singapore (median 9.6%). Most publications discussed patients treated with imatinib, a study from the USA had 34% of patients treated with second-generation tyrosine kinase inhibitors (TKIs) a study from Italy reported exclusively on patients with second-generation treatment. Further, six studies reported P-values for the cumulative incidence of CCyR, most of them indicating major differences between high and low risk group and half of them below 0.05. Six studies specified P-values for PFS, ranging from 0.01 to 0.73, four studies reported P-values of 0.05 or lower.

Summary and Conclusions: The validity of the EUTOS score was confirmed in the patients treated outside of prospective, protocol-based clinical studies. In addition, many independent studies from all over the world reported a substantial predictive value for cytogenetic remission and progression free survival. Even considering the comparatively small number of events in patients treated with TKIs the combined evidence indicates that the EUTOS score is able to reliably identify a small group of patients at high risk of suboptimal outcome.

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LONG-TERM SURVIVAL OUTCOME AND PROGNOSIS IN 2277 PATIENTS WITH CHRONIC MYELOID LEUKAEMIA ALLOCATED TO FIRST-LINE IMATINIB TREATMENT–NEW RESULTS FROM THE EUTOS IN-STUDY REGISTRY

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Background: In the European Treatment and Outcome Study (EUTOS) registry, individual data on patients with chronic myeloid leukaemia (CML) enrolled in prospective, controlled clinical trials are collected. Included were adult patients with Philadelphia chromosome-positive chronic-phase (CP) CML treated with any imatinib-based treatment within half a year after diagnosis. At first, focus was on the analysis of cytogenetic response resulting in the EUTOS score (Hasford *et al.*, 2011). Now, with each of six studies providing a median observation time of at least five years and altogether 2277 patients fulfilling the inclusion criteria, the EUTOS In-study registry allows a closer look on long-term survival outcome.
Aims: To describe long-term survival outcome and possibly identify variables with a prognostic influence on overall survival (OS).
Methods: Survival was censored at the time of allogeneic stem cell transplantation (SCT) in first CP. Time and frequency of progression were analysed by the cumulative incidence approach with death in CP as a competing risk. Definition of progression was in accordance with the ELN criteria (Baccarani *et al.*, 2009). Prognostic influence on OS was estimated with multiple Cox regression. Candidate variables were age, sex, spleen enlargement, haemoglobin, platelets, leukocytes, and percentages of blasts, eosinophils, and basophils in peripheral blood. Level of significance was 0.05.
Results: The 2277 patients came from study groups in Germany, France, Italy, Spain, the Netherlands, and from the Nordic study group. Median observation time was 6.25 years. Allogeneic SCT in first CP was performed in 99 patients (4%). Five-year survival probability was 90% [95%>confidence interval (CI): 88-91%]. Sufficient data on progression was available for three study groups. With death as a competing risk, five-year progression probabilities were 7% [Germany, 95%>CI: 5-9%], 5% [France, 95%>CI: 3-6%], and 6% [Italy, 95%>CI: 4-8%]. Assuming thus comparable progression evaluation, the 1821 patients of these three study groups could be merged and had a progression-free survival of 90% [95%>CI: 88-91%]. The learning sample for regression analysis, based on four studies with similar follow-up patterns, comprised 1884 patients with complete data for the candidate prognostic factors. Their median age was 52 years [range: 18-88], 60% were male. Besides age, multiple modelling also suggested that the interaction between sex and haemoglobin had an influence on survival (always: P<0.005). Females with lower haemoglobin values had less favourable survival probabilities while haemoglobin showed no effect in males. With patients only randomised within but not between the studies and the restriction that only a cut-out of the studies' patients was available, an unbiased investigation of OS differences between different imatinib-based treatments must be referred to the individual study groups. However, there was no apparent OS advantage for any of the treatments.
Summary and Conclusions: The median observation time of at least 6.25 years in 1884 patients with 181 events actually enabled prognostic modelling in imatinib-treated patients. Higher age significantly reduced OS probabilities. Though the age effect was considerable and a median observation period of 6.25 years suggests few deaths not directly related to CML, the next step will be to collect more information on the causes of death and also on disease progression. For further investigation, additional sensitivity analyses and a later validation in independent patients will be performed.

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NILOTINIB SHOWS SUSTAINED BENEFIT COMPARED WITH IMATINIB IN PATIENTS (PTS) WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): ENESTND 4-YEAR FOLLOW-UP (F/U)

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Background: In the Evaluating Nilotinib Efficacy and Safety in Clinical Trials–Newly Diagnosed Pts (ENESTnd) trial, nilotinib has demonstrated superior rates of major molecular response (MMR; BCR-ABL^{IS}≤0.1%), deeper molecular responses (MR^4, BCR-ABL^{IS} ≤0.01%; MR$^{4.5}$, BCR-ABL^{IS} ≤0.0032%) and reduced rates of progression compared with imatinib in pts with newly diagnosed, Philadelphia chromosome–positive (Ph+) CML-CP.
Aims: This analysis aims to explore the long-term efficacy and safety of frontline nilotinib *vs* imatinib in ENESTnd with a minimum of 4 y f/u.
Methods: Adult pts with newly diagnosed Ph+ CML-CP (N=846) were randomized to receive nilotinib 300 mg twice daily (BID; n=282), nilotinib 400 mg BID (n=281), or imatinib 400 mg once daily (QD; n=283) in this open-label, phase 3 study. Efficacy analyses were based on the intent-to-treat population, which included all randomized pts. Response assessments were collected during study treatment. Information on progression to accelerated phase/blast crisis (AP/BC) and overall survival (OS) was prospectively collected during f/u every 3 mo after discontinuation of treatment for the first 5 y and every 6 mo thereafter.
Results: With 4 y of f/u, nilotinib continued to demonstrate significantly higher rates of MMR, MR^4, and MR$^{4.5}$ *vs* imatinib (Table 1). Rates of MR$^{4.5}$ were consistently superior with nilotinib *vs* imatinib across all Sokal risk groups. Pts on both nilotinib arms had significantly lower rates of progression to AP/BC on study (including f/u after discontinuation of treatment) *vs* pts on the imatinib arm (n=9 [3.2%], 6 [2.1%], and 19 [6.7%] in the nilotinib 300 BID, nilotinib 400 mg BID, and imatinib arms, respectively). Of the pts who progressed on core study treatment (n=2 [0.7%], 3 [1.1%], and 12 [4.2%] in the nilotinib 300 mg BID, nilotinib 400 mg BID, and imatinib arms, respectively), the majority never achieved complete cytogenetic response (CCyR); no pt who has achieved MR$^{4.5}$has progressed (Table 1). The 4-y OS rates were 94.3%, 96.7%, and 93.3% for nilotinib 300 mg BID, nilotinib 400 mg BID, and imatinib, respectively. Treatment-emergent BCR-ABL mutations were less common in pts on nilotinib *vs* imatinib (detected in12,11, and 22 pts in the nilotinib 300 mg BID, nilotinib 400 mg BID, and imatinib arms, respectively); only 2 pts had newly emergent mutations in y 4 (1 pt with T315I on nilotinib 300 mg BID; 1 pt with F317L on imatinib). The safety profiles of both drugs were consistent with those previously reported, with few newly occurring hematologic/biochemical abnormalities or cardiac/vascular events in any treatment arm, and no new safety signals. By 4 y, peripheral arterial occlusive disease (PAOD) was reported in4,5, and 0 pts, and ischaemic heart disease (IHD) was reported in11,14, and 3 pts in the nilotinib 300 mg BID, nilotinib 400 mg BID, and imatinib arms, respectively. The annual frequency of these events has not increased with longer f/u (eg, in the nilotinib 300 mg BID arm, there were 4 PAOD events by 2 y, 0 from 2-3 y, and 0 from 3-4 y, and there were 5 IHD events by 2 y, 4 from 2-3 y, and 2 from 3-4 y). Based on a combination of baseline risk factors, 7 of the 9 pts with a PAOD event on nilotinib were at high risk for PAOD.

Table 1.

	Nilotinib 300 mg BID (n = 282)	Nilotinib 400 mg BID (n = 281)	Imatinib 400 mg QD (n = 283)
Still on active f/u[a] or died, %	94	95	93
Still on core treatment, %	66	69	57
Molecular response by 4 y (ITT), % (*P* value vs imatinib)			
MMR	76 (< .0001)	73 (< .0001)	56
MR^4	56 (< .0001)	50 (< .0001)	32
MR$^{4.5}$	40 (< .0001)	37 (.0002)	23
MR$^{4.5}$ by 4 y according to Sokal risk (ITT), % (*P* value vs imatinib)			
Low	38 (.17)	45 (.018)	29
Intermediate	48 (.0004)	34 (.11)	24
High	32 (.0040)	31 (.0066)	13
4-y freedom from progression to AP/BC,[b,c] % (*P* value vs imatinib)			
On study[a]	96.7 (.050)	97.8 (.0074)	93.1
Best response in pts who progressed to AP/BC on core treatment,[c] n			
< CCyR	2	1	8
CCyR	0	0	4
MMR	0	1	0
MR^4	0	1	0
≥ MR$^{4.5}$	0	0	0
4-y EFS on core treatment,[b,d] %	94.5 (.19)	97.4 (.011)	92.6
4-y PFS on core treatment,[b,e] %	96.1 (.20)	98.3 (.026)	94.7
4-y OS on study,[a,b] %	94.3 (.47)	96.7 (.050)	93.3
Pts with emergent BCR-ABL mutations on core treatment,[f] n			
Any mutation, n	12	11	22
Mutation category:			
T315I, n	4	2	3
Less sensitive to nilotinib,[g] n	6	9	4
Other mutation,[h] n	2	0	15

[a] Including core or extension treatment or f/u after discontinuation.
[b] Kaplan-Meier estimate.
[c] Progression to AP/BC events included progression to AP/BC or CML-related death.
[d] EFS events included: death from any cause, progression to AP/BC, loss of partial CyR, loss of CCyR, or loss of complete hematologic response.
[e] PFS events included progression to AP/BC or death from any cause.
[f] There were 120, 136, and 199 pts in the nilotinib 300 mg BID, nilotinib 400 mg BID, and imatinib arms, respectively, who had ≥ 1 postbaseline mutational analysis.
[g] Mutations clinically less sensitive to nilotinib included E255K/V, F359C/V, and Y253H.
[h] Includes all mutations other than less-sensitive mutations and T315I.
EFS, event-free survival; ITT, intention-to-treat; PFS, progression-free survival.

Summary and Conclusions: With 4 y of f/u, nilotinib continues to demonstrate significantly higher rates of molecular response, lower rates of progression to AP/BC, and fewer treatment-emergent BCR-ABL mutations *vs* imatinib. These data further support the use of nilotinib as frontline therapy in newly diagnosed pts with Ph+ CML-CP.

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DEFINING LOW AND UNDETECTABLE LEVELS OF DISEASE IN CHRONIC MYELOID LEUKAEMIA: PROGRESS TOWARDS STANDARDISATION IN EUROPE

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Background: The international, collaborative effort to standardise molecular testing for chronic myeloid leukaemia (CML) to date has focused largely on detectable residual disease, with a particular emphasis on determining whether (or not) a patient has achieved major molecular response (MMR). However, with new definitions of deep molecular responses (MR) a new problem arises in how to define assay sensitivity in a standardized manner when *BCR-ABL1* mRNA is undetectable.

Aims: We have previously established draft criteria to define deep molecular responses (MR^4, $MR^{4.5}$ etc) of residual disease in CML. To assess how well these definitions might work in practice and enable laboratories to benchmark their performance with regard to assay sensitivity in relation to others we sent out a series of cell line and armoured RNA (aRNA) samples along with a common (IRMM) plasmid dilution to testing laboratories. We evaluated assay sensitivity in two ways: (a) by directly evaluating the ability of labs to detect low level positive samples and (b) measuring absolute control gene (CG) transcript numbers as a surrogate for sample quality and thus theoretical sensitivity with which *BCR-ABL1* could be detected.

Methods: Cell line and aRNA samples were sent to EUTOS (European Treatment and Outcome Study) reference laboratories who agreed to participate (n=39). Laboratories tested two different levels of *e14a2* aRNA diluted in a background of *ABL1* aRNA only (MMR, MR^4) and three different cell line lysates (5%, 0.05%, 0.005% *BCR-ABL1*^{IS}). Absolute copy numbers of *BCR-ABL1* and *CG* and the *BCR-ABL1/CG* ratios before and after conversion to the international scale (IS) using a common plasmid standard curve and laboratory plasmid standard curve were recorded.

Results: Cell line and aRNA analysis indicated good comparability of%*BCR-ABL1* /*CG* results that was improved by use of the common plasmid. Analysis of the cell line lysates and aRNA samples indicated that all labs were able to detect *BCR-ABL1* at the lowest dilution (which corresponded to MR^4), however the median number of *CG* transcripts in the cell line samples (*ABL1*=74,509 (n=33); *GUSB*=84,762 (n=6)) was higher than would be expected from patient samples. Analysis of absolute numbers of *BCR-ABL1* and *ABL1* transcripts using the aRNA samples revealed that most labs are underestimating the absolute number of transcript copy numbers, even when using a common plasmid calibrator. All aRNA samples were predicted to contain 305,500 *ABL1* copies per µl aRNA but when laboratory data were corrected for the amount of aRNA added to the PCR, the median number of *ABL1* copies per µl aRNA reported was only 54,578 (n=31) using the usual laboratory standard curve and 28,458 (n=32) using the IRMM standard curve. These data suggest that there is substantial scope for most labs to improve the quality of their cDNA synthesis procedures and thereby increase assay sensitivity.

Summary and Conclusions: Good comparability was seen between the 39 participating labs for samples where *BCR-ABL1* was detectable, but assay sensitivity based on *CG* numbers was highly variable. Although the use of a common plasmid calibrator improved the comparability, it did not correct for differences in estimates of absolute *CG* numbers. The reasons for these differences are unclear but may relate to differences in the efficiency of reverse transcription. Further investigation will be required to determine if this is the case, and how to improve *CG* numbers/sensitivity where necessary.

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CLINICAL SIGNIFICANCE OF EARLY MOLECULAR RESPONSES (BCR-ABL1 ≤10% AT 3 MONTHS AND ≤1% 6 MONTHS) AS A PREDICTOR FOR LONG-TERM OUTCOMES IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH IMATINIB

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Background: Imatinib (IM) has been the standard of care for chronic phase (CP) chronic myeloid leukemia (CML). Complete cytogenetic response (CCyR) has been established as a predictor for outcome by the International Randomized Study of Interferon and STI571 (IRIS). Although recent studies have suggested that in IM-treated patients, measurements of BCR-ABL1 transcript levels at 3 and 6 months were able to identify high-risk patients, suggesting that the early switch to second-generation tyrosine kinase inhibitors may be beneficial for the high-risk patients, further validation is needed.

Aims: The aim of this study was to investigate clinical significance of early molecular responses (BCR-ABL1 ≤10% at 3 months and ≤1% 6 months) as a predictor for long-term outcomes in newly diagnosed CP CML patients treated with IM.

Methods: 620 consecutive patients were newly diagnosed as CP CML at Seoul St. Mary Hospital between January 2001 and December 2011, and started IM (400 mg/day) therapy without prior treatment except hydroxyurea or anagrelide. Among them, quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) data at 3 months from 146 patients were available for this study. Their molecular responses were monitored using qRT-PCR assay with 3 month intervals, and then 6 month intervals after achieving major molecular response (MMR). All qRT-PCR tests were performed in a single laboratory (Cancer Research Institute, The Catholic University of Korea, Seoul, Korea).

Results: A total of 146 newly diagnosed CP CML patients (including 80 men and 66 women) were analyzed. Their median age was 41 years (range, 18-74). The percentages of patients with low, intermediate and high Sokal risk scores were 30%, 23% and 34%, respectively with unknown Sokal risk scores in 13%. At 3 months, patients showed BCR-ABL1 ≤1% (n=48), >1 to ≤10% (n=54), or >10% (n=44). For 128 patients with qRT-PCR data at 6 months, they showed BCR-ABL1 ≤1% (n=73), >1 to ≤10% (n=39), or >10% (n=16). In patients with BCR-ABL1 ≤10% at 3 months, significantly higher rates of CCyR at 12 months (94% *vs* 78%, P=0.020) were observed, compared with that of patients with BCR-ABL1 >10%. They also had significantly better 7-y FFS (92.3% *vs* 69.2%, P<0.001), PFS (99.0% *vs* 81.2%, P<0.001), and OS (99.0% *vs* 93.2%, P=0.050). Patients with BCR-ABL1 ≤1% at 3 months had a better 7-y FFS (91.9% *vs* 82.5%, P=0.052) and PFS (100% *vs* 90.5%, P=0.041). However, there were no signifi-

cant differences in CCyR at 12 months and 7-y OS. Patients who had BCR-ABL1 >10% at 6 months had a trend for higher progression, compared with those who achieved ≤10% response at 6 months, which was translated into lower 7-y PFS (97.4% *vs* 86.5%, P=0.022). Patients with BCR-ABL ≤1% at 6 months had also significantly better PFS (100% *vs* 91.4%, P=0.028).
Summary and Conclusions: The achievement of ≤10% response at 3 months is predictive for long term FFS, PFS, and OS, and the ≤1% level of response at 3 months was associated with better FFS and PFS. Achieving a MMR or better at 6 months showed clinical significance in PFS. These results imply that early molecular response assessment may identify a group of patients requiring a change of treatment. In the meeting, we will show updated and extended data and predictors analyzed for achieving early molecular response, including Sokal risk score, IM dose intensity, and the result of IM blood level test.

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THE PERCENTAGE OF EFFECTOR POPULATIONS OF NK CELLS CORRELATED HIGHLY WITH SUSTAINED COMPLETE MOLECULAR RESPONSE WITH OR WITHOUT IMATINIB IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: Recently, it has been found that some chronic myeloid leukemia (CML) patients with complete molecular response (CMR) are able to maintain CMR after discontinuing imatinib (IM). Among patients with CMR lasting for at least 2 years, 41% maintained CMR conditions after discontinuing IM (Lancet Oncol. 2010;11:1029). We have recently reported that patients with sustained CMR after discontinuing IM had increasing numbers of natural killer (NK) cells (Br. J. Haematol. 2012;157:254). These findings indicate that immunological surveillance may have some effects in maintaining CMR after discontinuing IM.
Aims: To identify the long-term immunological effects on CML patients after discontinuing IM, we characterized their immunophenotypic profiles. We compared the profiles of CML patients who received IM with CMR for more than 2 consecutive years (CMR group), those who could not sustain CMR but maintained a major molecular response (fluctuating CMR group), those who sustained CMR after discontinuing IM (STOP-IM group), and healthy volunteers (control group). Some patients in the STOP-IM group were analyzed sequentially.
Methods: Immunophenotyping analysis of peripheral blood mononuclear cells (PBMCs) was performed using a 5-color flow cytometry panel including antibodies against the following cell surface antigens and effector molecules: CD3, CD8, CD45RO, CD56, CCR7, IFN-γ, granzyme-B, and perforin. After the PBMCs were stimulated with phorbol 12-myristate 13-acetate and ionomycin for 4 h in the presence of monensin, cell surface antigens were stained, fixed, and permeabilized.
Results: The percentage of effector populations of NK cells, such as interferon (IFN)-$\gamma^{+}CD3^{-}CD56^{+}$ cells, was significantly higher in the CMR and STOP-IM groups than in the fluctuating CMR group. In the STOP-IM group (maintaining CMR without IM), the elevated percentage (approximately >10%) of IFN^{+} NK cells was maintained for more than 12 months after discontinuing IM. By contrast, the percentage of effector populations of $CD8^{+}$ T cells in the CMR group did not differ significantly when compared with those in the STOP-IM group. However, we had 1 patient who showed reduction in NK cells concomitant with molecular relapse after discontinuing IM.
Summary and Conclusions: The STOP-IM group maintained higher percentages of functional NK cells than the fluctuating CMR group, and maintained elevated levels for more than 2 years even without IM, indicating that the elevation of functional NK cell population in CMR is not attributable to IM. Also, the elevation of functional NK cells in CMR patients, when compared with those in the fluctuating CMR group, suggests that NK cells may have some effects in maintaining CMR as immunological surveillance in CML therapy.

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HEPATITIS B VIRUS REACTIVATION IN CHRONIC MYELOID LEUKEMIA TREATED WITH VARIOUS TYROSINE KINASE INHIBITORS: A MULTICENTER RETROSPECTIVE STUDY

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Background: BCR-ABL1 tyrosine kinase inhibitors (TKI) are now a standard initial treatment for chronic myeloid leukemia (CML). Several cases reported that hepatitis B virus (HBV) reactivation was related to imatinib therapy. However, it is still unclear whether imatinib or other TKIs induce HBV reactivation in hepatitis B surface antigen (HBsAg)-positive patients.
Aims: The aim of this study is to investigate the incidence of HBV reactivation and analyze risk factors associated with HBV reactivation in CML patients who are treated with various TKIs.
Methods: We have retrospectively analyzed patients who had been treated with imatinib until February 2012, from 8 Korean university hospitals. All of CML patients diagnosed reviewed centrally. HBsAg-positive CML patients under imatinib or other TKI treatments were analyzed.
Results: 702 patients were diagnosed with CML from participating centers. The HBsAg-positive rate was 6.1% (43/702) at diagnosis. In the 43 HBsAg-positive patients, nine patients received prophylactic therapy and HBV reactivation rate was 34.9% (15/43) (95% CI: 21.0-50.9%). The cumulative incidence of HBV reactivation was 52.9% in patients without HBV prophylaxis. Patients who received prophylaxis did not develop HBV reactivation. The median age and the male to female ratio of the HBV reactivated patients were 47.0 years (range; 22-63) and 4:1, respectively. HBV reactivation according to each TKI treatment were: 12 cases under imatinib, 2 cases under dasatinib, and 1 case under nilotinib. Median time to HBV reactivation was 9.3 months (range; 2.3-68.8 months) (95% CI: 5.9–28.5 months). None of the patients died due to HBV reactivation, but one patient received liver transplantation due to hepatic failure. Prophylactic therapy and HBV DNA levels at diagnosis were the factors associated with HBV reactivation (P=0.011 and P=0.036, respectively). HBV DNA levels at diagnosis significantly affect the time to HBV reactivation from TKI treatment in univariate and multivariate analyses (P<0.001 and P<0.001, respectively).
Summary and Conclusions: To our knowledge, this is the first report that has analyzed HBV reactivation in HBsAg-positive CML patients during TKI treatment. Prophylaxis should be considered to prevent HBV reactivation during TKI treatment. Also, we recommend that HBsAg-positive patients with CML receiving TKI treatment be closely monitored.

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EARLY AND DEEP RESPONSES TO IMATINIB PREDICT NOT ONLY COMPLETE MOLECULAR RESPONSE BUT ALSO THE PROBABILITY FOR MAINTAINING THE RESPONSE.

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Background: Tyrosine kinase inhibitors (TKIs) have consistently proven a survival benefit in patients with chronic myeloid leukemia (CML). TKIs need to be taken for unlimited period of time in order to avoid the risk of relapse or transformation. It has been recently described how patients with a stable and complete molecular response (CMR), could safely quit treatment. Less is known about the real rates of patients under TKI to achieve CMR and which factors may play an important role maintaining the response.
Aims: To describe the percentage of patients who achieve a sustained complete molecular response and to identify factors associated with achieving this response.
Methods: We have analyzed retrospectively 578 patients treated with imatinib as first TKI in the Spanish RELMC registry. All patients were treated out of clinical trials. Follow up and treatment decisions were decided by hematologists according to their clinical judgment. Complete molecular response was defined as undetectable BCR-ABL transcript. Samples were not centralized but all PCR were done in the same laboratory for each patient during follow up.
Results: Out of the 578 patients analyzed, 124 patients (21%) had received interferon prior to imatinib. Sokal risk distribution was 42%, 47% and 11% for low, intermediate and high, respectively. 24% of the patients were treated with second generation TKIs (2GTKIs) (nilotinib or dasatinib) due to intolerance or inadequate response to imatinib. With a follow up of 85.59 months (8.93–130), the cumulative incidence of CMR for patients treated only with imatinib was 51%. Median time to CCR was 31 months. Probabilities for achieving CMR were higher for low and intermediate Sokal risk patients (58% *vs* 51% *vs* 14% for low, intermediate and high risk patients respectively (P=0.2).The cytogenetic response at 6 months was a strong predictor for CMR: 59% *vs* 36% for patients with and without complete

cytogenetic responses (CCyR) at 6 months (P=0.01). Once patients achieved CMR, the probability to maintain the response while on imatinib was 45% (23% of the entire cohort achieved and maintained CMR). Again, early response, defined as CCyR at 6 months, was a predictor factor for maintaining CMR: 68% *vs* 41% (P=.00). We found no association between probabilities of maintaining CMR and other prognostic factors such as Sokal risk index or previous interferon treatment. The probability to obtain CMR among patients who were changed to 2GTKIs was 25%, and this response was related to the indication for treatment changed: 50% *vs* 35% *vs* 19% (P=0.1) for intolerance, suboptimal response and treatment failure respectively. Among patients who achieved RMC after treatment changed, the probability for maintaining this response during follow up was 53%.

Summary and Conclusions: Our results show that complete and maintained molecular responses in patients treated with imatinib occurred in one fifth of the patients. The use of new treatments that enhance the rate of early responses could improve the number of patients candidate for discontinuation treatment clinical trials.

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EVOLUTION OF BOSUTINIB TOXICITY IN PATIENTS WITH PHILADELPHIA CHROMOSOME–POSITIVE LEUKEMIA AFTER RESISTANCE/INTOLERANCE TO PRIOR THERAPY

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Background: Bosutinib (BOS) is an oral dual Src/Abl tyrosine kinase inhibitor (TKI) approved for treatment of Philadelphia chromosome–positive (Ph+) chronic myeloid leukemia (CML) following resistance/intolerance to prior therapy. Prior reports from this phase I/II trial indicated the BOS safety profile was primarily characterized by myelosuppression, gastrointestinal events, and rash.
Aims: The current analysis compares the incidence of toxicity in Year 1 for patients on treatment ≤1 year and within Year 1 and Year 2 for patients on treatment for >1 year.
Methods: BOS 500 mg/day was evaluated in 3 cohorts: chronic phase (CP) CML after imatinib only (CP 2L cohort; n=286); CP CML after imatinib+dasatinib and/or nilotinib (CP 3L cohort; n=119); and accelerated/blast phase CML or acute lymphoblastic leukemia (ALL) after prior TKI therapy (ADV cohort; n=164).
Results: The most common treatment-emergent adverse events (TEAEs) in each cohort occurred more frequently within Year 1 than Year 2 (*Table*). The incidence for grade 3/4 events followed a similar pattern. AEs were the most common reason for BOS discontinuation in Year 1 (CP 2L, 53%; CP 3L, 32%; ADV, 41%). Of the patients whose primary reason for discontinuing BOS was an AE during the first 2 years, most did so during Year 1 (CP 2L, n=51/60 [85%]; CP 3L, n=22/24 [92%]; ADV, n=24/25 [96%]). Among all patients who discontinued BOS during Year 1 (due to any reason: CP 2L, n=97; CP 3L, n=69; ADV, n=125), the most common AEs leading to discontinuation were thrombocytopenia (12%; 9%; 4%), increased alanine aminotransferase (6%; 4%; 2%), neutropenia (3%; 6%; 0%), diarrhea (4%; 3%; 0%), and vomiting (3%; 4%; 1%). Serious AEs were more common among patients who discontinued BOS ≤1 year *versus* on treatment >1 year in the CP 3L and ADV cohorts, but similar in the CP 2L cohort (Table 1).

Table 1.

	CP 2L			CP 3L			ADV		
	BOS ≤1 y (n = 97)	BOS >1 y (n = 189)		BOS ≤1 y (n = 69)	BOS >1 y (n = 50)		BOS ≤1 y (n = 125)	BOS >1 y (n = 40)	
	Y1	Y1	Y2	Y1	Y1	Y2	Y1	Y1	Y2
TEAE									
Diarrhea	90%	81%	40%	83%	82%	50%	69%	90%	28%
Nausea	41%	45%	12%	51%	36%	18%	42%	58%	15%
Vomiting	40%	31%	7%	41%	32%	8%	41%	43%	8%
Thrombocytopenia	34%	28%	19%	28%	36%	20%	34%	30%	18%
Rash	29%	33%	12%	23%	24%	12%	22%	53%	13%
Abdominal pain	24%	21%	8%	19%	16%	14%	16%	30%	8%
Fatigue	23%	19%	10%	25%	16%	14%	19%	10%	10%
Increased ALT	23%	19%	7%	12%	18%	6%	9%	13%	5%
Anemia	22%	14%	13%	15%	12%	8%	36%	35%	18%
Pyrexia	21%	18%	10%	15%	12%	8%	40%	20%	18%
Headache	16%	12%	9%	29%	20%	6%	18%	20%	8%
Serious AE	25%	25%	23%	30%	20%	18%	60%	33%	33%

Summary and Conclusions: Discontinuation due to AEs was observed primarily in Year 1. For patients on BOS for >1 year, the incidence of common TEAEs decreased substantially after Year1, suggesting BOS tolerability improves after long-term exposure.

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MODULATION OF LEUKOCYTE POPULATION DURING FIRST LINE TREATMENT WITH NILOTINIB IN EARLY CHRONIC PHASE CML–FIRST RESULTS FROM THE ENEST1ST SUB STUDY CAMN107EIC01

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Background: We and others have shown that Imatinib and Dasatinib modulate immune responses *in vitro* and *in vivo*. Immunological surveillance in the MRD-situation might be of particular relevance for long-term control or even elimination of CML-repopulating stem cells. Little is known about potential immune-modulatory effects of nilotinib *in vivo*. Thus, a comprehensive immunological monitoring program of the patient's immune system going along with the clinical ENEST1st trial was performed.
Aims: Evaluate and characterize the immune-modulatory effects of nilotinib therapy in newly diagnosed CP-CML patients.
Methods: Peripheral blood was taken prior to treatment initiation and after 6 and12 months from 50 patients treated within the ENEST1st trial testing uPfront nilotinib in early chronic phase CML. Samples were analyzed by nine color flow cytometry employing six panels of antibodies to determine various leukocyte populations including lymphocyte subsets (*e.g.* T cell subpopulations including Treg and NKT cells, NK cells, B cells), myeloid populations (*e.g.* monocytes, MDSC) and dendritic cell subsets (*e.g.* mDC, pDC), respectively. Changes in immune cells were correlated to clinical endpoints.
Results: 55% of the patients included into this substudy achieved MMR at 6m and 75% at 12m of therapy. A high proportion of CD14+ monocytes with aberrant expression of CD56 present at baseline dropped to undetectable levels at 6 and 12 months after treatment. The most prominent immunological change from baseline to month 12 was an increase of the proportion of CD19+ B cells by about 50%. The abundance of CD19+ B cells at baseline was negatively correlated with SOKAL score. Among T-cell subpopulations, the proportion of CD56+ NKT-cells decreased over time. Similarly, the proportion of CD8 cells significantly decreased concomitant with increasing CD4+ T-cells during therapy. In parallel to a decrease of CD45RA+ expressing T cells among both CD8+ and CD4+ T cell subsets, the proportion of CD45R0+ memory cells increased. This increase was mainly due to an increase of the CD95+CD28+ central memory population. In contrast to our expectation, $CD25^{high}$ FoxP3+ Treg cells were even increased transiently 6 months after treatment initiation but at 12 months dropped back to levels seen at diagnosis. So far, we could not detect any significant correlation of immunological changes and clinical outcome variables.
Summary and Conclusions: Nilotinib therapy induces significant immunological changes. Most changes during therapy are likely due to normalization of the peripheral blood compartment. So far, correlation of a specific immunological signature with response to nilotinib could not be identified. More detailed data will be presented at the meeting.

P720

IN CML PATIENTS TREATED FRONTLINE WITH IMATINIB, WITH A BCR-ABL RATIO HIGHER THAN 10% AT 3 MONTHS, THE CHANGE TO A 2ND GTKI IS ASSOCIATED WITH IMPROVEMENT OF CYTOGENETIC RESPONSE, BUT NOT WITH MMR

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Background: A cut-off value of BCR-ABL ratio of more than 10% at 3 months discriminates patients with an ulterior worse outcome in treatments with Imatinib, Dasatinib, Nilotinib, and Bosutinib. There is limited data about how this outcome is affected by change to a 2GTKI. In our registry of Imatinib front-line treated patients, 156 out of 374 (41,7%) had been molecularly evaluated at 3 months.
Aims: To asses the influence of treatment change to 2GTKI in the outcome of patients with mollecular evaluation at 3 months.
Methods: Demographics. 93 M, 63 F, median age: 54,2 y (15-86). 7 received HD Imatinib upfront.
Results: Among 156 patients evaluable, 50 (32%) had a ratio of >10%. Sokal, Hasford, or EUTOS risk score were not significantly associated with this cut-off.
Response: The Probability of obtaining CCyR while on Imatinib was 89% for those patients with ratio of 10% or less (*vs* 66% with >10%) P<0.0001. The probability of CCyR, considering all TKI treatments was 92% for those patients with ratio of 10% or less (*vs* 77% with >10%) P=0.007. The probability of obtaining MMR while on Imatinib was 80% for those patients with ratio of 10% or less (*vs* 47% with >10%) P<0.0001. The probability of MMR, considering all TKI treatments was 88% for those patients with ratio of 10% or less (*vs* 58% with >10%) P<0.0001.
Response after changing therapy: 43 patients changed to a 2GTKI (30 to Dasatinib, 13 to Nilotinib). The median time to change was 15 months (3-82m). 19 patients changed during the 1 st year,and 13 beyond 2 years. Among the 106 patients with a ratio of 10% or less, 23 (22%) changed to a 2GTKI. The most frequent causes were intolerance (44%) and lost of response (39%). The probability of obtaining a CCyR and MMR after changing to a 2GTKI were 87% and 65%, respectively. Among the 50 patients with a ratio of more than 10%, 20 (40%) changed to a 2GTKI, mostly because of intolerance (40%) or primary resistance (55%). The probability of obtaining a CCyR and MMR after change were 75% and 45%, respectively.
Survival outcomes: PFS and OS were not significantly different across the 10% threshold at 3 months,(91,6 *vs* 92,5%) and (93,5 *vs* 02,8%) by 7 years, respectively (Figure 1.)

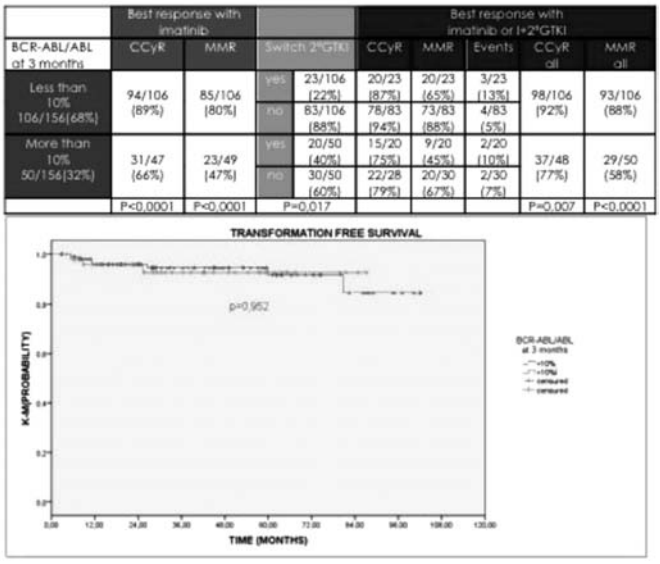

Figure 1.

Summary and Conclusions: In the setting of a multicentric, hospital based registry, only 42% of the CML patients treated with imatinib frontline were molecularly evaluated. The 10% BCR-ABL ratio threshold at 3 months discriminates the probability of obtaining CCyR and MMR thereafter, not only with imatinib, but with sequential TKI therapy. Although the reasons for changing to a 2GTKI in patients having £10% and >10% ratio differ, the improvement is small, and confined to cytogenetic response, whereas molecular response is not increased. But the proportion of transformation or death was not increased in any of the groups.

P721

CLINICAL AND BIOLOGICAL FEATURES OF BLAST CRISIS (BC) IN PATIENTS WITH PH+ CHRONIC MYELOID LEUKEMIA (CML) IN THE TKIS ERA: EXPERIENCES IN CLINICAL PRACTICE

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Background: Although TKIs have dramatically changed the outcome of CML, and despite improved knowledge of their biological and molecular mechanisms, the pathogenic processes and best treatment of the CML blast phase are still poorly understood. Most data on the use of Imatinib in patients with newly diagnosed Ph+CML are derived from results of the IRIS study: estimated 08-year rate of freedom from progression to accelerated phase (AP)/blast crisis (BC) was 92%. Data on the use of Nilotinib and Dasatinib in patients with newly diagnosed Ph+ CML are derived from results of the ENESTnd study [estimated 4-year rate of freedom from progression to AP/BC was 98.2% or 97.1% including clonal evolution] and from results of the DASISION study [estimated 3-year rate of freedom from progression to AP/BC was 97% or 95.8% including follow up beyond discontinuation]. Progression to BC seems to depend on genomic instability, resulting in an accumulation of additional genetic changes which lead to loss of differentiation and to a more aggressive clinical presentation.
Aims: Herein, we report clinical and biological features of a subset of patients who developed BC in the TKIs era.
Methods: We retrospectively evaluated 1100 patients (pts) with newly diagnosed Ph+CML receiving TKIs as first-line therapy, observed between January 2001 and December 2012; none of them had received prior therapies including Interferon before starting on TKIs and 33 of them (3%) developed BC.
Results: Sokal risk evaluation at the time of diagnosis of CML showed that 48.5% of patients were high risk, 24.3% intermediate and 27.2% low risk. With the EUTOS revised risk score 90% of patients were low risk and 10% high risk, so in this cohort of CML patients the EUTOS score appeared to have a low correlation with the risk of evolution to BC. Apart from one patient with a complex karyotype, all patients displayed the classic t(9;22) Ph chromosome according to standard cytogenetics. The BCR/ABL transcript at RT-PCR was b3a2 in 25 patients (76%) and b2a2 in 8 patients (24%). Median duration of the chronic phase was 15 months (range 4-144 months) and median time to best Cytogenetic Response (CyR), including minimal, minor, partial and complete CyR, was 8 months (range 3-32). Morphologic and flow cytometry characterization of BC revealed a myeloid subtype in 22 patients (67%) and lymphoid subtype in 11 patients (33%). BC was sudden in 3 patients (9%). Standard cytogenetics at the onset of BC revealed clonal chromosome abnormalities in 10 patients (10%). Three patients presented evidence of extramedullary disease. Nineteen patients were screened for mutational analysis and mutations were found in 8 of them (M318T, E255K(2), T315I(3), E359I, E279K). BC developed during imatinib therapy in 11 pts (34%), during dasatinib therapy in 16 pts(48%) (1 in first line, 12 in second line, 3 in third line), during nilotinib therapy in 6 pts (18%) (2 in first line, 3 in second line, 1 in third line). BC occurred in the first year of treatment with TKI in 15 pts (45%) and in the second year in 7 (21%). At the time of this report, after 112 months follow-up, 7 pts (21%) were alive (5 pts after HSCT) and 26 pts (79%) had died of disease progression, overall median survival being 19 months (range 1-112).
Summary and Conclusions: BC is a generally rare but possibly lethal phase that can develop in patients with CML. Further clinical and biological features should be considered for the purposes of early identification of patients at high risk of progression to BC.

P722

REVERSIBLE LYMPH NODE FOLLICULAR HYPERPLASIA ASSOCIATED WITH DASATINIB TREATMENT FOR CHRONIC MYELOID LEUKAEMIA IN CHRONIC PHASE

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Background: Dasatinib is a second generation dual SRC/ABL tyrosine kinase inhibitor (TKI) licensed in the treatment of chronic-, accelerated-, and blast-phase chronic myeloid leukaemia (CML). There is strong evidence suggesting that dasatinib exerts substantial cellular immunomodulatory effects both *in vitro* and *in vivo*.
Aims: We report on 6 chronic phase (CP)-CML patients who developed follicular lymphoid hyperplasia (FLH) apparently caused by dasatinib, a previously unreported adverse drug reaction.
Methods: Cases of FLH observed in CML patients treated with dasatinib were collected in a retrospective fashion among centers from the Fi-LMC group.
Results: All patients (3 males, 3 females) were diagnosed CP-CML. Sokal scores were low in 2 patients, intermediate in 1 patient, and high in 3 patients. Median age at FLH diagnosis was 49 years (range: 24-70). Dasatinib was given frontline (OPTIM dasatinib trial, n=2), after intolerance (n=1) or suboptimal response/resistance (n=2) to imatinib, or in the setting of a molecular relapse following an imatinib discontinuation attempt (n=1). Patients received dasatinib at 50mg/d (n=1) or 100 mg/d (n=5). Patients all presented with progressive cervical lymph node enlargement after a median duration of dasatinib treatment of 14 months (range: 9-32 months). At the time of lymph node enlargement discovery, 5 patients were in complete cytogenetic response (3 associated with a MMR) and 1 had no cytogenetic response. In 4 patients, exploration for viral infection (VIH, EBV, CMV, Parvovirus B19) failed to show any active viral replication. Search for toxoplasma gondii replication (performed in 2 patients) was negative. In 4 patients, autoantibodies assay was not in favor of autoimmune disorders. Chest, abdomen and pelvis tomodensitometry confirmed the absence of additional localizations. All patients underwent a lymph node biopsy and pathological analysis revealed FLH. FLH was characterized by an enlargement of lymph nodes with follicular (germinal) centers hyperplasia related to B lymphocytes-stimulation. Extramedullary blastic transformation of underlying CML was formally ruled out. Lymphocytes were CD10+, CD20+, and Bcl2. Complex chromosomal abnormalities without the Philadelphia chromosome (46XY,add(1)(q41),der(14)t(1;14)(q24;q32)) were identified in 1 patient, in association with a DJ-JH rearrangement, but no Igh/Bcl2 rearrangement. The occurrence of FLH under continuous dasatinib therapy in the absence of any sign of viral activation or autoimmune disease led to raise the hypothesis of a drug-induced adverse event. Dasatinib was thus discontinued in all patients, leading to a complete disappearance of node enlargement within a median time of 1 month (range: 15 days-2 months)
Summary and Conclusions: By targeting not only BCR-ABL but also SRC-family kinases, which are critical regulators of cell signaling in B-cells, dasatinib may be accompanied with off-target immune effects in CML. SRC targeting might have triggered FLH in our series. Studying the mechanisms by which dasatinib induces FLH such as abberant B-cell stimulation by activation of downstream AKT signaling pathway through SRC inhibition is warranted. Considering clonal cytogenetic abnormalities observed in 1 patient, further transformation into malignant B-cell lymphoma may not be ruled out. We thus recommend immediate dasatinib discontinuation in case of FLH associated with dasatinib in the absence of any other cause. The risk of developing dasatinib-associated FLH should now be assessed prospectively.

P723

MMR AND CMR IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS: ROLE OF SOKAL SCORE AND DIFFERENT TYROSINE KINASE INHIBITORS ON TIME OF THEIR OBTENTION, MAINTENANCE AND FAILURE-FREE SURVIVAL

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Background: Most patients with CML treated with Tyrosine Kinase Inhibitors (TKIs) achieve a major molecular response (MMR), that is, the absence of detectable Ph chromosomes. Even after this threshold is achieved, the disease burden continues to progressively decrease with continued treatment. Therefore, more sensitive polymerase chain reaction (PCR)–based molecular methods are required to detect and quantify levels of minimal residual disease leading to complete molecular response (CMR), especially at long time points after TKI initiation. Low levels of minimal residual disease, as measured by real-time quantitative PCR, have been shown to be an excellent surrogate marker for long-term prognosis.
Aims: Our main objective in this study was to describe the different treatments and factors involved in the first MMR occurence in chronic phase CML, its maintenance, and its conversion to CMR.
Methods: We analyzed 211 CML patients [124 males and 87 females; median age: 50 years (17-81)] in first chronic phase who received tyrosine-kinase inhibitors (TKI) followed at our institution between 2001 and 2011. Among 196 patients evaluated for Sokal score, 79 (40%) were low, 82 (42%) were intermediate and 35 (18%) were high. According to hasford score (153 evaluated), 46% were low, 46% intermediate and 8% were high.
Results: First MMR was obtained in: 61% of patients after first line treatment [Imatinib, n=102; Imatinib+Peg-interferon, n=11; Dasatinib, n=10; Nilotinib, n=6]; 26% after second line [Imatinib, n=32; Dasatinib, n=11; Nilotinib, n=12] and 13% beyond second line [Imatinib, n=14; Dasatinib, n=7; Nilotinib, n=6]. The median time of MMR obtention was 15 months (2.5-94) after Imatinib, 6.4 months (3-24) after Imatinib+Peg-interferon, and it was significantly shorter after both Dasatinib [HR=2.6 (1.7-3.9), P<0.001] and Nilotinib [HR=3 (1.8-5), P<0.001] with 6 months (3-23) and 5 months (3-56) respectively no matter the treatment line number was. Sokal score was an independent significant factor that impacted MMR obtention delay, with a median time of 9 months (2.5-98) for low score, 13 months (3-78) for intermediate [HR=0.7 (0.5-0.9), P=0.03] and 12 months (3-94) for high score [HR=0.7 (0.5-1.02), P=0.05]. Patients who converted to CMR were: 50 (34%) under Imatinib after a median time of 25 months (3-97); 7 (64%) under Imatinib+Peg-interferon after a median time of 8 months (3-15); 10 (42%) under Nilotinib after 25 months (13-32) and the only significant faster treatment was Dasatinib [n=13 (46%)] after a median time of 11 months (3-52) [HR=2.1 (1.1-4.2), P=0.02]; according to Sokal score, low (n=41, 52%) and intermediate (n=27, 33%), while patients with high score (n=11, 31%) were significantly slower [HR=0.53 (0.25-1), P<0.001]. The current rate of CMR at 5 years was not statistically different between the treatments [Imatinib (36%), Imatinib+Peg-interferon (40%), Nilotinib (43%), Dasatinib (56.5%)] nor according to sokal score [Low (49%), intermediate (43%) and high (40%)]. Treatment was discontinued in 42 patients who had 5 years failure-free survival of 56% (43-73) while patients under Dasatinib, Imatinib+Peg-interferon, Imatinib, and Nilotinib had 70.5% (50-99), 79% (56-100), 84% (77-91) and 93% (84-100) respectively.
Summary and Conclusions: Second generation TKI used at 1st, 2nd line treatment or beyond, showed an independent significant faster time to MMR, as well as patients with low sokal score. Conversion to CMR was significantly faster only in Dasatinib patients and slower in patients with intermediate and high sokal score. Treatment discontinuation did not have a benefit in terms of FFS while patients on TKI therapy seem to have better result.

P724

KIR HAPLOTYPE AA IS A POSSIBLE PREDICTIVE MARKER OF COMPLETE MOLECULAR RESPONSE TO TYROSINE KINASE INHIBITORS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: One of the most intriguing challenges in the management of patients with chronic myeloid leukemia (CML) is to decide for how long it is necessary to continue therapy with tyrosine kinase inhibitors (TKI). Although maintenance therapy with imatinib or a second generation TKI may be well tolerated, in many patients it has been associated with compromission of quality of life and reduced compliance.
Aims: In the literature, a growing amount of evidence suggests that killer immunoglobin-like receptors (KIR) could have a role in the development of chronic myeloid leukemia (CML) and response to therapy. This prompted us to investigate the prognostic and/or predictive role of KIRs and their human leukocyte antigen (HLA) Class I ligands in a cohort of 59 chronic-phase CML patients (mean age 53, range 23-81) and a group of 121 healthy controls.
Methods: The large majority of patients received frontline treatment with imatinib (59.3%), nilotinib or dasatinib (10.2%). Patients presenting side effects or inadequate response (25%) were moved to second line TKI therapy. Complete molecular response (CMR) was defined as repeatedly negative results on real-time quantitative polymerase chain reaction assay.
Results: The 2-year cumulative incidence rate for CMR was 51.2%. An increased frequency of the activating receptor KIR2DS1 (P=0.05) and a reduced frequency of the KIR-ligand combination KIR2DS2/KIR2DL2 absent/C1 present (P=0.001) were found associated with an unfavourable prognosis. Analysis of response to TKI treatment, revealed a positive association between CMR and a decrease in the frequency of the KIR2DL2 inhibitory gene (P=0.02), homozygosity for KIR haplotype A (P=0.01) and low numbers of inhibitory KIR genes (P=0.05).
Summary and Conclusions: It is well known that natural killer (NK) cell cytotoxicity is regulated through a balance of both activating and inhibitory KIR signals. Our data suggest that impairment in this delicate balance may lead to NK cell deficiency and thus contribute to the occurrence of CML. It is also possible that genetic imbalance between activating and inhibitory KIR genes limits recruitment of cells from the bone marrow niche, including mesenchymal stromal cells (MCS) which have a tendency to inhibit innate and adaptive immune response of a variety of cells interacting with NK cells. However, these hypotheses will need to be confirmed in larger cohorts of CML patients. What clearly emerges from our study is the potential value of KIR haplotype AA as a marker of durable CMR which, combined with the other factors capable of predicting cure of CML, should help us decide which patients are candidates for stopping TKI therapy (Figure 1).

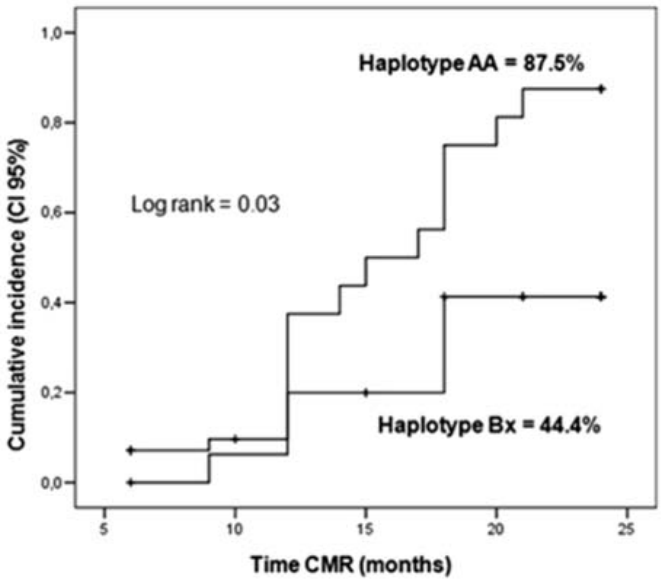

Figure 1.

P725

THE POSSIBLE PREDICTIVE ROLE OF THE IMATINIB PLASMA LEVELS AND POLYMORPHISMS ASSESSMENT IN PATIENTS AFFECTED BY CHRONIC MYELOID LEUKEMIA. THE TIKLET STUDY.

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Background: Major advantages of tyrosine kinase inhibitors (TKis), that changed the natural history of CML, are represented by high efficacy, oral administration of a standard daily dose and good tolerability. Moreover, the highest therapeutic benefit is expected when minimal plasma concentrations (Cmin) of imatinib are ≥900-1000 ng/mL. However, imatinib pharmacokinetics (PK) is influenced by several physiological, pathological, and pharmacogenetic factors (polymorphisms of transmembrane transporters), in addition to a possible poor adherence.
Aims: 1) Application of a therapeutic monitoring protocol for imatinib in order to evaluate its PK characteristics. 2) analysis of polymorphisms of gene controlling influx, efflux, and metabolism of imatinib. The TIKlet study was approved by the Pisa University Hospital Ethics Committee (ref. n. 46013, 08/01/2011).
Methods: Sixty-two patients, 35 men and 27 women affected by CML and treated with imatinib were enrolled. A commercially-available HPLC assay kit (Chromsystems, Munich, Germany) was used. A population PK analysis was performed based on values of imatinib plasma concentrations according to a nonlinear mixed-effects modeling approach, using the NONMEM software, version 7.1. Dataset checkout, model diagnostic and covariate testing were performed using Xpose and PsN softwares. The area under the plasma concentration-time curve from time zero up to infinity (AUC0to∞), terminal elimination half-life (t1/2) and minimum plasma concentration at steady state (Cmin,ss) were calculated. After DNA extraction, polymorphisms of the following genes have been assessed by real-time PCR; ABCB1, ABCG2, CYP3A4, CYP3A5, CYP2D6, hOCT1, OCTN1, and OATP1A2.
Results: After at least 3 determinations/patient, the average of imatinib Cmin was 1064±280 ng/mL, and the median value 1039 ng/mL. About 25% of patients showed Cmin<817 ng/mL. In the PK model, age, sex, height, weight, body mass, alpha1-acid-glycoprotein, smoking, renal and hepatic parameters were inserted and data were analysed on the basis of the interval between the last dose and the blood harvest. Only the alpha1-acid glycoprotein significantly correlated with imatinib plasma levels. The therapeutic effect of imatinib in terms of time to major molecular response (MMR) was analysed. Results demonstrated that patients with higher imatinib exposure (upper quartile distribution) achieved an earlier (but not statistically significant) MMR with respect those subjects who were characterized by lower drug plasma concentrations. On the other hand, values >1390 ng/mL resulted to significantly impact on the observed grade 3-4 toxicities. The introduction of hOCT1 c.480C>G polymorphism into the final model led to a significant decrease in interindividual variability in the apparent clearance of imatinib. In particular, imatinib clearance was significantly higher in patients homozygous for the wild-type C allele (12.2+/-2.3 L/h) with respect to other patients (9.4+/-1.6 L/h).
Summary and Conclusions: The present results confirm that variability of imatinib pharmacokinetics is not negligible among CML patients. The present PK model is characterized by a good performance, allowing the prediction of Cmin in the present patients regardless the time of blood withdrawal.
Finally, results from the present study suggest that patients' genotype with respect to the hOCT1 c.480C>G SNP may predict imatinib clearance.

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EUTOS CML PROGNOSTIC SCORING SYSTEM PREDICTS ELN-BASED EVENT-FREE SURVIVAL BETTER THAN EURO/HASFORD AND SOKAL SYSTEMS IN CHRONIC PHASE CML PATIENTS RECEIVING FRONTLINE IMATINIB MESYLATE

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Background: The validity of the three currently used chronic myeloid leukemia (CML) scoring systems (Sokal CML prognostic scoring system, Euro/Hasford CML scoring system, and the EUTOS CML prognostic scoring system) were compared in the CML patients receiving frontline imatinib mesylate.
Aims: The aim of this study was to perform a comparative assessment of the Sokal CML prognostic scoring system, Euro/Hasford CML scoring system, and the EUTOS CML prognostic scoring system in the CML patients receiving frontline imatinib mesylate. Validation of the novel EUTOS system is important for the clinical management of CML patients since there are some contradictions and discrepancies in the literature regarding the power of EUTOS score in CML.
Methods: One hundred and fourty-three chronic phase CML patients (71 males, 72 females) taking imatinib as frontline treatment were included in the study. The median age was 44 (16-82) years. Median total and on-imatinib follow-up durations were 29 (3.8-130) months and 25 (3-125) months, respectively.
Results: The complete hematological response (CHR) rate at 3 months was 95%. The best cumulative complete cytogenetic response (CCyR) rate at 24 months was 79.6%. Euro/Hasford scoring system was well-correlated with both Sokal and EUTOS scores (r=.6, P<0.001 and r=0.455, P<.001). However, there was only a weak correlation between Sokal and EUTOS scores (r=.2, P=.03). The 5-year median estimated event-free survival for low and high EUTOS risk patients were 62.6 (25.7-99.5) and 15.3 (7.4-23.2) months, respectively (P<.001). This performance was better than Sokal (P=.3) and Euro/Hasford (P=.04) scoring systems. Overall survival and complete cytogenetic response rates were also better predicted by the EUTOS score.
Summary and Conclusions: EUTOS CML prognostic scoring system, which is the only prognostic system developed during the imatinib era, predicts ELN-based EFS better than Euro/Hasford and Sokal systems in CML patients receiving frontline imatinib mesylate. This observation might have important clinical implications.

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FIRST-LINE THERAPY AND SEQUENTIAL APPLICATION OF TYROSINE KINASE INHIBITORS FOR CHRONIC MYELOID LEUKEMIA TREATMENT–A CLINICAL DECISION ANALYSIS

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Background: The introduction of the tyrosine kinase inhibitor (TKI) imatinib

about a decade ago dramatically extended the life span of chronic myeloid leukemia (CML) patients. Currently, there are several different TKIs approved for CML treatment. Long-term effectiveness and safety data for second-generation TKIs are not yet available. Despite this uncertainty about long-term effects, patients and physicians have to choose a first-line therapy.
Aims: The goal of this study is to develop a clinical decision-analytic model for the evaluation of the long-term effectiveness of different first-line therapy regimens for patients with CML.
Methods: We developed a Markov state-transition computer model for patients in the chronic-phase of CML treated with first-line TKI imatinib, dasatinib or nilotinib. Seven different strategies including different combinations of first and second-generation TKIs as well as chemotherapy or stem cell transplantation were evaluated. The model was parameterized using published trial data, data from the Austrian CML registry, and practice patterns estimated by an expert panel. (1-11). The model was analyzed as a cohort simulation over a lifelong time horizon. Health outcomes evaluated were life-years (LYs) gained and quality-adjusted life years (QALYs) gained. Several deterministic sensitivity analyses were performed.
Results: Nilotinib followed by dasatinib after failure is the most effective treatment in terms of both, remaining LYs (19.7 LY) and QALYs (17.1 QALYs). All strategies including a second-line TKI were superior compared to strategies without second-line TKI. Deterministic sensitivity analyses showed that the ranking of the strategies was mostly influenced by the duration of first- and second-line therapies.
Summary and Conclusions: Based on our clinical decision analysis, the most clinically effective strategy is first-line treatment with nilotinib followed by dasatinib as second-line therapy. As all three TKIs are approved as first-line therapy, our results may support clinicians and patients in their decision making regarding the sequential application of TKIs.
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BONE MARROW IMAGING OF HUMERAL AND FEMORAL MARROW BY MULTI-DETECTOR COMPUTED TOMOGRAPHY IN PATIENTS WITH APLASTIC ANEMIA AND HYPOPLASTIC MDS

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Background: Myelodysplastic syndrome (MDS) and aplastic anemia, a heterogeneous group of bone marrow disorders, are characterized by ineffective hematopoiesis with or without bone marrow dysplasia. It is often difficult to distinguish myelodysplastic syndrome (MDS) from severe aplastic anemia because both can present with profoundly hypocellular bone marrows.Multidetector CT (MDCT) of the humeral and femoral is a sensitive method for evaluating patients with bone marrow disorders that provides a better understandings of disease progression and remission.
Aims: In this study, we analysed the difference between the abnormalities by MDCT in patients with aplastic anemia and hypoplastic MDS. We also analysed the relationship between the abnormalities by MDCT and the development of leukemia and survivals of patients with MDS.
Methods: We evaluated MDCT of the humeral and femoral in 64 untreated adult patients with aplastic anemia (N=15) and MDS (N=49). We performed a retrospective chart review of hematologic and cytogenetics features, such as haemoglobin, platelets, bone marrow blast, poor cytogenetics, International Prognostic Scoring System (IPSS). Patients with MDS were classified according to WHO classification; RA (N=17), RARS (N=2), RCMD (N=9), RAEB (N=19) and MDS unclassified (N=2). Overall survival and leukemia-free survival were analyzed in 49 MDS patients by the Kaplan-Meier method and differences between curves were calculated by two-sided log-rank test. A multivariate analysis was performed to assess the impact of various prognostic factors including haemoglobin, platelets, bone marrow blast, poor cytogenetics, IPSS, WHO classification and MDCT patterns. Non-enhanced CT examinations were performed in supine position from the base of skull down to the knee joint by MS-CT scanner (AQUILION 64, Tohshiba, Tokyo, Japan). Images were reconstructed by medium smooth convolution kernel (B50f). Bony canal of humeral and femoral bone were visualized by coronal and sagittal axis image reconstruction. The effective radiation dose associated with whole body MD-CT was 10.1 mSv. (International Comission on Radiological Protection (ICRP) 26) The dose was comparable to whole body CT (2.4 mSv.).Bone marrow CT density was measured and results were expressed as Hounsfield unit (HU). As the normal adult bone marrow was composed of rich adipocytes and called yellow marrow, it is represented by low density CT value between -30 to -100 HU. The value above -30 HU observed in long bony canals was considered as high density lesions. The results of MDCT were evaluated by two observers, and the general patterns were categorized as follows: (1) fatty, showing a low signal density marrow on CT images; (2) focal, showing abnormally focal high density lesions: (3) diffuse, showing uniformly high density marrow.
Results: All 15 patients with aplastic anemia showed a fatty or focal pattern on their MDCT. No patients with aplastic anemia showed diffuse patterns. Meanwhile, among the 49 patients with MDS, 15 patients had fatty pattern, 21 patients had focal patterns,and 13 patients had diffuse pattern. This result indicate that diffuse patterns of MDCT support the diagnosis of MDS rather than aplastic anemia.Among the 12 patients who had diffuse patterns on MDCT, 6 patients developed acute myeloid leukemia. Leukemia free survival of 12 patients with diffuse patterns was significantly shorter than that of the 36 patients with fatty and focal patterns. (74% *vs* 29% at 3 years, P<0.01) Similarly, the overall survival was significantly shorter. (79% *vs* 40% at 3 years, P<0.01) On multivariable analysis, diffuse pattern on MDCT was emerged as independently negative prognostic predictors for PFS and OS in patients with MDS.
Summary and Conclusions: This study indicated that MDCT of the humeral and femoral is an important tool for the accurate diagnosis of patients with MDS and aplastic anemia. MDCT can also provide valuable information for assessment the prognosis and determining the most appropriate management with MDS.

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A CROSS-SECTIONAL ANALYSIS ON DEMOGRAPHICS AND POSSIBLE CAUSES OF ANEMIA IN THE ELDERLY

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Background: Late-life anemia is associated with increased morbidity and mortality.

Aims: To provide data on the demographic distribution and possible causes of anemia in the elderly.
Methods: Laboratory values from 19758 patients 64+ years treated at Innsbruck Medical University from 1.10.2004 to 29.9.2005 were analyzed (10917 women; 8841 men; median age 75 and 72 yrs, respectively; 10737 outpatients, 9021 inpatients).
Results: 19.3% of women and 23.4% of men suffered from anemia according to WHO criteria. In anemic patients, as compared to non-anemic patients, a decreased GFR <60 and <30mL/min/1.73m² (MDRD1 analysis) was observed in 45.1% (28.9%) and in 11.3% (2.1%), signs of inflammation as defined by elevated C reactive protein in 62.1% (31.4%), folic acid deficiency in 6.7% (3.0%), iron deficiency as defined by serum ferritin <30ng/mL in 14.4% (6.9%) and functional iron deficiency as defined by transferrin saturation <16% and serum ferritin <100 ng/mL in 22.1% (6.5%) of cases (P<0.001). Mean corpuscle volume (MCV) was seen to be weakly correlated with the various anemia subtypes, with normocytic anemia constituting the largest group. Macrocytic alterations without evidence for vitamin B12 or folic acid deficiency were observed in 16.4%, thrombopenia <100 G/l in 5.4% and leucopenia <4 G/l in 8.26% or <2 G/l in 1.1% of anemic cases, thus suggesting a Myelodysplastic Syndrome(MDS).
Summary and Conclusions: Anemia in the elderly is frequent in patients admitted to the hospital as well as in outpatients. Nutritional anemia, for which a specific treatment is available, is found in a relevant proportion of patients. In the majority of anemic elderly the pathogenesis of anemia is complex and includes a mixture of different causes including chronic inflammation and renal insufficiency. In a relevant proportion a hitherto undiagnosed Myelodysplastic Syndrome can be assumed.

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BONE MARROW SCREENING FOR MYELODYSPLASIA-RELATED CHANGES AND ABERRANT LYMPHOID POPULATIONS USING ONE TUBE, FOURTEEN ANTIBODY-TEN COLOR FLOW CYTOMETRY PANEL
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Background: Hematopathology laboratories often receive bone marrow (BM) samples from patients with cytopenia and no history of lymphoma or lymphocytosis, to investigate for both lymphoproliferative disorders and myelodysplastic syndromes (MDS). Flow cytometry (FCM) is a vital part of the integrated hematopathological diagnostics. However, large multicolor panels may be impossible to apply in times of economic constraints. Previous multinational study has applied a FCM score for MDS based on 4 parameters: CD34+ myeloblast and B-progenitor cluster size, myeloblast CD45 expression, and granulocyte/lymphocyte side scatter (SSC) value and reported that FCM score 2 or higher is suggestive of MDS (Della Porta *et al.*, Hematologica, 2012;97:1209).
Aims: To establish and validate an FCM antibody panel that would provide maximum immunophenotyping information on main lymphoid and myeloid cell subsets and also the FCM MDS score.
Methods: 14 monoclonal antibodies - 10 fluorochrome panel was applied: kappaFITC+CD4 FITC, LambdaPE+CD8 PE, CD3ECD+CD14 ECD, CD34 APC, CD20PC7+CD56 PC7, CD10 APC-A750, CD19 APC-A700, CD33 PC5.5, CD5 PB, and CD45 KO. This panel was accredited according to the Ontario Laboratory Accreditation requirements.
Analysis is illustrated in Figure 1. Various cell subpopulations were mapped on CD45/SSC plot (A). B-cells were gated on CD19/SSC plot (B). Kappa to lambda ratio was determined within the total B-cell population (C), CD10+ B-cells (D,E), and CD5+ B-cells (F,G). B-cell maturation pattern was evaluated using CD20 *vs.* CD10 expression (D). CD34+ cells were enumerated and fractions of myeloid and lymphoid CD34+ progenitors were dsetermined on CD19/CD33 plot (H,I). CD4+ and CD8+ T-lymphocyte subsets were enumerated after gating of CD3+ T-cells on SSC/CD3+CD14ECD plot (J,K). CD14+ monocytes were also counted (J). CD56+ NK cells were counted using CD3 v. CD20+CD56 plot after B-cells were removed from analysis using Boolean gating strategy (L). Maturation of granulopoietic and monocytic cells was studied using CD33 *vs.* CD14 and CD33 *vs.* CD10 expression (M,N). CD56 expression in non-lymphoid populations was investigated (not shown). MDS score was determined (Figure 1) using CD34+ myeloblast and CD34+ B-progenitor cluster size (O,P), myeloblast *vs.* lymphocyte CD45 expression (S,T), and granulocyte *vs.* lymphocyte SSC value (R). Results of MDS score were correlated with morphological and cytogenetic evaluation of BM samples in a selected group of 70 patients with full clinical information.
Results: In 13 of 226 studied BM samples (5.7%), a minor monotypic B-cell population was detected corresponding to previously undetected lymphoproliferative disorder or monoclonal B-cell lymphocytosis. All but one of 21 reactive BM samples had MDS score<2 (95%). Of 24 patients with cytopenia but no MDS, 7 (29%) had score 2. MDS score above 2 was not found in these groups. 22 of 35 (63%) patients where morphology, cytogenetic and clinical findings were consistent with myelodysplasia, had score 2 or higher. Differences in scores between the latter and two other groups were statistically significant (P<0.0001).

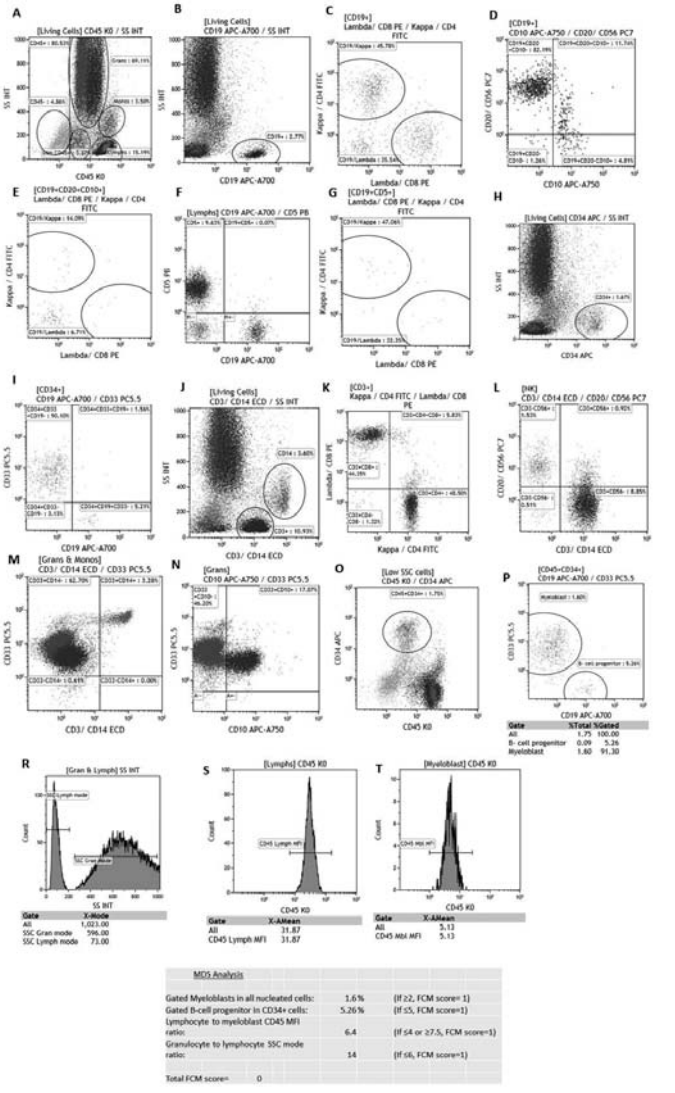

Figure 1.

Summary and Conclusions: Developed 14 antibody 10-color FCM panel is easy to apply for screening BM samples for aberrant lymphoid populations and to establish MDS FCM score. Our results confirm the previous reports that FCM score ≥2 should prompt further investigation for myelodysplastic syndrome. However, FCM score 2 has also been found in patients with cytopenia of other origin. Also, FCM score 2 or lower did not exclude possibility of MDS.

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PLATELET COUNT AND TRANSFUSION-DEPENDENCY AFFECT OVERALL SURVIVAL IN PATIENTS WITH REFRACTORY ANEMIA WITH RING SIDEROBLASTS AND THROMBOCYTOSIS (RARS-T)
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Background: Among the overlap myelodysplastic/myeloproliferative disorders, refractory anemia with ring sideroblasts and thrombocytosis (RARS-T) exists as a rare and provisional entity first defined in 2001 by the World Health Organization (WHO). Limited data exist on the impact of various clinical characteristics on patients' morbidity and overall survival (OS). Thus, in contrast to myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN) there is no validated prognostic model for RARS-T.
Aims: To evaluate clinical prognostic models for patients (pts) diagnosed with RARS-T.
Methods: A retrospective chart review of all pts diagnosed with WHO-defined RARS-T at Mayo Clinic Rochester between 2001 and 2013 was done. All demographic and clinical information including age, gender, hematological labs, and bone marrow biopsy results were obtained from medical records. IRB approval

was obtained in accordance with the Helsinki declaration. Comparison between medians was done using Wilcoxon test, between two groups using the t-test, and survival calculations per Kaplan-Meier estimates was done with JMP v.9.
Results: Nineteen pts meeting WHO criteria for RARS-T (with platelets >450×10^9/L) were identified. Median age at diagnosis was 72 years, 58% of pts were male. Median follow up was 367 days (range, 2-1815), hemoglobin 9.5 mg/dL, and platelets were 642×10^9/L (range, 470-1465). Eighty-three percent had diploid cytogenetics, median ring sideroblasts (RS) in the marrow were 50% (range, 20-90%). Out of 13 pts tested, JAK2V617F mutation was positive in 62%. Fifty-three percent of pts received exogenous erythropoietin, 37% received hydroxyurea and 16% received lenalidomide. All 3 pts who had lenalidomide responded with hematological improvement (at 6 and 11 months of therapy), and one had complete remission (at 6 months of therapy). Only 1 pt (5%) had a thrombotic event (myocardial infarction). Median overall survival was not reached, without anyone progressing to acute leukemia. JAK2V617F positivity in pts was associated with higher platelet count (764 *vs* 561, P=0.04), lower RS% (35 *vs* 58%, P=0.14), but had no effect on OS (P=0.08). On multivariate analysis, both platelet count at diagnosis (P=0.04), and transfusion-dependency (P=0.01) affected OS, but not percentage of RS (P=0.14). None of the existing models for MDS (international prognostic scoring system -IPSS, IPSS-revised) or myelofibrosis (IPSS) were able to predict OS in RARS-T pts.
Summary and Conclusions: JAK2V617F mutation was present in 62% of RARS-T pts, but did not affect OS. Platelet count at diagnosis and transfusion-dependency, but not percentage of RS in the bone marrow, affect OS in RARS-T. None of the existing prognostic models for MDS or myelofibrosis predicted OS in pts with RARS-T. Lenalidomide had favorable activity in some pts. These results support RARS-T being a distinct clinicopathologic entity.

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A PROSPECTIVE PHASE II STUDY OF ATG-CYCLOSPORINE A FOR ADULT SEVERE APLASTIC ANEMIA: MEDIAN 8-YEAR FOLLOW-UP DATA
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Background: Antithymocyte globuline (ATG) combined with cyclosporine A (CsA) is a standard immunosuppressive therapy (IST) for severe or transfusion-dependent aplastic anemia (sAA) in patients who are not suitable for allogeneic hematopoietic cell transplantation (alloHCT). Response rate to standard IST regimens seemed inferior and long-term follow-up data are rare in Korean population.
Aims: We conducted a prospective phase II study of ATG-CsA for adult sAA. Here we present long-term follow-up results.
Methods: Patients were idiopathic or secondary sAA over 15 years old. Thymoglobulin was infused at 2.5 mg/kg/d for 5 consecutive days. Horse ATG 2.5mg/kg/d for 4 days or horse ALG 10mg/kg/d for 5 days were used as alternatives of thymoglobulin. Cyclosporine administration was adjusted according to the plasma level and the change of renal function for three to six months.
Results: Total 83 patients were enrolled. The median age was 44 (range 15-78) years old. All patients were idiopathic sAA except for 2 patients (virus in one and drug in one patient). The median follow-up was 92.2 (range 15.72-136.1) months in survivors and 63.4 (range 0.1-136.1) months in all patients. Horse ATG, rabbit ATG and horse lymphoglobulin were used in 48 (57.8%), 29 (34.9%) and 6 (7.2%), respectively. Maximal responses were CR in 23 (27.7%), PR in 21 (25.3%), no response (NR) in 31 (37.3%) and not evaluable (NE) in 8 (9.6%) patients. Therefore maximal overall response rate (ORR) was 53.0%. There was no difference in terms of maximal ORR (59.3% *vs.* 41.4%; P=0.120), but significant difference in terms of maximal CR rate (37.0% *vs.* 10.3%; P=0.010) between horse ATG and rabbit ATG. Also there was significantly low cumulative incidence of maximal ORR in rabbit ATG (45.5%) compared with horse ATG (72.1%; P=0.009). ORRs in horse ATG were significantly higher compared with those in rabbit ATG at 3M (P=0.003) and 6M (P=0.005). However the differences were not significant at 12M (P=0.226), 18M (P=0.639) and 24M (P=0.359). Maximal ORR and CR were not affected by PNH clone (P=0.966 and P=0.151, respectively). There was a trend of better ORR in transfused-dependent AA (Tf-AA) compared with severe/very severe AA (S/V-AA; 69.6% *vs.* 46.7%; P=0.061). However, there was no difference in terms of CR and ORR at last follow-up between horse and rabbit ATG (P=0.586 and P=0.653, respectively). Also there was no difference between Tf-AA and S/V-AA in terms of CR and ORR (P=0.377 and P=0.653). There were no impact of ALC and ANC on ORR at various cut-off levels. Sixteen patients without any response received alloHCT. Relapse was found in 11/44 (25.0%) patients. Relapse rates seemed slightly higher in horse ATG (29.4%) than in rabbit ATG (10.0%) without any statistical significance (P=0.408). The 5-year and 10-year overall survival rates were 64.6% and 62.4%, respectively among patients who did not received alloHCT. There was no survival difference between horse and rabbit ATG (P=0.778).
Summary and Conclusions: Horse ATG seemed responding rapidly than rabbit ATG in adult sAA. However, overall response rates, relapse rates and overall survival were similar.

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IMPACT OF THE PROLIFERATION INDEX OF SPECIFIC BONE MARROW CELL COMPARTMENTS FROM MYELODYSPLASTIC SYNDROMES IN THE MONITORIZATION OF THE DISEASE: A PILOT STUDY
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Background: We have recently reported in MDS that the presence of decreased proliferation index (PI) among bone marrow (BM) non-lymphoid $CD34^+$precursors and nucleated red blood cells (NRBC) is significantly associated with adverse disease features, shorter overall survival and transformation to AML, even when low- and high-risk MDS patients are separately considered.
Aims: Here we study the PI of different BM cell compartments from MDS patients (n=28) both at diagnosis and at different follow-up controls in order to investigate the proliferation behaviour of these cells during patient monitorization.
Methods: A total of 24 patients with a final diagnostic of MDS were analysed both at diagnosis and at different follow-up BM when they had achieved (partial or complete) morphological and cytogenetic remission or they relapsed with more advanced disease at the time of follow-up. Initially, these 24 patients were diagnosed of RCMD, 7; RAEB1, 11; RAEB2, 6. Eleven of these 24 patients (RCMD, 3; RAEB-1, 6; RAEB-2, 2) had the same or less advanced WHO diagnostic subtype at follow-up compared to baseline control, while 13 (RCMD, 4; RAEB-1, 5; RAEB-2, 4) presented with more advanced disease than at diagnosis.
Results: Follow-up studies from all MDS patients showed decreased PI of overall BM cells –from 7% to 6%; P=0.04- due to decreased PI among non-lymphoid $CD34^+$ cells –from 14% to 6%; P=0.01-, $CD13^{hi}/CD11b^-$ neutrophil precursors –from 14.5% to 8%; P=0.005- and NRBC –from 25% to 15%; P=0.01- *vs.* those found at diagnosis. Of note, no differences were found in the PI from patients with similar WHO diagnostic at baseline and at follow-up (n=11) in any BM cell population. However, as expected, markedly decreased PI were found in cases with more advanced disease at the last follow-up (n=13), among non-lymphoid $CD34^+$ cells –from 15% to 4%; P=0.007-, $CD13^{hi}/CD11b^-$ neutrophil precursors –from 15.5% to 8%; P=0.03-, $CD13^{lo/int}/CD11b^-$ neutrophil precursors –from 20.5% to 12%; P=0.03- and NRBC –from 27% to 13%; P=0.003- *vs.* those found at diagnosis. Furthermore, in detail, among patients with an initial diagnostic of low-risk MDS (n=9), those who evolved to intermediate-risk MDS (n=4) depicted at follow-up a significant decrease of the PI among whole BM cells –from 9% to 5%; P=0.05-, non-lymphoid CD34+ cells –from 17% to 6%; P=0.03-, $CD13^{hi}/CD11b^-$ neutrophil precursors –from 18% to 8%; P=0.03- and NRBC –from 30.5% to 14.5%; P=0.03-. In line with this, low-risk patients evolving to advanced MDS/AML (n=5) showed at follow-up a more marked decrease of the proliferation of non-lymphoid $CD34^+$ cells (P<0.001), $CD13^{hi}/CD11b^-$ and $CD13^{lo/int}/CD11b^-$ neutrophil precursors (P=0.02) and NRBC (P=0.003). Conversely, in patients with advanced MDS (RAEB-2; n=4) at diagnostic evolving to AML at follow-up, no differences were found in the PI of any BM cell population due to achievement of minimal proliferation rates already at baseline. However, those cases with advanced MDS (RAEB-2) who evolved *vs.* those not evolving to AML depicted already at diagnosis a tendency toward lower PI among whole BM cells (4.6% *vs.* 8.5%), non-lymphoid $CD34^+$ cells (3% *vs.* 8%) and NRBC (16.5% *vs.* 26%), although due to the low number of cases statistical significance was not reached.
Summary and Conclusions: The assessment of the PI among specific BM cell compartments may help identifying MDS patients with progression-associated proliferation features, even among cases presenting with decreased BM blast cells at follow-up and might also be useful to identify MDS patients with favourable *vs.* unfavourable response to different treatment strategies.

P734

BENEFITS OF THE USE OF AZACITDINE IN PATIENTS WITH AML REFRACTORY OR RELAPSED AFTER INTENSIVE CHEMOTHERAPY
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Background: Azacitine (AZA) is a demethylating agent approved for the treatment of patient with intermediate-2 (Int-2) or high-risk myelodysplastic syndromes (MDS), chronic myelomonocytic leukaemia, acute myeloid leukaemia (AML) with 20-30% blasts. Subcutaneous therapy with AZA, compared with

conventional care regimens (CCRs), prolongs median survival, delays progression to AML, reduces transfusion needs in MDS in the Int-2 and high risk groups. AZA prolongs median overall survival (OS) in old patients with low BM blasts AML and reduces toxicity.
Aims: Our intent is to suggest as AZA therapy could prolong OS, reduce adverse events and improve quality of life in patients with AML refractory/relapsed after intensive chemotherapy (IC). As well known, median OS in this subset of patients is about 3 months, when treated with CCRs, with few chances to achieve complete remission and a high risk to experience great toxicity.
Methods: Here we report our experience about the AZA therapy in 17 patients (8 MDS and 9 AML) 6 MDS patients were in the Int-2 risk group, 2 in the high, 2 AML were *de novo*, 2 secondary to MDS, 3 refractory and 2 relapsed after IC; three refractory patients have been treated with Clofarabine and Aracytin (Ara-C-), Fludarabine, Ara-C and Idarubicine and low dose Ara-C respectively; two patients relapsed one after Ara-C and Daunorubicine therapy and one after Ara-C, Ethoposide, Daunorubucine treatment and Au-BMT. All AML patients were unfit for other CCRs. Median age was 77 years and BM blasts count was 34% (range3,5-96%); all patients were transfusion dependent (median Hb8,5 g/dl, range7,0-10 g/dl), granulocyte count was 1896/mmc (+/-3142)and Plt 92400/mmc (+/-73800). AML and MDS patients received AZA subcutaneously for 7 days every month at a dose of 75 mg/m^2 daily until disease progression.
Results: As expected OS in MDS patients was 16 months. One patient with denovo AML obtained a CR that persists after 7 months, the other one obtained a PR and died after 9 months. Two patients with secondary AML achieved PR after 3 courses, one showed disease progression after 12 months and one is still in PR after 4 months. After three courses of therapy all refractory/relapsed AML patients (5) showed increased Hb concentration (median9,1 g/dl, range 8-10,6 g/dl) withreduction of transfusion requirements and two of them became subsequently transfusion indipendent; all patients also achieved improvement in Plt count (median 138000/mmc,range 39000-350000/mmc). Median BM blasts countafter 3 courses of AZA therapy was 6%(range 0-21%): 1 patient obtained CR that persists after 8 courses; 4 patients achieved PR and 2 of them are still alive in good PR after 4 courses, while 2 patients showed disease progression after 3 and 9 courses, respectively. During AZA therapy no patient had relevant infections and none needed hospital admission. After a median follow-up of 7 months, 7 patients are still alive (1 denovo AML, 2 secondary AML, 4 refractory/relapsed AML).
Summary and Conclusions: AZA is an effective treatment and has an acceptable safety profile for the treatment of patients with refractory/relapsed AML unfit for IC. AZA provides a clinical benefit reducing transfusion requirement, improving quality of life and extending OS. Furthermore, patients treated with AZA have a lower rate of infectious complications and low toxicity with subsequently fewer hospital admissions compared with CCRs-treated patients.

P735

CURRENT MANAGEMENT OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES IN A REAL-WORLD SETTING: A SURVEY FROM A REGIONAL ITALIAN REGISTRY

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Background: Myelodysplastic Syndromes (MDS) are commonly associated with a poor prognosis in "higher-risk" (HR) patients, and an indolent course in "lower-risk"(LR) patients. Current international Guidelines indicate that management's strategy should be based on evaluation of prognostic score systems such the International Prognostic Score System (IPSS); however, several different reasons might largely influence in the real life the actual clinical management of these patients.
Aims: To examine the "real word" current management strategies and survival of MDS patients in an unselected Italian population.
Methods: We prospectively collected data regarding management of MDS patients from Ligurian MDS database, a regional registry established within the framework of the Italian Network of regional MDS registries.
Results: Data are presented for 293 patients (158 (54%) males and 135 (46%) females), with a median age of 76 years (range 42-98 years), registered into the database from 2010 to 2012. Regarding WHO categorization, 32% patients had RA, 34% RCMD, 5% RARS or RCMD-RS, 3% 5q- syndrome, 6% RAEB-1, 8% RAEB-2 and 1% MDS-unclassified; 11% remained undetermined. IPSS was available in 226 (77%) of the patients; within this group, LR (low and intermediate-1 risk) patients were 87% of the total, while HR (intermediate-2 and high-risk) were 13%. The recently revised form of IPSS (r-IPSS) was calculated in 225 (77%) of the patients, and it stratified them as follows: very low risk 15%, low risk 43%, intermediate-risk 10%, high-risk 6% and very high-risk 3%. Transfusion-dependent patients at diagnosis were 56 (19%). Applying WPSS stratification patients were categorised as follow: 33% very low, 42% low, 13% intermediate, 9% high and 3% very high risk. As first line treatment, 127 (39,5%) patients received no treatment or only supporting therapy (mainly transfusions); 138 (48%) received ESAs, alone or with GCSF. Twenty-six (9%) patients were treated with Azacytidine, 3 (1%) –all with a 5q-syndrome- with lenalidomide; the remaining 2,5% were treated with immunosuppression or AML-like chemotherapy. Among the patients who whenever during the course of the disease required transfusions, 26 (9%) received iron chelation therapy. On December 2012, after a median follow-up of 19 months, the overall survival was 85,2% in LR group and 54% in HR group, respectively.
Summary and Conclusions: Despite well-established clinical guidelines suggest that treatment of MDS should be based on a reliable prognostic score system, this survey shows that in the "real life" MDS remains a disease that is not always managed accordingly. Almost a quarter of the patients, especially older individuals from peripheral centers, are not categorizable according to IPPS because of a lack in diagnostic evaluation (mainly cytogenetics). A large proportion of them received no treatment or supporting transfusion only and very few were treated with iron chelation therapy. A small number received aggressive chemotherapy.

P736

LONG DURATION OF RESPONSE TO AZACITIDINE IN MYELODYSPLASTIC SYNDROMES. MULTICENTER RETROSPECTIVE STUDY OF 36 PATIENTS

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Background: Although azacitidine (AZA) has proven effective in myelodysplastic syndromes (MDS), the duration of haematological response is limited (median 13.6 months) *(Fenaux, 2009)*. The French Group *(Itzykson 2011)* identified some clinical and haematologic parameters (poor ECOG performance status, IPSS intermediate and poor risk cytogenetics, circulating blasts, high transfusion need) independently associated with a a poorer outcome, and these 4 criteria were integrated in a 3-group prognostic score, validated in other cohorts *(van der Helm 2011; Breccia 2012)*. Moreover, a complex karyotype was also predictive of a shorter duration of response.
Aims: These data prompted us to retrospectively analyse our MDS pts treated with AZA who showed a favourable long-lasting response (i.e: duration of response ≥20 months), in order to enucleate the clinical and haematologic features of long-responder pts.
Methods: The type of response was defined according to IWG criteria *(Cheson 2006)*: Complete Remission (CR), Marrow CR, Partial Remission (PR), Hematologic Improvement (HI). The response duration was measured from the date of achievement of a first response, until the date of disease progression or death. Overall Survival (OS) was measured from the start of AZA treatment.
Results: Thirty-six pts (M/F: 21/15), from nine Institutions, with a median age of 72 (range 52-84) yrs, showed a response duration ≥20 months. At AZA onset, WHO diagnosis was: refractory anemia (RA): 2 pts; refractory cytopenia with multilineage dysplasia (RCMD): 1 pt; RCMD with ringed sideroblasts (RCMD-RS): 1 pt; refractory anemia with excess blasts (RAEB)-1: 8 pts; RAEB-2: 16 pts; cronic myelomonocytic leukemia(CMML): 4 pts; AML with 20-30% blasts: 3 pts, MDS with fibrosis (MDS-F): 1 pt. Six pts had therapy-related MDS. IPSS risk was: low: 3 pts; intermediate-1: 7 pts; intermediate-2: 21 pts, high: 5 pts. IPSS cytogenetic risk was: low: 23 pts (63.9%); intermediate: 7 pts (19.4%); high: 6 pts (16.7%) (3 with complex karyotypes and 3 with isolated -7 or 7q-). ECOG-PS was poor (≥2) in 2 pts (5.5%). Transfusion need was high (≥4 RBC units/8 weeks) in 17 pts (47.2%). Three pts (8.3%) presented circulating blasts. Following Itzykson's AZA prognostic scoring system, the risk was low in 14 pts (38.9%), intermediate in 21 pts (58.3%), and high in 1 pt (2.8%), respectively. The pts received a median of 23.5 cycles of AZA (range: 8-59). The median number of cyles to any first response was 4 (range: 2-10). The best response achieved was: CR in 21 pts (58.3%), marrow CR: 4 pts (11.1%), PR in 2 pts (5.5%), and HI in 9 pts (25%). Cytogenetic remission was achieved in 7 pts (19.4%). The median duration of response was 24.5 (range: 20-88) months. A significant toxicity (grade >2) was observed in 6 (16.7%) pts. Twenty-two pts (61.1%) are still maintaining hematologic response, 9 pts (25%) are still alive but discontinued treatment because of disease progression, and 5 pts

died, for AML (2 pts), infection (1 pt), myocardial infarction (1 pt),cachexy (1 pt), respectively. Median OS from the start of AZA was 35.5 (range: 22-120) months.
Summary and Conclusions: Although our data confirm the finding of other Authors, as the majority of long-responder patients showed pre-treatment favourable prognostic factors, a long-lasting hematologic response can be achieved even in a significant fraction of pts presenting one or more poor risk features (IPSS intermediate or high risk cytogenetics, high transfusion need).

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PROLONGED LOW-DOSE AZACITIDINE SCHEDULE IN HIGH-RISK MDS PATIENTS: LONG TERM EFFICACY AND RELATIONSHIP WITH MOLECULAR RESPONSE

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Background: The currently approved azacitidine (AZA) regimen for myelodysplastic syndromes (MDS)is 75 mg/sqm/die subcutaneously (SC) or intravenously (IV) for 7 days every 28 days. Recently 3 different AZA dosing regimens, which avoid week-end dosing, have shown to induce therapeutic responses consistent with the currently approved schedule *(Lyons, 2009)*. In particular, one of them, *i.e.* the AZA 5-2-5 regimen (50 mg/m2/d subcutaneously for 5 days, followed by 2 days no treatment, then 50 mg/m2/d for 5 days) allows the administration of a nearly equivalent monthly total dose of AZA.Moreover, some data suggest the possibility that prolonged exposure to lower doses of AZA may increase the response rate *(Gore, 2006)*. However, the community-based study of Lyons mainly involved lower-risk MDS patients (pts).
Aims: These data prompted us to investigate the therapeutic effect of the more convenient AZA 5-2-5 regimen in higher-risk MDS pts (*i.e.*: IPSS risk: high or intermediate-2).
Methods: From December 2007, in our Institution, 29 IPSS high-or-intermediate-2 risk MDS pts. (20 males), with a median age of 70 (37-83) yrs, were treated with the AZA 5-2-5 regimen. Moreover, as our group *(Follo, 2009)*previously demonstrated that the inositide signalling pathways, in particular phosphoinositide-phospholipase C (PI-PLC) beta1, may represent a target for AZA, we quantified the degree of PI-PLCbeta1 methylation and gene expression before and during AZA administration in this group of pts, and in a control group of high-risk pts previously treated with the conventional AZA regimen.
Results: At AZA onset, IPSS risk was: intermediate-2: 23 pts; high: 3 pts; 3 pts, with intermediate-1 IPSS risk, showed other high-risk features (therapy-related MDS: 2 pts; complex karyotype: 1 pt). IPSS cytogenetic risk was: low: 14 pts; intermediate: 5 pts; high: 10 pts (8 with complex karyotypes and 2 with isolated -7 or 7q-). Revised IPSS *(Greenberg, 2012)* was: intermediate: 1 pt; high: 6 pts; very high: 22 pts. 5 ptspresented circulating blasts. Transfusion need was high (≥4 RBC units/8 weeks) in 13 pts. ECOG-PS was poor (≥2) in 7 pts. Itzykson's prognostic risk for outcome after AZA *(Blood 2011)* was: low: 4 pts; intermediate: 24 pts; high: 1 pt. The pts received a median number of 8 (1- 26) AZA cycles. 26 pts (89.6%) received at least 6 cycles of AZA and were considered evaluable for response, while 2 pts prematurely discontinued AZA because of toxicity or worsening of their clinical conditions, and 1 pt. is still on treatment. Among the 26 evaluable pts, 19 (73%) showed a favourable response, following IWG criteria *(Cheson, 2006)*: 5 (19.2%) Complete Remission (CR), and 14 (53.8%) Hematologic Improvement (HI). First response occurred after a median of 3 (2-7) cycles. 4 pts maintained a stable disease (SD), while the 3 remaining pts showed treatment failure. A significant toxicity (grade >2) was observed in 13 (44.8%) pts. The median duration of response was 12 (1-48) months. Four pts showed a long-lasting response (≥20 months). The median OS from the start of AZA was 16 (5-51) months. 16 pts died, 8 because of evolution into Acute Myeloid Leukemia, and 8 for other causes (infection, haemorrhage, heart failure, stroke, suicide). PI-PLCbeta1 methylation and gene expression appeared to be related to the therapeutic response, but not to the dose schedule.
Summary and Conclusions: Our data seem to confirm, in a population of high-risk MDS, the effectiveness, in terms of hematologic and molecular response, of the more convenient AZA 5-2-5 regimen.

P738

THE ASSOCIATION OF FREE LEPTIN INDEX AND INSULIN LEVELS WITH THE OCCURRENCE OF MYELODYSPLASTIC SYNDROMES

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Background: Recent evidence suggests that obesity may be implicated in the etiology of hematologic malignancies,including myelogenous leukemia and myelodysplastic syndromes (MDS). Leptin, and, in particular, free leptin, the biologically important form of leptin, reflecting accurately the body fat mass, regulates glucose and lipid metabolism by improving insulin sensitivity and reducing intracellular lipids. We thus attempted to explore whether altered secretion of free leptin in association with insulinemia, a hormonal system linked with several obesity and insulin resistance associated malignancies including leukemia, may underlie this association.
Aims: In thiscase-control study, we investigated the potential role of free leptin, soluble leptin receptor in the etiopathogenesis of MDS after adjusting for a potential confounding effect of body mass index (BMI), height, weight, family history of lymphohematopoietic cancer (LHC), serum insulin and adiponectin. We also explored associations between free leptin and established MDS prognostic factors.
Methods: Blood samples were collected from 101 cases with incident, histologically confirmed primary MDS, and 101 hospital controls, admitted for non-neoplastic and non-infectious conditions, matched on gender, age and year/month of diagnosis (±1 month) between 2004 and 2007. Serum insulin and serum leptin were determined by radioimmunoassay (Millipore and Linco Research, Inc. respectively). Serum leptin receptor levels (sOB-R) levels were measured using a commercially available ELISA (BioVendor Laboratory Medicine). Free Leptin Index (FLI) was calculated as the ratio of leptin to sOBR. The statistical analysis of the data was performed using IBM-SPSS® version 20 for Windows.
Results: Cases presented significantly higher height and weight than control subjects (P<0.001), while differences of BMI were only of borderline significance (P=0.12). Seruminsulin was significantly higher in cases than in controls (P=0.005). MDS cases exhibited significantly lower serum levels of sOB-R and adiponectin than controls (P=0.04 and P<0.001 respectively). Furthermore, MDS cases presented hypoleptinemia, though not statistically significant at α=0.05 (P=0.07). In univariate analysis, FLI was similar in both cases and controls (P=0.35). Serum leptin was higher in RAEB and RAEB-t, in high and intermediate-2 risk category of IPSS, and in MDS with an intermediate and poor prognosis karyotype (P<0.001). In multivariate analysis, subjects in the second and fourth quartile of FLI presented significantly lower odds for MDS (2nd quartile: OR=0.36, 95% C.I. 0.13-0.95 and 4th quartile: OR=0.08, 95% C.I. 0.02-0.29) after adjusting for age, gender, date of diagnosis, BMI, family history of LHC, smoking history, adiponectin and insulin levels.
Summary and Conclusions: This study raises the hypothesis that the association of lower FLI reflecting overall fat mass, hypoadiponectinemia reflecting visceral obesity and hyperinsulinemia are associated with higher risk of MDS. Leptin's major physiological role is to signal inadequate rather than excess energy stores, and hypoleptinemia found in a small but significant percentage of obese humans is associated with hyperinsulinemia and impaired T-cell function. Further mechanistic and interventional studies are needed to confirm these findings and to explore whether these hormones mediate the effect of body fat distribution on insulin resistance and myelodysplasia risk.

P739

REAL WORLD USE OF AZACITIDINE IN ELDERLY PATIENTS WITH MDS/AML–THE SCOTTISH EXPERIENCE

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Background: The management of elderly patients with MDS and AML poses significant challenges despite considerable attention focused at improving treatment. Even those who can tolerate standard induction treatment have significantly inferior outcomes (AML 5yr overall survival rates of 3-8% in over 60s compared to 50% in younger patients).[1] Azacitidine has shown promise in this context with reports of superior response rates and improved survival when compared to standard therapy.[2,3]
Aims: Azacitidine received National Institute of Clinical Excellence (NICE) endorsement in the United Kingdom in March 2011 and its use was facilitated in Scotland following approval by the Scottish Medicines Consortium (SMC) in September 2011. However, lack of consensus remains regarding the value of Azacitidine given that it confers significant toxicity. This study was performed to investigate the use of the drug in a "real life" setting.
Results: We report a retrospective analysis of 42 patients with MDS (n=19), AML (n=19) and CMML (n=4) treated with Azacitidine in Scotland between 2008 and 2012. Median age of patients was 72 yrs. 45.2% (n=19) patients received the drug as first line treatment and 26.2% (n=11) as maintenance therapy following remission induction using standard chemotherapy of which 7 patients received Azacitidine in the context of the National Cancer Research Institute (NCRI) AML 16 trial (www.aml16.bham.ac.uk). 26.2% (n=11) patients were treated with Azacitidine as second/third line therapy, with 4.8% (n=2) treated as a bridge to stem cell transplant. Patients received a median of 4 cycles of treatment. Overall response rate was 31% (n=13); complete response in 9.5% (n=4) and mono- or bilineage haematological improvement

in 21.5% (n=9). 50% (n=21) showed no response or progressed whilst on therapy. 54.7% (n=23) patients required admission to hospital for management of drug-related complication. Moderate or severe gastrointestinal and dermatological toxicity was reported in 14.3% (n=6) and 19% (n=8) cases respectively. 61.9% (n=26) patients experienced infective complications and sepsis-associated deaths occurred in 14.3% (n=6). Median survival had not been reached at a median follow-up of 6 months (range 1-44 months) and all cause mortality was 45.2% (n=19).

Summary and Conclusions: In the "real world" setting Azacitidine is used in multiple therapeutic contexts. Consequently published studies may not reflect the drug's effect on unfit, often heavily pre-treated, patients. Whilst Azacitidine undoubtedly improves the quality of life of a small cohort of patients, its durability is often short-lived and its toxicity should not be underestimated. The significant levels of sepsis reported in this analysis suggest that Azacitidine should be used with caution in patients perceived to be at particular risk of infection. Its role as a consolidation treatment for older patients following successful induction therapy is currently being explored.

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P740

MODELLING THE LONG-TERM SURVIVAL OF PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS) DEL (5Q) TREATED WITH LENALIDOMIDE

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Background: Myelodysplastic syndrome (MDS) are a group of malignant hematological disorders in which the bone marrow function is abnormal and produces insufficient numbers of mature blood cells necessitating frequent blood transfusions in some cases. Lenalidomide is an immunomodulatory (IMiD) derivative, approved in the US in 2005 for the treatment of patients with transfusion-dependent anemia due to low or int-1 risk MDS associated with a del5q cytogenetic abnormality with or without additional cytogenetic abnormalities. The aim of treatment with lenalidomide is to achieve transfusion independence (TI). A similar indication is currently under review for approval in Europe. In UK, where there are no licensed treatments, current options consist of: blood transfusions, growth factors and antibiotics - best supportive care (BSC). The efficacy and safety of lenalidomide (10mg and 5mg doses) has been demonstrated in two trials in MDS patients with del5q, MDS004: a randomized placebo-controlled trial and MDS003: a single arm trial. Results previously presented from analysis of the MDS003 and MDS004 trials showed that patients who achieved TI for at least 26 weeks had a 64% reduction in risk of death.

Aims: In order to carry out an economic evaluation mean survival is required. This work aims to estimate the impact of lenalidomide on long-term mean survival of low and int-1 risk MDS del5q transfusion dependent patients compared to patients on BSC, based on MDS003 and MDS004 trial data and published literature. BSC is defined, based on UK treatment patterns, as 72% of patients receiving only transfusions and 28% receiving ESA followed by G-CSF if there is no response to ESA.

Methods: As TI has previously been identified as a prognostic factor in MDS, for this analysis overall survival (OS) was modeled based entirely upon the probability of becoming TI, regardless of the treatment received. The probability of achieving TI for ≥182 days was taken from MDS004 for lenalidomide 10mg and transfusion only (placebo) patients and from Hellström-Lindberg's predictive model for ESA and G-CSF. OS is modeled for BSC and lenalidomide, for patients who did not progress to AML, using parametric curves based upon whether patients achieved TI. Weibull, exponential, lognormal, loglogistic and extreme value curves were fitted to combined trial data across all trial arms. Weibull curves were found to provide the best fit (based on AIC, BIC and integrated brier score). The rate of progression to AML was also modeled using parametric curves based upon whether patients achieved TI with weibull curves again providing the best fit. For patients who progressed to AML OS curves were taken from published literature, as not enough patients progressed to AML and died within the trials to enable this to be modeled directly.

Results: Consistent with the trials, within the model more patients were predicted to become TI with lenalidomide: 60.9% *vs.* 8.4% for BSC. Based upon this increase in TI the model predicts a mean OS of 5.7 years for patients treated with lenalidomide (median 4.7 years) *vs.* a mean OS of 4.6 years for patients receiving BSC (median 4.5 years). A comparison of model and trial results is shown in the Figure 1. Using probabilistic analysis sampling10,000 model iterations lenalidomide improved survival in 99.9% of cases.

Summary and Conclusions: TI has been demonstrated to be an independent prognostic factor in MDS patients. This analysis shows that the increase in TI associated with lenalidomide treatment of MDS del5q patients results in an increase in mean survival of over 1 year when compared to BSC.

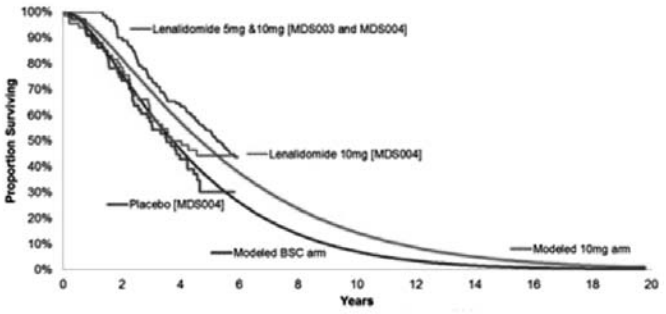

Figure 1.

P741

ANALYSIS OF RESPONSE TO ERYTHROPOYESIS STIMULATING AGENTS (ESAS) IN 114 MYELODYSPLASTIC SYNDROME (MDS) PATIENTS FROM REGISTRO CAMPANO DELLE MIELODISPLASIE

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Background: Anemia is one of the main problems of MDS patients even in absence of transfusion need. The great majority of MDS patients are old and numerous comorbidities characterize their clinical features, anemia significantly worsens their quality and expectancy of life. ESAs is considered first line therapy in anemic MDS patients and there is an increased tendency to its employment in the earlier stage of the disease.

Aims: The aim of this work is to evaluate clinical response to ESAs therapy in a cohort of anemic MDS patients, no transfusion dependent, enrolled in a regional retrospective register, RECAMDS (Registro Campano delle Mielodisplasie), a subgroup of the Italian MDS Register, focusing on WHO classification and IPSS risk stratification.

Methods: We have retrospectively considered 114 anemic MDS patients (M/F 55/59, median age 77±9.1, WHO 43 RA, 17 RARS, 39 RCMD, 8 RAEB, 7 MDS with del 5q, risk assessment:53 low,58 Int-1, 3 Int-2), not transfusion-dependent and treated with ESAs (α or β EPO 40000/80000 or 30000/60000 U/weekly respectively) from 2006 to 2012. We analyzed data at three and six months of treatment: responses were evaluated according to IWG criteria2006; in the responders patients we evaluated median interval from diagnosis, serum erythropoietin concentration at the beginning of therapy, time to response, duration of response and relationship with WHO subgroups. Statistical analysis was performed applying ANOVA, t Student and c2 tests.

Results: 28 patients under study were treated with standard dose of α erythropoietin (40.000 IU weekly) and 12 received a high dose (80000 weekly), the remaining 70 were treated with β erythropoietin: 56 with a weekly standard dose of 30.000IU and 14 with high dose (60000IU weekly). ESA therapy was started at mean Hb concentration of 9.50 g/dl (±1.5) with EPO serum level of 64mU/L±79.6 after a mean of six months (1-118) from diagnosis. The global response rate was 84% (96/114): 88/114 patients achieved response after three months, other 8 patients achieved a significant response after six months of therapy (4 AR, 1RARS,2 RCMD, 1 MDS 5q-). 40 patients (38%) lost the response after a mean of 18 months (3-84) while 74 (65%) are still on treatment without transfusion need after a median time of 25 months (3-96). WHO classification of non responders patients identified 7 AR,4 RARS,5 RCMD, 1 AREB, 1Del 5q and their EPO level was 173 mU/L ±143.93 while in the responders subgroup it was 47.28 mU/L ±48.6, with statistical difference between the two groups. The WHO subgroups did not exhibited different profiles of response. It is noteworthy that in a subgroup of anemic patients (7) with 5q deletion, not transfusion dependent, we observed a good response to ESA treatment in the majority of them (6/7), 5/6 achieved response after three months and 1 after six, all responder 5q- patients (3 5q- syndromes,3 RA, 1RCMD) are still responsive with a median time of 34 months (range 8-96 months).

Summary and Conclusions: As well known ESAs therapy can be considered a successful treatment in MDS patients in low risk group where, as first line therapy, delays the need for RBC transfusion hypothetically by slowing the disease course thus suggesting their employment in the earlier stages of the disease. Here we report a good response to ESA treatment also in more advanced stage of MDS (INT1 and INT 2) and in a subgroup of 5q- MDS generally reported as bad responders to such treatment, further studies will evaluate if this good response will delay transfusion requirement and subsequent lenalidomide therapy.

P742

INFLAMMATION INDICES IN MYELODISPLASTIC SYNDROMES AT DIAGNOSIS

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Background: Myelodisplastic syndromes (MDS) are a group of heterogeneous diseases characterized by peripheral cytopenias and hypercellular bone marrow. MDS usually affect elderly patients, and they often had other comorbidies. The role of inflammation in MDS is still under explored.It has been recently observed a correlation of inflammation indexes with outcome.
Aims: We evaluated the impact of 5 common inflammation index, ferritin, alpha 1 glycoprotein (α1g), alpha 1 antripsin (α1a), C reactive protein (CRP) and aptoglobin (apto) on overall survival (OS) and their correlation with WHO 2008 classification, IPSS risk and presentation CBC.
Methods: We collected data from 79 consecutive MDS outpatients diagnosed in the Hematology Unit of the University of Florence. Patients were classified according to WHO classification as follow: 17 RA, 3 RARS, 30 RCMD, 11 RAEB1, 5 RAEB2, 8 AML <30% of blasts and 5 CMML, and prognostic score was evaluated according to IPSS as follow: 34 low risk, 33 intermediate1, 4 intermediate 2 and 8 high risk. Median age was 75 years (range...), 43% were female (34/45 F/M). Median follow up was 11 months.
Results: Median value of all the indexes we analysed was within the limits of our laboratory exam. Median value of α1g was 0.89 ±0.33 g/L, 12 patients had α1g level above normal value (n.v.) (n.v 0.5-1.2 g/L), median value of α1a was 1.6 ±0.44 g/L, 12 patients had α1a above n.v. (n.v. 0.9-2 g/l), median value of apto was 0.82±0.72 g/L, 5 patients had apto level above n.v. (n.v.0.3-2 g/L), median value of ferritin was 229±826 ng/mL, 28 patients had ferritin level above n.v. (20-300 ng/mL), only 17 patients had CRP level above the normal limit (9 mg/L. Analysis of variance among different WHO subtypes and in IPSS indicated that there was no statistical difference among different subtypes of MDS. The average value of parameters were uniformly distributed among WHO and IPSS subtypes. There were some interesting correlations. Ferritin was inversely correlated with hemoglobin levels (P<0.001, r=-0.366), apto was directly correlated with hemoglobin level (P=0.045 r=0.236) and with α1g (P=0.000, r=0.461), α1g was also directly correlated with absolute neutrophils count (P=0.045 r=0.236) and with α1a (P=0.000, r=0.536). Regarding the association with outcome, we compared MDS patients with normal to those with abnormal values. We observed that MDS patients with abnormal ferritin had a median OS of14,6 month while median was not reached at 40 months for the group with normal value of ferritin (P=0.041). We had the same observation for CRP (14 months median OS *vs.* not reached) (P=0.018) and for α1a (OS 12 months *vs* 32 months) (P=0.005). There was no significant difference in OS between the MDS patients with normal and abnormal α1g and apto.
Summary and Conclusions: Although the increase of inflammation markers is not a common feature of MDS at diagnosis our basic analysis indicated that in all MDS, irrespective of IPSS risk, there is a strict correlation with survival for ferritin, alfa 1antripsin and C reactive protein, possibly indicating a role of inflammatory cytokines in determining the history of the disease Further studies are warranted to support this observation and implement our aramamentarium to define MDS prognosis.

P743

RISK SCORING SYSTEM USING IPSS-R, LDH AND PERFORMANCE STATUS TO PREDICT THE OUTCOMES OF PATIENTS WITH LOWER RISK MYELODYSPLASTIC SYNDROME TREATED WITH HYPOMETHYLATING AGENTS

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Background: Prognosis of patients with myelodysplastic syndrome (MDS) can be calculated using a number of scoring systems. The most commonly used system is the International Prognostic Scoring System (IPSS), and the treatment decision using hypomethylating agents (HMA) has been based on IPSS risk groups. HMA has been known to improve the treatment outcomes of patients with the IPSS lower risk [low and intermediate-1 (INT-1)] groups. However, some of the patients showed the deteriorating course even though they were categorized to lower risk IPSS.
Aims: In the current study, we revised the original IPSS lower risk to know whether it could further discriminate the prognosis of the IPSS lower risk MDS patients treated with HMA, and to propose a 'Risk scoring system' using the variables known to be related with the outcome of lower risk MDS patients.
Methods: The data of 68 patients with lower risk MDS receiving HMA treatment were retrospectively reviewed. The IPSS risk groups were revised into IPSS-R. The overall survival (OS) rate among the IPSS-R risk groups and other clinical variables were analyzed, and prognostic power was calculated using Cox-hazard models.
Results: HMA were treated with median 4 cycles (range 1-22). Azacitidine was used in 57 patients (83.8%) and decitabine in 11 (16.2%). Baseline LDH were increased in 16 patients (23.5%), and ECOG performance status (PS) were 0-1 in 51 patients (75.0%) and 2 in 17 (25.0%). IPSS low risk and INT-1 risk were 7 patients (10.3%) and 61 (89.7%), respectively. The patients with IPSS were revised into IPSS-R as followes; 15 patients (22.1%) as low, 33 (48.5%) INT, 19 (27.9%) high, and 1 (1.5%) very high. The OS rate were not different between original IPSS-low and INT-1 (P=0.400). However, OS of the low risk group defined by IPSS-R was significantly better than other risk groups [hazard ratio (HR) 3.128, P=0.033]. Among the other clinical variables, female sex (HR 0.453, P=0.037), ECOG PS 2 (HR 2.130, P=0.042), and increase LDH (HR 3.049, P=0.003) were related to OS in the univariate analyses. In the multivariate analysis, increased LDH (HR 2.868, P=0.008), IPSS-R low risk (HR 2.976, P=0.051), and ECOG PS 2 (HR 2.159, P=0.051) affected the outcomes of patients treated with HMAs.
Finally, weighted risk scores were designated based on HR and significance as folllows: IPSS-R low (score 1), ECOG-PS 2 (score 1), and increased LDH (score 2). According to this 'Risk scoring system', 10 patients (14.7%) were score 0, 30 (44.1%) score1, 14 (20.6%) score2, 12 (17.6%) score3, and 2 (2.9%) score 4. The 3-yr OS rate were significantly different between each groups: 88.9±10.5% in score 0 (n=10), 28.3.±9.7% in score 1-2 (n=44), and 0% in score ≥3 (n=14) (Figure 1).

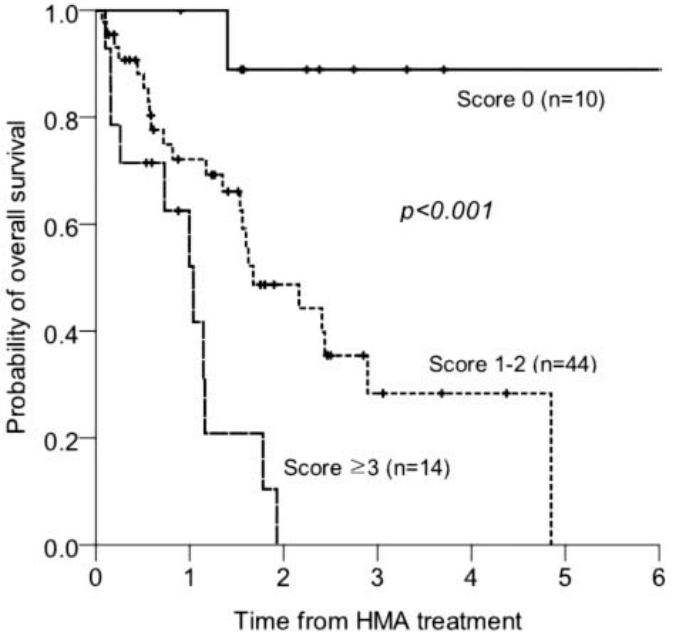

Figure 1.

Summary and Conclusions: The risk scoring system based on IPSS-R, baseline LDH, and ECOG PS could discriminated further among the original IPSS lower risk patients, which need to be validated in the larger cohorts. Furthermore, to define the beneficial effect of HMA for each risk groups should be evaluated by comparing the treatment-naive patients with comparable patient populations.

P744

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA CLONE: DIAGNOSTIC AND CLASSIFICATION OF 103 BRAZILIAN PATIENTS

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired chronic hemolytic anemia, which often manifests with peripheral blood cytopenias and thrombosis. The patients with PNH have acquired mutations in the phosphatidylinositol glycan class-A (PIG-A) gene, which causes the lack of Glycosylphosphatidylinositol-anchored complement regulatory proteins (GPI-AP) on hematopoietic cells. The clinical course of the disease is highly variable.
Aims: We retrospectively reviewed 103 PNH cases referred to our hospital from December 1999 through December 2011 to assess clinical presentations, thrombosis, survival, difference among subcategories, and clinical significance of PNH clone size.
Methods: The diagnosis of PNH was established by detection of the positive PNH clone by Flow Cytometry (FCM). The proteins studied were CD55 and CD59 on erythrocytes, neutrophils and monocytes, CD16, CD24 and CD66b on neutrophils and CD14 on monocytes.The presence of a clone was defined as the presence of GPI-AP greater than 0,1% of neutrophils or red cells, and the size of the clone was defined by the highest level of red blood cells and neutrophils lacking GPI-anchored proteins. Patients with severe and moderated AA, MDS or AML were subclassified according to literature.

Results: 103 patients with PNH clone were included into this study. The median age at presentation was 24.1 years (5.5–62 years). Hemoglobinuria as the initial symptomatic manifestation were seen in 23.2% of the patients, while the frequencies of infectious (46.6%) and bleeding symptoms (47.1%) were higher than other series. 17 (16,5%) patients developed thrombosis during monitoring. 101 patients presented with peripheral blood abnormalities: 83 (80,6%) had pancytopenia, 12 (15,2%) anemia and thrombocytopenia, and 4 (3,9%) anemia with leucopenia. The median hemoglobin level were 88g/L (38-145 g/L; normal range, 120-160 g/L), median ANC of 0.94×10^9/L ($0.26\text{-}1.45\times10^9$/L; normal range; $2\text{-}11\times10^9$/L), and with median PLT of 25×10^9/L ($2\text{-}294\times10^9$/L; normal range, $120\text{-}400\times10^9$/L). The median LDH were 328U/L (30-7970U/L, normal range 190-240U/L). 86,4% had hypocelular bone marrow. The cohort of patients were divided into subcategories of classic PNH (10 patients), PNH/AA (39 patients), and PNH-sc/AA (54 patients) based on the proposed PNH working clinical classification. 26 patients developed hemolysis after 2.35 years (median) after AA diagnosis. The overall survival at 10 years after diagnosis estimated by Kaplan–Meier was 81.7%, but PNH-sc/AA patients had lower OS (76,5%). There are significant differences in terms of median age, size of PNH clones, clinical symptoms, and peripheral blood cell counts among the three subcategories, especially between classic PNH and PNH-sc/AA subcategories. PNH clone size in erythrocytes and granulocytes were significantly different among the three subcategories (P<0.001), since the median percentages were respectively: 0,04% (0-10%) and 7.3% (6.4-19.0%) in PNH-sc/AA;15,8% (14-52%) and 63% (56-78%) in PNH/AA, and 82,2% (89.7-92.2%) and 98.0% (86.7-98.4%) in classic PNH. There were statistical difference in the variables platelets (P=0,001), LDH (P=0,002) and in the median percentages of PNH clone in neutrophils and erythrocytes among patients with or without thrombotic event, respectively neutrophils=92.7% (3.4%>100%) *versus* 21.5% (0.25%>99.9%), P<0,001, and erythrocytes=31.8% (0-100%) *versus* 1.2% (0-100%), P=0,008.

Summary and Conclusions: In conclusion, these retrospective review 103 PNH patients over a 10-year period represented the largest collection of such patients based on a single center in Brazil. It provided us useful information to understand the disease since our results confirmed the suggestion that large PNH clone are associated with increased risks for hemolytic form and thrombosis, even in patients with bone marrow failure, whereas small PNH clone is associated with a non-hemolytic form of the disease.

P745

ERYTHROPOIETIC ASPECTS IN MDS PATIENTS RESPONDERS TO ESAS TREATMENT

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Background:
ESAs are the first line therapy in low risk anemic MDS patients and an early inception of this therapy can delay the need for RBC transfusion, hypothetically by slowing the disease course. It is a matter of debate whether the clinical response is a result of proliferation and maturation of the dysplastic clone or stimulation ofresidual normal erythropoiesis by ESAs. Evidence has been provided to support both views.

Aims: Macrocytosis is one of the cytological hallmarks of dyserithropoiesis in MDS: in this work we have analyzed erythropoietic response to ESAs therapy in a cohort of anemic MDS prevalently "low risk" patients enrolled in a regional retrospective register, RECAMDS (Registro Campano Mielodisplasie), subgroup of the Italian MDS register. We focused on cytometric differences in Mean Corpuscolar Volume of erythrocytes during the observation period in order to speculate on the target of such therapy in responsive patients.

Methods: 114 anemic MDS patients (43 RA, 17 RARS, 39 RCMD, 8 RAEB, 7 MDS del5q) not transfusion dependent, under standard ESA treatment ($\alpha$and βEpo, 40000/80000 or 30000/60000 U/weekly respectively), were analyzed at the baseline, after three and six month of continuous therapy. The response rate was evaluated following IWG criteria. Statistical analysis was performed with χ_2 and Anova tests.

Results: ESA therapy was started at Hb concentration 9.56 g/dl±1,5, global response rate was 84% (96/114), no difference among WHO subgroups was found. 88 patients responded after three months, 8 after six. In the responsive cohort, MCV was higher than normal at baseline in 52/96 (54%) patients, while 14/18 (77%) non-responsive patients exhibited macrocytosis. During the response at ESAs treatment, after 6 months from beginning of EPO therapy, 45/52 (86%) macrocytic patients showed permanently elevated values of MCV whereas 7/52 (13%) macrocytic responsive patients became permanently normocytic. In the group of 44 initially normocytic responsive patients 7/44 (15%) became macrocytic and contemporarily 4 of them showed an increase in their neutropenia and/or thrombocytopenia.

Summary and Conclusions: These very preliminary data can suggest that in the majority of MDS patients responsive to ESA treatment the increase of hemoglobin level occurs mainly stimulating erythroid production in MDS clones; in the minority of patients probably it happens recruiting residual polyclonal erythropoiesis. It is interesting to note that stimulating effects of ESA last even when the expression of dysplasia progresses.

P746

COMPARISON OF IPSS AND R-IPSS IN A SINGLE UNIT PATIENT COHORT

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Background: The International Prognostic Scoring System (IPSS) is the most widely used prognostic tool in Myelodysplasic Syndrome (MDS). A revised IPSS (R-IPSS) has been proposed, differing from IPSS by including finer cytogenetic grouping and depth of cytopenias.

Aims: In a MDS patient cohort diagnosed between January 2005 and December 2012 in our institution we retrospectively applied the R-IPSS and compared it to the IPSS risk groups.

Results: Complete data at diagnosis were available for 104 patients. Median age was 70.5 years, with male predominance (66/38); the most common WHO 2008 subtype was refractory cytopenia with multilineage dysplasia (33%) and 53% of patients were transfusion dependent. According to the IPSS, 78% were lower and 22% higher risk. Using the R-IPSS, patients were stratified as follows: very low 10%, low 48%, intermediate 14%, high 15% and very high 13%. Table 1 summarizes the reclassification of each IPSS group according to the R-IPSS. All low IPSS remained low risk in the R-IPSS, distributed between low and very low, while the great majority of high risk IPSS fall into the very high risk group. Intermediate-1 spanned from low to high, while intermediate-2 from high to very high. During a median follow-up of 17months, 17 patients (16%) progressed to acute myeloid leukaemia (AML), 6% in the low, 20% in the intermediate, 27% in the high and 54% in the very high risk group. Thirty-seven patients died (36%), 6% in the low, 46% in the intermediate, 53% in the high and 77% in the very high risk group. All 3 deaths in the low risk group were due to AML progression and the majority of deaths in the high and very high risk groups were AML/disease related. In the intermediate groups deaths were either due to infection or comorbidities.

Table 1.

	R-IPSS					
	VERY LOW	LOW	INT	HIGH	VERY HIGH	TOTAL
IPSS						
LOW	10	25	0	0	0	35 (34%)
INT-1	0	25	14	7	0	46 (44%)
INT-2	0	0	0	7	5	12 (11%)
HIGH	0	0	1	2	8	11 (11%)
TOTAL	10 (10%)	50 (48%)	15 (14%)	16 (15%)	13 (13%)	104

Summary and Conclusions: In our cohort most patients maintained their original risk stratification but 15% of those in IPSS-1 were re-stratified as high risk by R-IPSS. This suggests that this group is that in which therapeutic decision based on the R-IPSS might be more relevant.

P747

DOES REVISED INTERNATIONAL PROGNOSITIC SCORING SYSTEM (IPSS-R) HAVE ANY IMPACT ON CLINICAL OUTCOMES IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES TREATED WITH HYPOMETHYLATING AGENTS?

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Background: Currently, stem cell transplant (SCT) remains the only curative therapy for myelodysplastic syndromes (MDS). Newer treatments with hypomethylating agents (HMAs) such as azacitidine and decitabine have proven to alter the disease course of MDS. In particular, azacitidine improves survival in higher-risk MDS patients compared with conventional treatment. International prognosis scoring system (IPSS) has recently been refined into the revised IPSS (IPSS-R) which predicts the clinical outcome of patients with untreated MDS. Whether the IPSS-R retains its value in the prognostication of patients with MDS treated with HMAs remains unknown.

Aims: We aim to determine the correlation between IPSS-R and clinical outcomes of MDS patients who received HMAs in our institution.

Methods: We performed a retrospective analysis on 47 patients with MDS (n=39) and low blast count acute myeloid leukaemia (AML; n=8) who received HMAs from November 2007 to November 2012. Clinical, haematological and

cytogenetic data were collected. Eleven and 2 of these patients underwent SCT and intensive chemotherapy respectively after HMAs. Their data were censored at the time of SCT or chemotherapy. Response criteria were based on the modified International Working Group (IWG) response criteria for MDS. Treatment response and survival data were analyzed using SPSS version 17.
Results: The median age of the whole cohort was 66 years (range 18-82). Twenty-eight of the patients were male and 19 were female. They received azacitidine (n=28) or decitabine (n=19). The median number of treatment cycles received was 4. Among these patients, the IPSS scores were intermediate-1 (n=13), intermediate-2 (n=19) and high (n=15) and the IPSS-R risk scores were low (n=3), intermediate (n=5), high (n=15) and very high (n=23). Cytogenetics risk stratification for these patients were good (n=21), intermediate (n=9), poor (n=2) and very poor (n=15). Median follow-up duration was 21.8 months. Overall response rate was 46.8% with complete remission (CR), marrow CR (mCR) and haematological improvement only (HI only) attained in 19.1%, 19.1% and 8.5% of patients respectively. Overall response was not influenced by IPSS-R (P=0.34). Median overall survival (OS) for the whole cohort was 20.3 months. The median survival for IPSS-R low, intermediate, high and very-high risk groups was 21.9, 24.4, 26.2 and 9.8 months respectively (P=0.039).
Summary and Conclusions: Many MDS patients are elderly and not eligible for intensive chemotherapy or SCT. HMAs with low toxicity have become an attractive treatment option for these MDS patients. HMAs have also been used to reduce disease burden in MDS prior to SCT in clinical trials. Our analysis showed that HMAs were able to induce good overall response rates of 46.8% and a median OS of 20.3 months. Significantly, IPSS-R has been shown to have an impact on the survival outcomes in our small series. The clinical outcome of MDS patients with very-high risk IPSS-R scores remains particularly poor, with a median survival of 9.8 months, despite treatment with HMAs. New treatment strategies will be needed to improve the survival of this group of MDS patients who are not eligible for SCT.

P748

THE CLINICAL RELEVANCE OF MINOR PAROXYSMAL NOCTURNAL HEMOGLOBINURIA CLONES IN REFRACTORY CYTOPENIA OF CHILDHOOD–A PROSPECTIVE STUDY BY EWOG-MDS

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Background: Minor paroxysmal nocturnal hemoglobinuria (PNH) clones are frequently present in adults with myelodysplastic syndrome (MDS) and are predictive of response to immunosuppressive therapy (IST) in some studies. We recently reported that in refractory cytopenia of childhood (RCC), the most common subtype of childhood MDS, IST might be effective. Data on the frequency and clinical correlates of PNH clones in RCC are lacking, and the value of the presence of a PNH clone as response predictor to IST in RCC is unknown.
Aims: In a prospective multicenter study of the European Working Group of MDS in Childhood (EWOG-MDS), we determined the clinical relevance of PNH clones in RCC.
Methods: 87 previously untreated primary RCC patients, diagnosed between June 2005 and December 2011, were evaluated for the presence of PNH clones. Diagnosis of RCC was based on WHO criteria for pediatric MDS and confirmed by central review of bone marrow morphology and histology. Peripheral blood was analyzed by high-sensitivity flow cytometry, defining PNH-type granulocytes as $CD24^{-}FLAER^{-}$ and PNH-type erythrocytes as $CD55^{-}CD59^{-}$. Patients were considered PNH positive when a GPI-deficient population larger than 0.01% in the erythroid and/or a population larger than 0.03% in the granulocytic lineage were present. Of 87 RCC patients, 28 hypocellular patients were subsequently treated with IST, consisting of horse- or rabbit-ATG and CsA.
Results: The median age of included patients was 10.2 years (range: 1-18 years). Bone marrow was hypocellular in 84% of patients; cytogenetic analysis was normal in 85%, monosomy 7 was present in 7%, and other cytogenetic aberrations in 8%. PNH clones were detected in 36 of 87 (41%) RCC patients at diagnosis. Median clone sizes were 0.06% (range: 0.01-58%) in erythrocytes and 0.9% (range: 0.03-86%) in granulocytes. PNH positive patients, compared to PNH negative patients, were significantly older (median: 12.7 *versus* 7.5 years, P=0.005), had lower leukocyte counts (median: 2.7 *versus* 3.3×10^9/L, P=0.029), lower platelet counts (median: 20 *versus* 44.5×10^9/L, P=0.008), lower hemoglobin levels (median: 7.7 *versus* 9.5 g/dL, P=0.006), tended to have a higher MCV (above the 97th percentile in 81 *versus* 62% of patients, P=0.073), and were more often HLADR15 positive (42% *versus* 21%, P=0.051). Hypocellular RCC patients with a PNH clone >0.1% were more likely to respond to IST than PNH negative patients (7 of 8 (88%) *versus* 8 of 20 (40%) patients responded at six months, respectively, P=0.038) and tended to have a better event-free survival. PNH clone size remained relatively stable in sequentially followed patients, both in patients under a watch-and-wait strategy, and in patients treated with IST, independent of response.
Summary and Conclusions: We conclude that PNH clones are frequently present in RCC, predict response to IST, and might indicate an immune-mediated pathophysiology in at least a subset of RCC patients.

Multiple myeloma - Biology 2

P749

NEW STRATEGIES OF ANALYSIS WITH FLOW CYTOMETRY FOR THE DIAGNOSIS OF MINIMAL RESIDUAL DISEASE IN MULTIPLE MYELOMA

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Background: The use of intensive therapy and novice agents has increased the quality of responses in multiple myeloma (MM), necessitating the development of more sensitive techniques to monitor the disease.
- In the last years, the study of minimal residual disease (MRD) by multiparameter flow cytometry 4 colour and PCR has shown that obtaining MRD <0.01% is an independent prognostic factor for survival in patients undergoing ABMT.
- However, the selective loss of plasma cells (PC) manipulating the sample, changes in the immunophenotype of residual neoplastic PC, and the reappearance of an increasing number of normal PC may complicate the identification of small pathological populations, limiting the sensitivity of the technique.

Aims: Optimizing a 8 colours panel, restricted to surface antibodies that have most frequently aberrant expression in neoplastic plasma cell.
- Check our systematic of study, and propose a simple and reproducible way to identify residual populations with high sensitivity.

Methods:
- Bone marrow aspirate in EDTA tube (1 cm^3).
- Multiparameterflowcytometer8 colours.
- Panel as recommended by the European Myeloma Network, and validated by our own experience (two tubes: CD38/CD27/CD200/CD19/CD28/CD10/CD45/CD138 and CD38/CD20/β2m/CD56/CD117/CD81/CD45/CD138).
- The use in studies analyzed with 8 fluorescences identifies a real "fingerprint" the neoplastic population).

We analize:
- 30 healthy controls.
- 60 MGUS.
- 60 patients with MM at diagnosis.
- 40 patients with MM in complete remission post-treatment.

Results: We superimpose the reference image of the initial diagnostic study, and make a selection of the study population by similarity to the original. Select all PC by expression of CD38 and CD138, and analyzed with the APS option. The APS clearly separates into two subpopulations. Through the combination of the selected antibodies, we clearly identify aberrant residual population (red), which is distinguished from the normal PC (green).

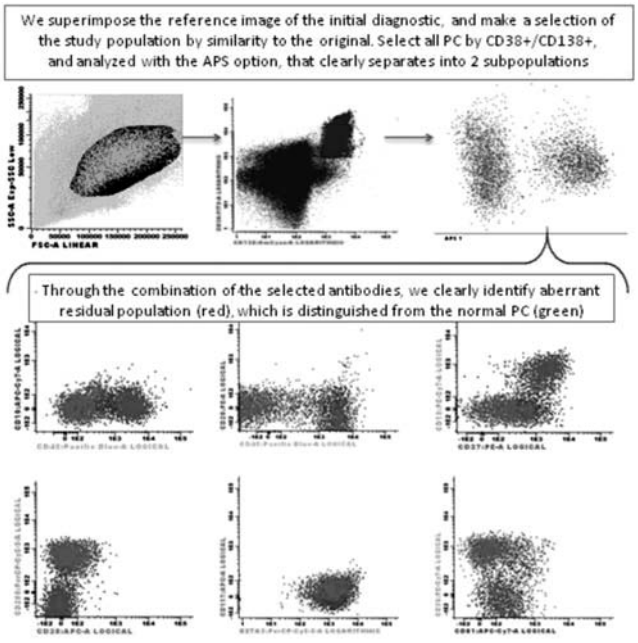

Figure 1.

Summary and Conclusions: The strategy described allows to detect and discriminate normal populations of pathological in a simple and reproducible manner, even in cases with immunophenotypic changes after the relapse.
In contrast with most combinations of four colours, the strategy does notrequire detection of cytoplasmic immunoglobulins and, therefore, avoids selective cell losses by manipulation and permeabilization.
Given the high sensitivity and the possibility of detecting very small populations, we believe feasible to increase the sensitivity of the technique of $10^{-4a10-5}$.

P750

FREQUENT MUTATION OF THE MYD88 GENE IN MACROGLOBULINEMIA

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Background: Waldenström's macroglobulinemia (WM) is an IgM-secreting lymphoplasmacytic lymphoma (LPL). Nuclear factor (NF)-κB signaling is important for the growth and survival of WM cells. Myeloid differentiation primary response gene 88 (MYD88) is an adaptor protein that mediates toll and interleukin (IL)-1 receptor signaling. After stimulation, Toll/IL-1 receptor (TIR) domains activate MYD88. Subsequently MYD88 dimerizes and induces phosphorylation of IL-1 receptor-associated kinase (IRAK)4, IRAK1, and IRAK2, leading to inhibitor κBα (IkBα) phosphorylation and activation of NF-κB. The MYD88 gene was mutated in activated B-cell-like subtype of diffuse large B-cell lymphoma (DLBCL). The most frequent mutation of the MYD88 gene was a T to C transition at nucleotide 978 resulting in a leucine to proline substitution at amino acid position 265 (L265P). This mutation is located in the TIR domain and triggers IRAK-mediated NF-κB signaling. The L265P mutation was recently identified in 49 of 54 patients with WM, and 3 of 3 non-IgM-secreting LPL. Inhibition of MYD88 signaling reduced IκBα, NF-κB p65 phosphorylation, and NF-κB nuclear staining in WM cells expressing the L265P. More recently, the mutation was found in 18 of 27 WM (67%).
Aims: To determine the relevance of the L265P mutation and its association with clinical characteristics in lymphoid neoplasms, we analyzed on the MYD88 gene in WM, B-cell lymphoma, and myeloma.
Methods: The L265P mutation was analyzed in 23 WM, 1 non-IgM-secreting LPL, 1 low grade B-cell lymphoma with IgG M-protein, and 38 myeloma. Mononuclear cells were separated from bone marrow after obtaining written informed consent. PCR products covering the TIR domain were purified and ligated into the pGEM-T vector. Sequencing was performed in both directions on a MegaBase sequence system. Direct sequencing was performed using the purified PCR products. The L265P mutation was also examined with BSiE1 restriction enzyme digestion. The PCR products were purified, digested with BSiE1, and subject to electrophoresis through a 2% agarose gel. The current study was conducted within the guidelines and with the approval of the institutional ethical committee. Correlations between the categorical variables were analyzed using the chi-square test or Fisher's exact probability test. Statistical analysis was performed with the Wilcoxon's signed-ranks test. Ordinal variables were compared with the use of the Mann-Whitney's U- test. The analysis was performed using Dr SPSSII (version 11.01) or Statcel 3 software. P-value of less than 0.05 was considered statistically significant.
Results: We first selected 10 patients including 8 WM, 1 non-IgM-secreting LPL, and 1 B-cell lymphoma with IgG M-protein for cloning and sequencing. At least four clones were examined. The L265P was detected in 2 of 9 clones from 1 patient with WM, while it was absent in other 9 patients. Because of the low frequency of the mutation, we next performed direct sequencing in the 10 patients and additional 15 patients with WM. The L265P was detected in 8 of 25 patients (32%). It was found in 7 of 23 WM (30%) and the low grade B-cell lymphoma with IgG M-protein. All of the 8 patients with the transition also had wild-type sequences. We next tested for the mutation with BSiE1 digestion. Sensitivity of the L265P mutation is 5% in our study. Aberrant bands corresponding to the mutation were detected in 19 of the 25 patients: 18 of the 23 WM (78%) and the B-cell lymphoma with IgG M-protein but not in the non-IgM-secreting LPL. All of the 8 patients with the L265P (by sequencing) showed the aberrant bands. We also examined with BSiE1 digestion in the 38 patients with myeloma, however no aberrant bands were observed. The incidence of the mutation was significantly higher in WM than in myeloma (P<0.00001). In the 23 WM patients, the L265P mutation was more frequently detected by the BSiE1 digestion (78%) than direct sequencing (30%) (P=0.0010). The L265P mutation was significantly more frequent in males (15/16, 94%) than females (3/7, 43%) (P=0.017). The percentage of lymphocytes was larger in the L265P group than wild-type group (P=0.041). It was larger in male than female patients (P=0.030).
Summary and Conclusions: This study shows the L265P mutation to be frequent in WM and absent in myeloma. Our results reveal that the BSiE1 digestion is more sensitive than direct sequencing. Since some patients have only a small amount of the mutant allele, the contribution of the mutation remains to be clarified in these cases.

P751

THE ROLE OF FLT3-LIGAND IN THE PROGRESSION OF MULTIPLE MYELOMA; FLT3-LIGAND RELEASED FROM BONE MARROW STROMAL CELLS ACTIVATED PI3K/AKT PATHWAY AND WNT SIGNALING

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Background: The concerted action of cytokines secreted locally in the bone marrow controls the maintenance, expansion, and differentiation of hematopoietic stem cells (HSCs). Given that the progression of multiple myeloma (MM) is regulated by growth factors released from bone marrow stromal cells, aberrant cytokine signaling contributes to oncogenic transformation during multilineage hematopoietic cell differentiation. The flt3-ligand is capable of inducing multilineage hematopoietic cell differentiation. However, the exact role of flt3-ligand still remains unknown in MM.
Aims: To determine the dynamic interplay between MM cells and microenvironment, the role of flt3-ligand released from BMSCs is examined in relation with the progression of MM
Methods: To identify potential, functionally relevant flt3-lgand in MM, we measured, by ELISA, bone marrow plasma flt3-ligand concentrations in 127 MM patients. We also determined the role of flt3-ligand in the growth of MM cell lines. Specifically, we investigated flt3-mediated cell signaling in the growth of MM cells and the expression of adhesion molecules in MM cells, to emphasizing the important role of flt3-ligand as a mediator in the interaction of MM cells with their microenvironment.
Results: The steady-state level of flt3-ligand in MM patients (n=127) was 67 +/- 4 pg/mL and was higher than in case-control groups (AML, CML, and ALL). Association between the levels of flt3-ligand and clinical parameters (poor prognostic marker and remission duration) of MM patients was examined. The levels of flt3-ligand showed a significant difference between the two groups with or without IgH spilt (14q32 FISH) (*P=0.004*). They also differed according to remission duration (within 6 months and more than 6 months; *P=0.027*). To examine the dynamic interplay between MM cells and bone marrow stromal cells (BMSCs), flt3-ligand was measured in 7 different MM cell lines and 12 different BMSCs ex-vivo cultured from MM patients' bone marrow. Flt3 ligand was detected in cultured soup of 12 BMSCs but not detected in cultured soup of MM cell lines. Levels of flt3-ligand was variable in the cultured soup of 12 BMSCs. Levels of flt3-ligand from BMSCs obtained from 5 MM patients relapsed within 6 months were higher than those from 7 MM patients (*P=0.021*) relapsed after 6 months. Flt3-ligand levels were higher in cultured soup of MM cells co-cultured with BMSCs than in controls (either MM cells alone or BMSCs alone). In the presence of flt3-ligand in U266 cells and MOLP8 cells, phosphorylation of AKT and STAT5 was dose-dependently increased as well as flt-3 ligand-induced DKK1 secretion was observed. Since DKK1 expression was regulated by wnt-signaling, we then examined a set of genes related with wnt signaling after U266 cells and MOLP8 cells were treated with flt3-ligand for 2 hours. We found that flt3-ligand effectively activated *wnt* signaling and induction of DKK1 was detected in both cell lines treated with flt3-ligand. Partial knockdown of flt-3 expression by short interfering RNA also resulted in inhibition of DKK1 secretion. These results indicated that flt-3 signaling plays a central role in the growth of MM cells and the regulation of DKK1 secretion.
Summary and Conclusions: Our results indicated that the activation of flt3-ligand signaling play a critical role in the progression of MM.

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CIRCULATING MICRORNA AS POTENTIAL BIOMARKERS OF MGUS AND MM

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Background: MicroRNA, a family of small non-coding regulatory RNA, is implicated in deregulation of critical pathways involved in multiple myeloma (MM). Since they are also present in serum of MM and monoclonal gammopathy of undetermined significance (MGUS) patients (pts) with high stability, they could serve as effective biomarkers for such pts. We have identified a specific serum miRNA profile in newly diagnosed MM and MGUS pts, correlated it with clinical parameters, FISH and survival data.
Aims: 1. To identify a specific profile of serum miRNAs characteristic for MM and MGUS pts. 2. To evaluate the association between serum miRNAs and clinical parameters, FISH data, overall survival (OS) and time to progression (TTP).
Methods: 190 serum samples obtained from MM pts, MGUS pts and healthy donors (HD) were evaluated for this study. Screening analysis of 667 miRNAs was performed on 4 MM, 4 HD and 5 MGUS samples with TaqMan Low Density Arrays (TLDA). Levels of 6 differentially expressed miRNAs (*P*<0.05) between MM and HD were confirmed by quantitative real-time PCR using an absolute quantification approach on 103 MM, 57 MGUS and 30 HD samples. Receiver Operating Characteristic (ROC) analysis was used to calculate specificity and sensitivity of each miRNA and their combination. Univariate Cox proportional hazards survival model was used to evaluate prognostic impact of miRNAs. Biochemical data and cytogenetic characteristics were also available for MM and MGUS pts. P values <0.05 were considered as significant.
Results: MiRNA TLDA arrays analysis showed 14 differentially expressed miRNAs between MM and HD, but no significantly deregulated miRNAs between MM and MGUS. Therefore, miR-222, miR-130a, miR-34a, miR-744, let-7d and let-7e were further validated on a larger cohort of MM, MGUS and HD samples. Overall reduction of circulating serum miRNAs was observed in MM and MGUS compared to HD. MiR-744, miR-130a, let-7d and let-7e were significantly downregulated and miR-34a was upregulated in MM and MGUS cohort of pts (all *P*<0.001). ROC analysis showed highest sensitivity (80.6%), specificity (86.7%) (AUC=0.898) for combination of miR-34a and let-7e to distinguish MM from HD and sensitivity (91.1%), specificity (96.7%) (AUC=0.976) for miR-34a and let-7e combination to distinguish MGUS from HD. Significant positive correlation between low levels of serum miR-744, let-7d, let-7e and levels of hemoglobin (r_S=0.543; 0.508 and 0.585 resp., all *P*<0.0001), thrombocytes (r_S=0.555; 0.500 and 0.515 resp., all *P*<0.0001) and albumin (r_S=0.355; 0.385 and 0.355 resp., all *P*<0.0001) was observed in MM. These miRNAs significantly negatively correlated with levels of creatinine (r_S=-0.415; -0.372 and -0.406, resp., all *P*<0.0001) and beta$_2$-microglobulin (r_S=-0.504; -0.447 and -0.529 resp., all *P*<0.0001). In MGUS, similar correlation pattern with these parameters was observed, except for correlation between miRNA and thrombocytes, where no correlation was found. However; neither in MM, nor in MGUS, the levels of miRNAs correlated with PCs infiltration. Only in MM pts, let-7d and let-7e correlated with del (13q14) (*P*=0.045 and *P*=0.019 resp.). Levels of miR-744, let-7d and let-7e showed an inverse correlation with ISS stage (all *P*<0.0001). Lower levels of miR-744 were found to be significantly connected with worse OS (HR 0.998 [HR95%CI: 0.997; 0.999); *P*=0.001) and TTP (HR 0.998 [HR95%CI: 0.998; 0.999]; *P*=0.001).
Summary and Conclusions: Our observations demonstrate that circulating serum miRNA may be promising biomarkers for patients with monoclonal gammopathies, such as MGUS and MM.
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P753

TGR-1202: A NOVEL PI3K-DELTA SPECIFIC INHIBITOR IN MULTIPLE MYELOMA

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Background: The bone marrow microenvironment contributes to the pathogenesis of Multiple Myeloma (MM) by promoting the oncogenic process, including drug resistance. High expression levels of the PI3K-δ isoform in patient MM cells implicate this target as a novel and attractive interventional strategy aimed at attenuating the progression of the disease.
Aims: Herein, we describe the biological and pharmacokinetic properties of TGR-1202, a novel small molecule PI3K-δ inhibitor currently under clinical development for patients with relapsed and refractory hematological malignancies, with potential to be developed as a clinical candidate for patients with relapsed and/or refractory MM.
Methods: Activity of TGR-1202 on individual PI3K isoforms was determined in enzyme, cell, and whole blood based assays. Potency of TGR-1202 was confirmed *via* MM cell viability and apoptosis assays in addition to testing for inhibition of pAkt, a downstream kinase regulating cell survival and growth. Additionally, TGR-1202 was tested for potency in viability, apoptosis, and Akt phosphorylation assays using immortalized and MM patient derived primary cells. ADME and pharmacokinetic properties of the molecule were also determined.
Results: TGR-1202 demonstrated significant potency against PI3K-δ (22.2 nM) with high selectivity over the α (>10,000 fold), β (>50 fold), and γ (>48 fold) isoforms. Additionally, TGR-1202 inhibited B-cell proliferation (24.3 nM) and FcεR1 induced CD63 expression in human whole blood basophils (68.2 nM) indicating specificity towards the delta isoform. The addition of 5 μM TGR-1202 to dexamethasone (0.55 μM) caused a dramatic shift in GI_{50} of MM-1S cells compared to that of either TGR-1202 (9.7 μM) or dexamethasone (18.3 μM) alone. Further viability testing demonstrated that TGR-1202 caused a dose-dependent inhibition (>50% @ 1-3 μM) in growth of immortalized (U266B1 and MM-1R) as well as patient-derived MM cells. Reduction in viability was accompanied by a reduction

in pAKT (>80% @ 3 μM) along with induction of apoptosis in both cell lines and patient samples. Pharmacokinetic studies across species indicated good oral absorption (>40% bioavailability for mice, rat, and dog) with favorable plasma concentrations (3-10 μM @ 20 mg/kg for mice, rat, and dog) relevant for efficacy.
Summary and Conclusions: Data demonstrate the therapeutic potential of TGR-1202 in MM *via* inhibition of the PI3K-δ pathway. Data from immortalized as well as primary patient-derived cells justify further evaluation of TGR-1202 in MM. Additional studies, including combination therapy of TGR-1202 with anti-MM agents in mouse xenograft models are currently ongoing.

P754

DEF124B, A MEMBER OF THE β-DEFENSINS, IS DOWNREGULATED IN DIFFERENTIATING OSTEOBLASTS BY EXPOSURE TO IMIDS AND MAY MEDIATE THE SUPPRESSIVE EFFECT OF THESE AGENTS ON OSTEOGENESIS

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Background: Osteoblastic activity is severely impaired in most myeloma cases, which contributes to the development of osteolytic lesions. Although several drugs reducing osteoclast mediated bone degradation are in clinical use, approaches to specifically augment bone formation are at an early stage of development. Novel anti-myeloma drugs not only directly act on myeloma cells, but impact on the microenvironment as well. Bortezomib was previously shown to exert bone anabolic properties. In contrast, the influence of immunomodulatory drugs (IMiDs) on osteogenesis is less well defined.
Aims: IMiDs were shown to influence various cell types including osteoclast and granulocyte differentiation as well as activity of T- and NK-cells. Data on the impact of IMiDs on osteoblast development is very limited so far. In this study we aimed to investigate the differential effects of IMiDs alone or in combination with bortezomib on osteogenesis.
Methods: An *in vitro* model for osteoblast differentiation was used employing immortalized or primary human bone marrow-derived stromal cells to study the effect of thalidomide and lenalidomide on osteogenesis. Alkaline phosphatase activity and matrix mineralization were used as markers of early and late osteoblast development, respectively. Genes known to be important for osteogenesis were studied by qPCR. Furthermore, gene expression profiling was applied to identify additional genes of importance in mediating the effects of IMiDs.
Results: Treatment with thalidomide and lenalidomide significantly inhibited osteoblast development, as reflected by a 43% (P<0.05) reduction of alkaline phosphatase activity on day 14 and a 70.4% (P<0.05) reduction of matrix mineralization on day 21 of osteogenesis. This observation was accompanied by downregulation of osteogenic transcription factors Runx2 and Dlx5. The same effects were noted when IMiDs were combined with bortezomib. Furthermore, DKK1 and inhibin beta A expression was induced up to 8.6 and 1.9 fold (P<0.05), respectively, by both IMiDs. Neither DKK-1 nor activin A inhibition rescued BMSCs from the OB inhibitory action of the IMiDs, suggesting that other mechanisms are involved in IMiD related suppression of osteogenesis. Microarray analysis revealed deregulation of several OB associated genes including *Runx2*, *MMP3*, *COL5A3*, *PTN* and *GREM1*, with different patterns depending on the IMiD used. *DEFB124*, a poorly characterized member of the family of ß-defensins, was the only gene affected by both IMiDs, being downregulated approximately 5- fold (P=0.02).
Summary and Conclusions: We show a significant inhibitory effect of thalidomide and lenalidomide on osteoblast development. This finding is likely due to IMiD induced downregulation of *DEFB124,* although other osteoblast inhibitors such as DKK1 and inhibin beta A were downregulated as well. Modulation of ß-defensin expression might mitigate untoward bone specific side effects of IMiDs, which may become particularly important during periods of prolonged drug exposure such as maintenance therapy.

P755

IDENTIFICATION AND CHARACTERISATION OF THE ROLE OF SAMSN1 GENE EXPRESSION IN A MURINE MODEL OF MYELOMA AND MYELOMA PATIENTS

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Background: Multiple myeloma (MM), a haematological malignancy characterised by the clonal growth of malignant plasma cells in the bone marrow, is preceded by the benign, asymptomatic condition, monoclonal gammopathy of undetermined significance (MGUS). Several genetic abnormalities have been identified as critical for the development of myeloma, however these abnormalities are also found in patients with MGUS, indicating that there must be other as yet unidentified factors that contribute to the onset of myeloma disease.
Aims: To identify novel genetic factors that play a role in the development or progression of MM.
Methods: Unlike the related C57BL/6 mouse strain, the C57BL/KaLwRij mouse strain displays an inherent ability to develop MM. We used comparative gene expression profiling analysis to identify differences between these two strains as a strategy to identify genes that may play roles in MM disease development.
Results: We identified the adaptor molecule, *Samsn1,* as having significantly decreased expression in all tissues and haematopoietic cell subsets of the C57BL/KaLwRij mice compared to C57BL/6 mouse strain. Over-expression of *Samsn1* in the 5TGM1 murine myeloma plasma cell line resulted in complete inhibition of myeloma disease development *in vivo*. *SAMSN1* expression was also found to be decreased in human myeloma cell lines and in the bone marrow plasma cells from MM patients compared to MGUS and normal controls.
Summary and Conclusions: *Samsn1* therefore represents a key gene, whose loss of expression may facilitate the progression of MM disease, and hence may represent a novel therapeutic target.

P756

IDENTIFICATION AND CHARACTERIZATION OF LAMBDA MYELOMA ANTIGEN, LMA, AS A THERAPEUTIC TARGET IN LAMBDA MULTIPLE MYELOMA

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Background: Clinical studies have shown that the monoclonal antibody (mAb), MDX-1097, specific for kappa myeloma antigen (KMA), has anti-myeloma activity in kappa multiple myeloma (κMM) and an excellent safety profile as demonstrated by early stage clinical trials**.**
Aims: The aim of this study was to verify whether a λ equivalent of KMA, *i.e.* lambda myeloma antigen (LMA), existed on the cell surface of λ myeloma cell lines and λMM patient samples and characterize this novel antigen.
Methods: Two mAbs and human myeloma cell lines (HMCLs) of divergent λ isotype were analysed by flow cytometry. Surface plasmon resonance and Western blotting were used to characterize the reactivity of the two mAbs against a range of lLCs and select a prototypic candidate. Confocal microscopy using the selected mAb was utilized to further characterize the presence of LMA on the cell surface of RPMI8266 λHMCL cells. Epitope excision experiments were conducted to identify the epitope of this mAb and additionally LMA was recreated on the cell surface of HEK cells using recombinant expression approaches.
Results: Flow cytometry demonstrated the presence of LMA on the cell surface of λHMCLs and on primary BM samples from λMM patients. Confocal microscopy also demonstrated the cell surface location of LMA. Only one of the mAbs tested showed pan-reactivity against all λLCs tested. The epitope of this mAb was identified, shown to be of a conformational nature, and mapped to the variable (V)-constant (C) proximal region of λFLC. LMA expression on the cell surface was characterized by recombinant expression of l free light chain (λFLC) in CHO and HEK host cells. Both host cells secreted soluble λFLC, however, LMA was only detected on the surface of HEK cells.
Summary and Conclusions: These data identify LMA as a novel antigen that is specific to malignant B cells of λLC istoype. We have generated a number of human anti-LMA mAbs which are currently undergoing pre-clinical evaluation for clinical development. We believe this targeted approach to treatment would be beneficial in l type multiple myeloma and in particular for patients with POEMS syndrome.

P757

NEUTROPHILS ARE IMPAIRED IN MULTIPLE MYELOMA BUT NOT IN MGUS

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Background: In Multiple Myeloma (MM) the immune function is impaired but the role of microenvironment on this dysfunction is unknown.
Aims: Evaluation of immunosuppressive properties of mature neutrophils in MM and MGUS.
Methods: In 60 consecutive newly diagnosed MM, 70 MGUS and 30 healthy subjects we evaluated in neutrophils (N) from peripheral blood the phagocytic activity using a commercially available kit (Phagotest R), and the expression of molecule arginase-1 (Arg-1) and PROK-2. We tested the immunosuppressive properties with functional assay, based on *in vitro* co-culture of N isolated from patients and T-lymphocytes from healthy subjects.
Results: Despite no differences in the absolute number of neutrophils between MM, MGUS and healthy donors, we found a functional impairment in MM not evident in MGUS patients.
The capability of phagocytosis of MM-N was significantly reduced compared to healthy subjects (P<0.001) and MGUS (P<0.0001), and partially restored after

induction chemotherapy. MM-N exhibited an increased expression of ARG-1 compared to MGUS and healthy controls (25.5 *vs* 6.2 *vs* 1 fold changes in gene expression, P=0.003), confirmed by functional assay of enzymatic activity of ARG-1, positively correlated with advanced disease. Similarly, MM-N exhibited an increased expression of PROK-2 compared to MGUS and healthy controls (13.8 *vs* 1.5 *vs* 1 fold changes in gene expression, P=0.001). After PHA-P stimulation, T-lymphocytes isolated from healthy donors missed the expression of activation markers such CD71, CD69, CD25, CD3ζ in presence of MM-N for 72 hours, and in a less extensive way in presence of MGUS-N.
Summary and Conclusions: Compared to controls, neutrophils obtained from MM patients and to a lesser extend from MGUS patients, have a reduced phagocytic activity and increased ability to suppress lymphocyte activation. These alterations may contribute to impairment of immune functions that characterizes MM patients.

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IDENTIFICATION OF INSULIN LIKE GROWTH FACTOR BINDING PROTEIN 7 (IGFBP7) AS TUMOR SUPPRESSOR IN MULTIPLE MYELOMA LINKED TO THE PRESENCE OF OSTEOLYTIC LESIONS AND OVERALL SURVIVAL
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Background: Direct interactions between myeloma cells and bone marrow stromal cells (BMSCs) as well as the crosstalk through various soluble factors play a major role in the pathophysiology of MM leading to clinical features such as myeloma bone disease. Insulin like growth factor binding protein 7 (IGFBP7) has been described as secreted tumor suppressor protein in melanoma and in various other solid tumors. Methylation dependent silencing of IGFBP7 correlated with outcome in lung-, breast-, hepatocellular- and pancreatic ductal cancer. Recent studies also indicate a possible role for IGFBP7 in hematological malignancies.
Aims: Here we aimed to analyze microenvironmental factors possibly interfering with BMP signaling in the bone marrow environment and to elucidate the role of insulin like growth factor binding protein 7 (IGFBP7) in the pathophysiology of multiple myeloma (MM).
Methods: BMP antagonist expression pattern in myeloma was analyzed by gene expression profiling. IGFBP7 gene expression in MMCLs was analyzed by qPCR after treatment with 5-aza-2´-deoxycytidine and Trichostatin A. Pyrosequencing was performed by Varionostic® GmbH. Viability was assessed after treatment with recombinant human IGFBP7 for 96 hours. Immortalized human BMSCs were co-cultured with MMCLs and primary MM cells for 72 h. Primary human BMSCs were kept in osteogenic differentiation medium for 7-14 days.
Results: Microarray analysis of BMP signaling antagonists revealed downregulation of IGFBP7 in MGUS (n=22) and MM patient samples (n=329) as well as MM cell lines (MMCLs)(n=17) compared to normal plasma cells (n=10)(P<0.02). IGFBP7 silencing in MM cells was found to be regulated by methylation, confirmed by pyrosequencing of the IGFBP7 promoter region in primary MM cells and by upregulation of expression after 5-aza-2-deoxycytidine treatment in 4 of 7 MMCLs (P<0.05). Treatment with recIGFBP7 decreased viability in all cell samples testes, reaching statistical significance in 6 of 7 MMCLs (P<0.05) and 2 of 4 primary MM cell samples (P<0.05) tested. This effect was due to an impairment of proliferation confirmed by BrdU assay and upregulation of p21 (P<0.05), while no increase in apoptosis could be detected. Focusing on the BM microenvironment, we observed decreased expression of IGFBP7 in BMSCs after co-culture with 4 of 5 MMCLs (P<0.001) and 3 of 3 primary MM cell samples (P<0.001). Treatment with recIGFBP7 stimulated osteoblast activity up to 1.8 fold (P<0.001) and decreased DKK1 expression (P<0.05). In line with this, we found an association of low IGFBP7 expression with bone disease (P<0.05) in an independent series of newly diagnosed myeloma patients (n=62). Furthermore, high IGFBP7 expression tended to predict longer OS (median survival 62 *vs.* 20 months, P=0.06).
Summary and Conclusions: Our data show significant downregulation of IGFBP7 in MM cells. This effect is mediated by hypermethylation, and likely contributes to disruption of cell cycle control and enhanced proliferation of myeloma cells. IGFBP7 seems also to be suppressed in stromal cells in the vicinity of MM cells, which might be involved in the impairment of osteoblast development and contribute to myeloma bone disease. Moreover, IGFBP7 expression tends to predict overall survival in MM. Upregulation of IGFBP7 might therefore be a useful therapeutic intervention in the treatment of MM.

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HIGH RISK CYTOGENETIC ABNORMALITIES AND PLASMA CELLS PHENOTYPE INDUCE SIGNIFICANT ALTERATIONS IN BONE MARROW B-CELL COMPARTMENTS IN MONOCLONAL GAMMOPATHIES
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Background: Multiple Myeloma (MM) is a hematopoietic neoplasm characterized by an accumulation of malignant plasma cells in the bone marrow (BM). The disease is considered incurable. Immune dysfunction is an important finding in MM. Decreased levels of these cells and immune paresis are usual findings in symptomatic MM (s-MM). Many cytogenetic abnormalities are associated with MM. t(4;14), t(14;16) or del(17p) are associated with poor prognosis. Aberrant CD117 expression is associated with higher numbers of normal bone marrow plasma cells in monoclonal gammopathies (MG) and a favorable outcome in MM. Co-expression of CD28 by clonal plasma cells might attenuate the positive impact of CD117, leading to poor prognosis and disease progression. CD27 is homogeneously expressed on normal plasma cells, plasma cells from MGUS patients and in MM patients in complete remission; its loss is thought to be associated with progression/relapse of MM and poor prognosis.
Aims: We aimed to compare the distribution of BM B-cell compartments between MGUS, smoldering MM and s-MM patients according with cytogenetic risk and phenotype prognostic markers.
Methods: We studied rectrospectively a total of 85 patients with MG: 31 MGUS, 8 smoldering MM and 46 s-MM. As control group we studied 8 age-matched normal BM samples. Clinical and laboratory data were collected. Staging with International Scoring System (ISS) was performed. A 8-color flow cytometry technique was used to perform BM immunophenotyping and five major B-cells compartments were identified: CD34+ B cells, hematogones, immature B cells, memory and naïve B cells. Cytogenetic abnormalities with more relevant prognostic impact in MG were carried out after plasma cell FACS sorting purification by fluorescence *in situ* hibridization (FISH). Accordingly to cytogenetic risk abnormalities, we divided our population in 4 groups: MGUS, smoldering MM, s-MM with high cytogenetic risk, s-MM with standard cytogenetic risk. Patients were also grouped based on the expression of CD27, CD28 and CD117 prognostic markers. Statistical analysis was made using SPSS software Version 20.
Results: Our population had a median age of 74 years old. 54,8% were males and 45,2% were females. BM B-cell frequency (%) from MGUS, smoldering MM and s-MM were within the normal range and similar to that observed in control group. Concerning different B-cell compartments, we found higher frequency of naïve B-cells in high cytogenetic risk s-MM than in low risk ones and smoldering MM, with statistical significance. Although not achieving statistical significance, we observed lower frequencies of memory B-cells in s-MM with high cytogenetic risk than in standard risk groups. When we analyzed B-cell compartments by phenotypic prognostic markers, we observed that MGUS patients with aberrant BM plasma cells CD117+ have higher frequency of total B cells, CD34+ B cells, hematogones and immature B cells than MGUS patients with plasma cells CD117-, reaching statistical significance.
Summary and Conclusions: Lower frequency of memory B-cells in s-MM high risk cytogenetic patients may correspond to a loss of immunologic memory and a consequent decrease in humoral immunologic response against tumor cells. Aberrant expression of CD117 in plasma cells of MGUS patients appears to promote an increased B-cell precursor differentiation. Plasma cells expressing this marker may compete in BM myeloid precursor niches with normal myeloid precursors and may keep more quiescent, conferring a more favorable prognosis to these patients.

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ANGIOPOIETIN-2 AND MACROPHAGE INFLAMMATORY PROTEIN-1 ALPHA HAVE DUAL EFFECT ON BONE INVOLVEMENT AND ANGIOGENESIS IN MULTIPLE MYELOMA
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Background: The development of the malignant clone in multiple myeloma is enhanced by the production of various cytokines with several properties. Angiopoietin-2 (Ang-2) participates mainly in the angiogenic process, whereas macrophage inflammatory protein-1alpha (MIP-1alpha) acts as an osteoclast activating factor.
Aims: The purpose of the study was to evaluate their role in myeloma patients and to determine possible correlation of them with markers of bone disease and angiogenesis, such as receptor activator of nuclear factor-κB ligand (RANKL), osteoprotegerin (OPG), amino-terminal collagen type-I telopeptide (Ntx) and interleukin-1beta (IL-1beta).
Methods: We studied 54 newly diagnosed myeloma patients (27 male and 27 female, with mean age 66.9±9.4 years). None of the patients had received any myeloma-related therapy prior to initial sampling. According to international staging system (ISS), 12 were in stage I, 21 in stage II and 21 in stage III. Concerning the immunoglobulins' types, 31 were IgG, 17 IgA and 6 light chain disease. Concerning the bone disease, estimated by skeletal ×-rays, 27 had grades 0-2 (low grade), whereas the rest 27 had grades 3 and4, (high grade). Twenty-five, age and sex matched healthy volunteers were used as controls. Serum samples were collected, stored at -70ºC after collection, and assayed

at the end of the study. Serum levels of Ang-2, MIP-1alpha, IL-1beta, RANKL and OPG were measured by ELISA, according to the manufacturer's instructions. Ntx was measured by ELISA, using a monoclonal antibody for Ntx labeled with horse radish peroxidase.
Results: All values were higher in myeloma patients compared to controls (P<0.03 for OPG, P<0.001 for the other cases). The values of Ang-2, MIP-1alpha, IL-1beta, RANKL and Ntx were increasing with the stage of the disease (P<0.002 for Ntx, P<0.001 for the other cases), whereas the levels of OPG were not. Similarly to ISS, the values of Ang-2, MIP-1alpha, IL-1beta, RANKL and Ntx were higher in high skeletal grade patients compared to those with low grade (P<0.001 for all cases), whereas the levels of OPG were not. Serum levels of Ang-2 correlated positively with levels of MIP-1alpha (r=0.658), IL-1beta (r=0.541), RANKL (r=0.609) and Ntx (r=0.607) (P<0.0001 for all cases). Serum levels of MIP-1alpha correlated positively with levels of IL-1beta (r=0.436 P<0.001), RANKL (r=0.472 P<0.0001) and Ntx (r=0.394 P<0.003). Serum levels of IL-1beta correlated positively with levels of RANKL (r=0.524) and Ntx (0.647) (P<0.0001 for both cases) and moreover, negatively with OPG (r=-0.347 P<0.01). Serum levels of RANKL correlated positively with levels of Ntx (r=0.702 P<0.0001) and negatively with OPG (r=-0.307 P<0.02). At last, urine levels of Ntx showed a trend for negative correlation with OPG serum levels (r=-0.263 P=0.054).
Summary and Conclusions: Our data demonstrate that MIP-1alpha, Ang-2 and the other measured parameters are among the main factors that promote the extensive bone disease in multiple myeloma. Moreover, they may be also involved in the mechanisms of angiogenesis, linking the two processes in myeloma disease. Consequently, they possess multiple roles in the complex pathogenesis of the disease.

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GENE EXPRESSION OF BONE MODULATORS IN WHOLE BONE MARROW BIOPSIES IN CORRELATION TO DYNAMIC BONE MARKERS IN MULTIPLE MYELOMA BONE DISEASE

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Background: The multiple myeloma (MM) plasma cell is dependent on the bone marrow (BM) microenvironment. Most gene expression studies have been performed upon BM aspirates, which differ in cellular composition from the BM biopsy, the latter being more representative for the total bone marrow microenvironment. The osteoclast regulatory factors RANK/RANKL/OPG and MIP-1-alpha have been shown to be important in the pathogenesis of osteolytic bone disease. RUNX2 and osterix are known osteoblast transcription factors. Dynamic markers of bone resorption (CTX-1) and bone formation (PINP) have been shown to offer information about the ongoing activity in bone degradation and formation. The ratio of bone resorption and bone formation (CTX-1/PINP) provides information about whether unbalanced bone remodeling is present.
Aims: To study the correlation between quantitative gene expression of the osteoclast regulators *RANK/RANKL/OPG* and *MIP-1-alpha* and osteoblast transcription factors *osterix* and *RUNX2* in whole BM biopsies and dynamic markers of bone resorption (CTX-1) and bone formation (PINP) in an unselected MM patient cohort (N=39).
Methods: During the diagnostic BM procedure we obtained an extra BM core biopsy after obtaining informed consent. The biopsies were snaP-frozen bed-side. Biopsies were cut, homogenized and RNA was purified using the MagNa Pure Robot (Roche). cDNA was loaded for QPCR on 384-wells micro-fluidic cards (Applied Biosystems). Using three internal reference genes (*ABL, GAPDH* and *GUSB*) the relative quantitative gene expression was calculated. All patients were untreated and did not receive bone-remodeling medicine. CTX-1 and PINP were measured on simultaneously obtained peripheral blood plasma by commercial kits (Roche Diagnostics). Due to renal excretion of CTX-1, patients with S-Creatinine above 176umol/L where excluded from CTX-1 data analysis.
Results: PINP and *RUNX2* gene expression correlated significantly (rho=0.51, P=0.027), while *RANKL* and *osterix* gene expression trended towards a significant correlation with PINP (rho=0.33, P=0.055 and rho=0.39, P=0.12, respectively). CTX-1 correlated inversely with *OPG* (rho=-0.35, P=0.03), but not with *RANKL* expression. The CTX-1/PINP ratio correlated significantly with gene expression of *RANKL* and *OPG* (rho=-0.49, P=0.0062 and rho=-0.39, P=0.012, respectively) and *RUNX2* gene expression trended towards significance (rho=-0.42, P=0.094). *RANKL* and *OPG* expression correlated significantly (rho=0.68, P=0.0001). *MIP-1-alpha* and *RANK* gene expression did not correlate with PINP, CTX-1 or CTX-1/PINP ratio (Figure 1).
Summary and Conclusions: Using snaP-frozen biopsies, as a surrogate for the *in vivo* situation in the BM microenvironment in MM patients, and dynamic bone markers we were able to demonstrate that down-regulation of *OPG* is correlated with increased CTX-1 levels, and that uP-regulation of *RUNX2* is associated with increased levels of the bone formation marker PINP. These observations underscore the likely importance of these two factors in the pathogenesis of MM bone disease. In our study, the gene expression of *RANKL* is co-regulated with *OPG* gene-expression, rather than correlated to the MM osteolytic bone disease itself.

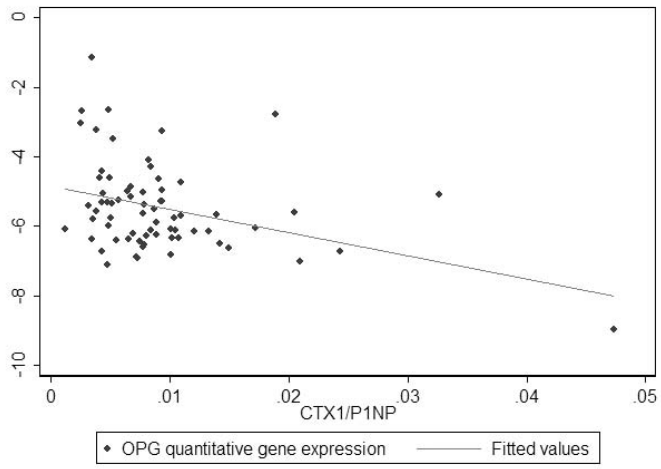

Figure 1.

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P15 AND DAPK METHYLATION IN MULTIPLE MYELOMA AND MGUS: COMPARISON BETWEEN BONE MARROW AND PERIPHERAL BLOOD

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Background: DNA methylation status was the earliest discovered epigenetic regulator and aberrant DNA methylation is associated with cancer development and progression. There are several types of specimens from which DNA methylation pattern can be measured and evaluated. Blood-based specimens may be a potential source of noninvasive cancer biomarkers. Blood leukocytes from patients with solid tumors exhibit complex and distinct cancer-associated patterns of DNA, which might be seen as epigenetic biomarkers with significant clinical potential. Peripheral blood cell methylation profiles are largely unknown in hematopoietic cancers. We have focused on DNA methylation changes in death-associated protein kinase (DAPK) and in p15 genes in monoclonal gammopathies (MG): multiple myeloma (MM) and monoclonal gammopathy of uncertain significance (MGUS).
Aims: Our aim was to compare DNA methylation status of two genes (p15 and DAPK) in MGUS and in MM at diagnosis in bone marrow (BM) aspirate and in peripheral blood (PB).
Methods: For this purpose we have examined the methylation pattern of p15 and DAPK genes, in genomic DNA obtained from bone marrow (BM) and peripheral blood (PB) samples collected at diagnosis from 99 patients, M/F=51/48, median age 71 years (36-86) with MGUS (n=57) and MM (n=52). Samples were collected after informed consent was obtained in accordance with the Declaration of Helsinki. Diagnosis of MM followed International Myeloma Working Group criteria (Br J Haematol 2003). Genomic DNA was isolated by standard protocols and modified by sodium bisulphite. The MS-PCR for p15 and DAPK promoter genes was performed using two sets of primers, one for methylated DNA and other for unmethylated DNA.
Results: Overall, 42% of pts with monoclonal gammopathy presented at least one hypermethylated gene in BM samples (54% of MM pts and 23% of MGUS pts, *P<0,05*) and 39% in PB (52% of MM pts and 21% of MGUS pts, *P<0,05).* The frequency of hypermethylation for individual genes in MM was: *p15*, 21% in BM; 17% in PB; *DAPK*, 38,5% in BM and PB. In MGUS patients, the frequency of *p15* and *DAPK* methylation was, respectively, 14% in BM, 17,5% in PB and 14% in BM, 7% in PB. Only 4 out of 47 (8.5%) samples in MGUS and 8 of 52 (15.4%) in MM had discrepant results in p15 methylation between BM aspirate and PB (P=NS). Discrepancies were bidirectional, with 6 of 12 cases presenting with demethylated PB and methylated BM aspirate, and the remaining 6 presenting with methylated PB and demethylated BM. 6 out of 47 (12.8%) samples in MGUS and 8 of 52 (15.4%) in MM had discrepant results in DAPK methylation between BM and PB (P=NS). Discrepancies were also bidirectional, with 5 of 14 cases presenting with demethylated PB and methylated BM aspirate, and the remaining 9 presenting with methylated PB and demethylated BM.
Summary and Conclusions: Aberrant hypermethylation of p15 and DAPK genes is a common event in MM and in MGUS pts. We conclude that there is a correlation between methylation patterns of p15 and DAPK in peripheral blood and in bone marrow aspirate. Although DNA methylation patterns measured in peripheral blood have great potential to be useful and informative biomarkers of cancer risk and prognosis, large systematic and prospective studies will be needed.

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MECHANISTIC STUDIES ON THE SYNERGISTIC CYTOTOXICITY OF THE NUCLEOSIDE ANALOGS GEMCITABINE AND CLOFARABINE IN MULTIPLE MYELOMA; RELEVANCE OF P53 AND ITS CLINICAL IMPLICATIONS

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Background: In spite of newer agents, both conventional and high-dose chemotherapy with hematopoietic stem cell transplantation (HSCT) are important treatments for multiple myeloma (MM). In order to identify improved therapy regimens, we investigated the cytotoxicity of gemcitabine (Gem) and clofarabine (Clo) combinations toward MM cell lines and explanted patient cell samples.

Aims: To investigate the mechanisms for possible synergistic cytotoxicity of nucleoside analogs in human multiple myeloma cell lines, such that these agents can be investigated as part of reduced-toxicity pretransplant conditioning therapy in patients with multiple myeloma.

Methods: Examining cytotoxic effects at low concentrations of gemcitabine and clofarabine in the human cell lines NCI-H929, MM.1R,

Cytotoxicity was assessed over 48 hours in the MTT assay, and apoptosis was assayed by flow cytometry (Sub-G1 fraction and Annexin V). Gene expression was assayed using the appropriate primers in western blotting.

Results: A strong synergism of the two nucleoside analogs was observed, when they were combined at ~IC_{10} concentrations. This synergism may be partly due to the observed Gem-mediated phosphorylation and activation of deoxycytidine kinase, resulting in enhanced phosphorylation of both Gem and Clo. Their cytotoxicity further correlated with a robust activation of the DNA damage response pathway. [Gem+Clo] decreased the mitochondrial membrane potential with a concomitant release of pro-apoptotic factors into the cytoplasm and nucleus, as well as activation of apoptosis. Exposure of MM cells to [Gem+Clo] also decreased the level of ribosomal RNA (rRNA), which may result in nucleolar stress and, as reported previously, cause p53-dependent cell death. A reduction (by ~50%) in the cytotoxicity of Gem and Clo was observed in the presence of pifithrin α, a p53 inhibitor. Furthermore, MM cell lines with mutant p53 exhibited greater resistance to Gem and Clo, supporting a role for the p53 protein in these cytotoxic responses.

Summary and Conclusions: Our results 1) give a mechanistic explanation for the observed synergisitc toxicity of two combined nucleoside analogs in multiple myeloma and 2) provide a rationale for (a) clinical trial(s) using [Gem+Clo] combinations as part of conditioning therapy for high-risk MM patients undergoing HSCT. 3). The p53 status of patients undergoing such therapy may include prognostic information and should be followed in a clinical setting.

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PHASE (PH) I/II STUDY OF ELOTUZUMAB PLUS LENALIDOMIDE/DEXAMETHASONE (LEN/DEX) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RR MM): UPDATED PH II RESULTS AND PH I/II LONG TERM SAFETY

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Background: Elotuzumab (Elo) is a humanized anti-CS1monoclonal antibody that enhances natural killer cell mediated antibody dependent cellular cytotoxicity of CS1 expressing myeloma cells. This study included a dose finding Ph I cohort (N=28) and a Ph II cohort (N=73).

Aims: To update the Ph II safety/efficacy data and provide long term safety data from both cohorts.

Methods: Patients (pts) treated with ≥1 (Ph I) or 1–3 (Ph II) prior therapies received Elo+Len/dex as described previously (Lonial JCO 2012; Richardson ASH 2012) until disease progression, unacceptable toxicity, or death. All pts received a premedication regimen including methylprednisolone, diphenhydramine or equivalent, ranitidine or equivalent, and acetaminophen to mitigate infusion reactions. Adverse events (AEs) in Ph I/II pts occurring ≤18 months (mo) (N=98) were compared to AEs with a >18 mo onset in a subgroup of pts treated >18 mo (n=49). This safety analysis excluded 3 Ph I pts treated with Elo 5 mg/kg, since Ph III studies are evaluating a 10 mg/kg dose.

Results: In the Ph II cohort (median 63 yr), objective response rate (ORR) was 84%; 92% with 10 mg/kg (n=36) and 76% with 20 mg/kg (n=37). At a median follow-up of 20.8 mo, median progression free survival (PFS) was not reached (10 mg/kg) and 18.6 mo (20 mg/kg). The most common treatment emergent grade ≥3 AEs were lymphopenia (19%), neutropenia (18%), thrombocytopenia (16%) and anemia (14%). The most common grade 3/4 AEs emerging ≤18 *vs* >18 mo in Ph I/II cohorts are shown (Table 1). 15 pts discontinued due to AEs; none after 18 mo of treatment. There were 4 second primary malignancies; none were reported after 18 mo.

Table 1.

	Onset	
	≤18 mo	>18 mo
Grade 3/4 AEs*	N=98	n=49
Neutropenia	21%	2%
Thrombocytopenia	18%	2%
Lymphopenia	15%	2%
Anemia	12%	2%
Hyperglycemia	9%	0%
Fatigue	8%	0%
Diarrhea	7%	2%
Leukopenia	7%	2%
Hypokalemia	6%	4%
Pneumonia	6%	2%

*In >5% of pts.

Summary and Conclusions: Elo 10 mg/kg+Len/dex was generally well tolerated and resulted in a high ORR and encouraging PFS in pts with RR MM. AEs emergent after 18 mo of therapy were consistent with AEs emergent during the initial 18 mo. Updated Ph II safety/efficacy data and long term safety data from Ph I/II cohorts will be presented.

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NEW DRUG PARTNER FOR COMBINATION THERAPY IN MULTIPLE MYELOMA (MM): DEVELOPMENT OF ACY-1215, A SELECTIVE HISTONE DEACETYLASE 6 INHIBITOR ALONE AND IN COMBINATION WITH BORTEZOMIB OR LENALIDOMIDE

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Background: First generation HDAC inhibitors (HDACi) active in combination with standard agents in MM are limited by fatigue, vomiting, diarrhea, and myleosuppression. ACY-1215, the first oral next generation selective HDACi in the clinic is 11 fold selective for HDAC6 over class 1 HDACs, is well tolerated preclinically and synergizes with bortezomib (Bort) in MM cell lines and animal models (Blood, V20(210):4061) and synergizes with lenalidomide (Len) *in vitro* with or without dexamethasone (D). Two clinical trials are ongoing: ACY-100 with ACY-1215 monotherapy and in combination with Bort and D, and ACE-MM-101 with ACY-1215 in combination with Len and D.
Aims: The aims of the development program are to characterize safety, pharmacokinetics (PK) and pharmacodynamics (PD) of ACY-1215 alone and in combination with D and Bort or Len and to identify phase 2 combination regimens.
Methods: ACY-100 is a single arm open label study with dose escalation in a 3+3 design. Relapsed/refractory (R/R) patients (pts) who received at least two lines of therapy (tx) including a proteasome inhibitor and an immunomodulatory agents, refused or were ineligible for autologous stem cell transplant (ASCT), had adequate marrow reserve and hepatic function, and creatinine clearance (CrCl) >30, were eligible for the phase 1a and 1b portions after informed consent. Pts received 40 mg to 360 mg of ACY-1215 orally on days 1-5 and 8-12 of a 21 day cycle in part 1a, and in 1b with Bort 1.0-1.3 mg/m^2 on days1,4, 8 and 11 and D 40 mg/week. Peripheral blood samples were obtained for PK and PD assessment of acetylated tubulin and histones in peripheral blood mononuclear cells (PBMC). ACE-MM-101 R/R pts received at least one prior rx and have CrCl>60. Len, 25 mg after an initial 15 mg safety cohort, is given orally daily on 21 days of a 28 day schedule with 40 mg D/week, and if well tolerated a third week of ACY-1215 days 15-19 will be added. ACY-1215 doses of 40 to 240 mg will be explored. PK and PD assessment is similar to ACY-100.
Results: ACY-100: In 15 pts on ACY-1215 monotherapy, no dose limiting toxicity (DLT) was observed, and creatinine elevations, anemia, fatigue, hypercalcemia and respiratory infection, the most common toxicities, were most low grade and not attributed to ACY-1215. Possibly related grade 3 toxicities were anemia and low white blood cell counts (n=1 each). Stable disease was observed in 6 pts, up to 10 cycles of therapy. Doses of ≥160 mg gave similar PK with μM concentrations at C_{max} in all pts with $t_{1/2}$ of 3 hr and no accumulation. At least doubling of acetylated tubulin was seen in PBMCs at ≥160 mg, while increases in acetylated histones were observed only at 240 mg. Two cohorts with Bort (1.0 and 1.3 mg/m^2) are enrolled and one cohort was expanded due to a DLT, asymptomatic elevation in amylase that appeared temporally associated with rx. No further DLTs occurred. One partial response (PR) ongoing at 9 cycles and enrollment is ongoing at 80 mg. ACE-MM-101: The first 3 cohorts, up to 80 mg ACY-1215 and 25 mg Len and 40 mg D enrolled 9 pts, five of whom had previously received an immunomodulatory agent. No DLTs were observed. Four episodes of neutropenia and one ALT elevation were probably attributed to Len. Three pts have withdrawn, two due to progressive disease and one to persistent neutropenia leading to missed L doses. There are four PRs, one VGPR, and one minimal response in the first 6 pts.
Summary and Conclusions: ACY-1215 is well tolerated as monotherapy at doses leading to PD activity and can safely be combined with full doses of B or L with D at ACY-1215 doses explored in combination. Responses have been seen in both studies and dose escalation continues.

P766

HIGH RATES OF PROLONGED MOLECULAR REMISSIONS AFTER TANDEM AUTOLOGOUS-NONMYELOABLATIVE ALLOGRAFTING IN NEWLY DIAGNOSED MYELOMA

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Background: Myeloablative allografting induces high rates of persistent molecular remissions (MR) in multiple myeloma (MM) (Corradini, J Clin Oncol 1999) and greatly reduces the risk of relapse. Similar results have also been reported after reduced-intensity conditioning (Kröger, Biol Bone Marrow Transpl 2012). Long term data on minimal residual disease (MRD) kinetics after combined autologous and nonmyeloablative allografting (auto-allo) are lacking. We here present the results of MRD analyses by nested qualitative PCR (Nested-PCR) and real time quantitative (RQ)–PCR.
Aims: To performe MRD analysis by Nested-PCR and RQ-PCR on newly diagnosed MM patients treated with auto-allo on a prospective clinical trial [ClinicalTrial.gov, NCT-00702247].
Methods: 26 patients (pts) with stage II-III MM and a diagnostic bone marrow (BM) specimen suitable for immunoglobulin heavy-chain gene rearrangement (IGH) sequencing, were evaluated for MRD by PCR methods. Auto-allo consisted of an autograft followed by 200 cGy TBI and an allograft. BM samples were collected at diagnosis, after the autograft, at month1,3, 6 after the allograft and then every 6 months. Nested-PCR and RQ-PCR analyses were carried out using patient-specific primers as previously described (Voena, Leukemia 1997; Ladetto, Biol Bone Marrow Transpl 2000). For outcome analysis pts were grouped according to reported criteria (Ladetto, ASH 2011): FullMR and StandardMR indicated MRD negativity on two consecutive samples by nested-PCR or RQ-PCR respectively, the latter standardized according to European Study Group on MRD detection guidelines (van der Vendel, Leukemia 2007).
Results: 19/26 pts had a molecular marker. At a median follow-up of 10 years (4.4-12) from diagnosis and 8.9 years (3.5-11) from the allograft, overall survival (OS) was 61% and median time-to-progression (TTP) 5.6 years. Transplant-related mortality occurred in 3/19 pts (16%), while 4/19 pts (21%) died of disease progression. MRD analysis showed that after the autograft 3/19 pts (16%) were negative by nested-PCR. After the autograft 3/19 pts (16%) were PCR-negative. After allograft, the rate of PCR negativity remained low at month 1 (5/19, 26%) and 3 (3/19, 16%). Starting from month 6 PCR-negativity occurred up to 44% (8/18) at 6 months and 47% (7/15) at one year post-transplant. Overall, 8 pts achieved FullMR at a median time from allograft of 6 months (1-12) and for a median duration of 33 months (6-102). Overall, 8 relapses occurred, 6 among 11 pts who never achieved FullMR and 2 in 8 pts who reached FullMR. Of these one has incomplete follow up and in the other one clinical relapsed was heralded by a molecular relapse. Pts in FullMR had better median TTP (not reached *vs* 1.6 years, P=0.043) and OS (not reached *vs* 3.3 years, P=0.008) than pts who did not achieve FullMR (Figure 1). StandardMR occurred in 12/19 pts (63%) during the first 24 months post-transplant, at a median time of 2 months (1-18) and for a median duration of 27 months (3-102). Pts in StandardMR showed better median TTP (not reached *vs* 1 year, P=0.005) and OS (not reached *vs* 3.3 years, P=0.031) as compared to pts with positive PCR (Figure 2). There was no correlation between MR and chronic graft-*vs*-host disease, suggesting specific graft-*vs*-myeloma.

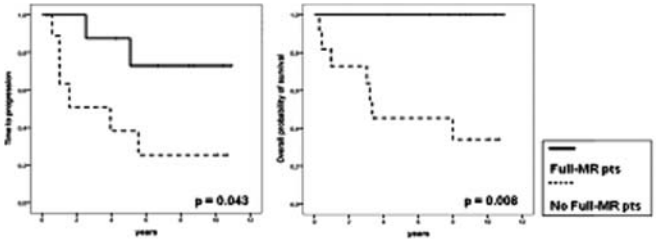

Figure 1. TIP (A) and OS (B) by FullMR status.

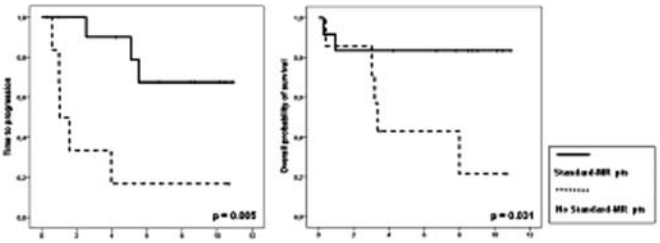

Figure 2. TIP (A) and OS (B) by StandardMR status.

Summary and Conclusions: Auto-allo induces high molecular remission rates, significantly associated with better TTP and OS, clearly documenting the existence of an effective and persistent graft-*vs*-myeloma effect, potentially curative in a subset of patients.

P767

INTEGRATED ANALYSIS OF DATA FROM PHASE 3 RANDOMIZED, CONTROLLED TRIALS OF BORTEZOMIB-BASED VS NON-BORTEZOMIB-BASED INDUCTION PRIOR TO ASCT IN PATIENTS WITH PREVIOUSLY UNTREATED MULTIPLE MYELOMA (MM)

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Background: A total of four large, multicenter, cooperative group studies have investigated bortezomib-based regimens compared with non-bortezomib-based previous standards of care as induction prior to autologous stem cell transplant (ASCT) in previously untreated MM: IFM 2005-01, bortezomib-dexamethasone *vs* vincristine-doxorubicin-dexamethasone (VAD); HOVON-65/GMMG-HD4, bortezomib-doxorubicin-dexamethasone *vs* VAD; PETHEMA GEM2005 MENOS65, bortezomib-thalidomide-dexamethasone (VTD) *vs* thalidomide-dexamethasone (TD) *vs* combination chemotherapy followed by bortezomib; and GIMEMA MM-BO2005, VTD *vs* TD.

Aims: This integrated analysis aimed to characterize the overall impact of bortezomib-based *vs* non-bortezomib-based induction therapy on efficacy, outcomes, and safety using data from these phase 3 studies in patients with previously untreated MM. The two key efficacy endpoints were post-transplant complete plus near-complete response (CR+nCR) rate and progression-free survival (PFS).

Methods: Patient-level data were pooled in a thorough integrated analysis of efficacy and safety from the IFM 2005-01, HOVON-65/GMMG-HD4, and PETHEMA GEM2005MENOS65 studies. Patient-level data were not available from GIMEMA MM-BO2005 due to local legal restrictions, but study-level data were used to supplement the integrated analysis.

Results: The integrated analysis incorporated data from 787 and 785 patients treated with bortezomib-based and non-bortezomib-based induction, respectively; demographics and disease characteristics were well balanced between the groups. Post-transplant CR+nCR rate and all other response rates post-induction and post-transplant were significantly higher in the bortezomib-based *vs* non-bortezomib-based group (Table 1). With inclusion of study-level data from GIMEMA MM-BO2005, the pooled odds ratio for post-transplant CR+nCR rate remained similar, at 1.96. The significant improvement in post-transplant CR+nCR rate was seen across patient subgroups, including patients with high-risk features. The median PFS was 35.9 *vs* 28.6 months in the bortezomib-based *vs* non-bortezomib-based groups (hazard ratio [HR] 0.75, P<0.0001); 3-year PFS rates were 50.0% and 41.1%, respectively. HRs for PFS were consistent across studies. After a median follow-up of ~37 months, 3-year overall survival (OS) rates were 79.7% and 74.7%, respectively (HR 0.81, P=0.0402). Median duration of induction therapy was 11 weeks in both groups. During bortezomib-based and non-bortezomib-based induction, respectively, 63% and 59% of patients had grade ≥3 adverse events (AEs), 41% and 37% had serious AEs, 6% and 5% had AEs resulting in discontinuation, and 3% and 4% of patients died. Overall rates of peripheral neuropathy (PN) during induction were 34% *vs* 17%, respectively, including 6% *vs* 1% grade ≥3 PN.

Table 1.

Response rate, n (%)	Btz-based (n=775)	Non-btz-based (n=772)	Odds ratio
Post-induction			
CR	105 (14)	32 (4)	3.92
CR+nCR	175 (23)	63 (8)	3.45
≥VGPR	362 (47)	139 (18)	4.03
ORR	646 (83)	480 (62)	3.05
Post-ASCT			
CR	199 (26)	106 (14)	2.21
CR+nCR	298 (38)	182 (24)	2.05
≥VGPR	463 (60)	315 (41)	2.16
ORR	615 (79)	526 (68)	1.81

For all comparisons, p<0.0001 by Cochran-Mantel-Haenszel chi-squared test. Btz, bortezomib; CR, complete response; nCR, near-CR; ORR, overall response rate; VGPR, very good partial response

Summary and Conclusions: The findings of the integrated analysis demonstrate that bortezomib-based induction results in a consistent and robust benefit in terms of response rates, PFS, and OS compared to non-bortezomib-based induction. Bortezomib-based induction was generally well tolerated, with a higher rate of PN but no increase in the risk of death during induction *vs* non-bortezomib-based induction.

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SAFETY AND EFFICACY OF POMALIDOMIDE WITH OR WITHOUT LOW-DOSE DEXAMETHASONE IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA: LONG-TERM FOLLOW-UP OF PATIENTS ENROLLED IN THE MM-002 PHASE 2 TRIAL

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Background: MM-002 is a randomized, open-label, multicenter, phase 2 trial evaluating the safety and efficacy of pomalidomide (POM) with or without low-dose dexamethasone (LoDEX) in advanced relapsed and refractory multiple myeloma (RRMM) patients (pts) who have received prior treatment with lenalidomide (LEN) and bortezomib (BORT).

Aims: The objective of this analysis of MM-002 trial data was to assess the safety and efficacy of POM with or without LoDEX with extended follow-up.

Methods: Pts who had received ≥2 prior therapies, including LEN and BORT, and were refractory to their last treatment were randomized to POM+LoDEX (POM 4 mg/day, days 1–21 of a 28-day cycle; LoDEX 40 mg/week) or POM alone. Refractory disease was defined as documented progression during treatment or within 60 days of the last dose of treatment. At progression, pts receiving POM alone could receive POM+LoDEX at investigator's discretion. Pts aged >75 yrs received LoDEX, 20 mg/week. All pts received mandatory thromboprophylaxis (daily low-dose aspirin). End-points included progression-free survival (PFS), response rate (according to EBMT criteria and investigator assessment), response duration, overall survival (OS), and safety. The efficacy outcomes are based on the intent-to-treat population (POM+LoDEX, n=113; POM, n=108). Data with a median follow-up of 14.2 mos is presented here.

Results: The median number of prior therapies in each group was 5 (range 1–13). All pts (100%) had prior exposure to LEN, BORT, and steroids; 62% of pts were LEN and BORT dual-refractory. In the POM+LoDEX arm, 30 (27%) pts had high-risk cytogenetics, including del(17p13) and/or t(4p16/14q32). The overall response rate (ORR; defined as at least partial response) was 34% and 15% with POM+LoDEX and POM, respectively, with a median duration of 8.3 mos (95% confidence interval [CI]: 5.8–10.1) and 8.8 mos (95% CI: 5.5–11.4), and at least a minimal response was observed in 45% and 31% of pts, respectively. Median PFS was 4.6 mos (95% CI: 3.6–5.5) and 2.6 mos (95% CI: 1.9–2.8); with a median follow-up of 16.0 and 12.2 mos, median OS was 16.5 mos (95% CI: 12.4–18.5) and 13.6 mos (95% CI: 9.6–18.1), with POM+LoDEX and POM, respectively. Among pts with LEN and BORT refractory disease, ORRs were 33% with combination therapy and 17% with POM alone, with median response duration of 6.5 mos (95% CI: 3.7–8.3) and 8.3 mos (95% CI: 2.8–13.1); 46% and 33% of pts achieved at least minimal response, respectively. Median PFS was 3.8 mos (95% CI: 2.8–5.8) and 2.0 mos (95% CI: 1.8–2.9); median OS was 13.4 mos (95% CI: 11.0–17.6) and 12.4 mos (95% CI: 8.2–18.0), with POM+LoDEX and POM, respectively. The most common treatment emergent grade 3/4 adverse events (AEs) reported in the safety population (n=219) were neutropenia (44%), anemia (23%), thrombocytopenia (21%), and pneumonia (18%); there were no reports of grade 3/4 peripheral neuropathy. The incidence of deeP-vein thrombosis was low (2%). In this overall safety population, AEs were managed through dose reductions or interruptions (30% and 64% of pts with at least one, respectively), and supportive care with granulocyte colony-stimulating factor (53%), and red blood cell (48%) and platelet transfusions (18%). Discontinuations of POM due to AEs were 10%.

Summary and Conclusions: POM with or without LoDEX represents a new clinical option for pts with advanced RRMM, including pts with LEN and BORT refractory disease. AEs were predictable and manageable. Updated data with extended follow-up will be presented at the meeting.

P769

CONSOLIDATION WITH HD- MELPHALAN AND AUTOTRANSPLANT (ASCT) AFTER SECOND-LINE TREATMENT INCREASES RESPONSE RATE AND PROGRESSION-FREE SURVIVAL IN MYELOMA PATIENTS RELAPSED AFTER FIRST-LINE ASCT

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Background: Therapeutic options for patients relapsing after first-line HD-Mel and ASCT are not standardized. Few studies have addressed the potential role of ASCT consolidation after II-line treatment in patients relapsed after first-line ASCT.
Aims: To compare the outcome of MM patients relapsed after first-line ASCT who received second-line therapy with or without consolidation with ASCT
Methods: In 159 MM patients treated at two Italian Institution between 1997 and 2010 and relapsed after first-line HD-Mel and ASCT, second-line therapy was given to 98 pts (61%) with bortezomib-or Imids-or thalidomide-based regimens only (ND group) and to 61 pts (38%) with ASCT consolidation after ND-based regimens (45 pts) or chemotherapy (16 pts) (ASCT group). ND and ASCT groups did not differ in baseline characteristics, including age (median 59), type of first-line therapy, median follow-up from diagnosis.CR/VGPR rates and ORR obtained after first-line treatment (ND 52% and 89%; ASCT 62% and 90%, respectively) were also similar. Median duration of first response and time to second treatment were 14 and 24 months in both group respectively. Proportion of patients receiving a double ASCT at first line therapy was 21% in ND and 33% in ASCT group (*P*=0.079).
Results: After second line therapy ORR(CR+VGPR+PR) was 77% in ASCT group, significantly better than ND group (48%)(*P*=0.0001).The second CR/VGPR rate was significantly higher after ASCT (41%) than after ND (21%)(*P*=0.012), independently from the type of second line treatment received before consolidation ASCT (ORR:ND 91% *vs* CT 75%; *P*=0.21).After a median follow-up from second-line treatment of 28 months (range 1-128 mo), 2-year PFS was 17% after ND (median 12 mo) and 26% after ASCT (median 19mo)(*P*=0.013).Two-year OS was 62% (median 33mo) and 80% (median 44mo) after ND and ASCT group, respectively (*P*=0.3). PFS after salvage did not differ between pts receiving single or double first-line ASCT (P=0.066).
Summary and Conclusions: The use of ASCT as consolidation after second-line treatment increased both ORR and CR/VGPR rates, independently from the type of debulking treatment used (chemotherapy *versus* novel drugs) and significantly impacted on PFS when compared with second-line ND-based regimens without ASCT.

P770

TOTAL THERAPY 3 (TT3)-BASED TREATMENT FOR MULTIPLE MYELOMA–AN EXTENDED SINGLE CENTER EXPERIENCE

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Background: Total therapy 3 (TT3) is an intensified treatment for multiple myeloma (MM) introduced by the Arkansas group, designed for newly diagnosed patients. Originally, this regimen incorporates a component of the novel agents bortezomib and thalidomide plus dexamethasone (=VDT) to PACE (cisplatinum, doxorubicin, cyclophosphamide, and etoposide) chemotherapy for 2 cycles, followed by consolidation with high dose melphalan tandem autotransplantation. Thereafter, additional 2 cycles of VDT-PACE are given followed by VDT maintenance. This protocol has not been adopted by many centers and its results have rarely been reported. In 2010 we described a short-term follow up of 23 patients treated TT3-based protocol (Ann Hematol 89:53-9, 2010).
Aims: to review an extended group of MM patients treated with TT3-based protocol, administrated mainly to patients with aggressive clinical course and/or high risk cytogenetic abnormalities at different treatment settings (induction; induction failure; relapse; relapsed-and-refractory MM (RR-MM)].
Methods: We retrospectively analyzed all sequential patients (n=80) from 2004 to 2012 that were given at least one cycle of (V)DT-PACE. For efficacy evaluation, we used the IMWG response criteria.
Results: median age was 58 years (range 35-75). ISS score at diagnosis was I, II and III in 22%, 44% and 29% of patients, respectively (5% undetermined). 53% of patients (32/60) had poor cytogenetic abnormalities. (V)DT-PACE was given in 44 patients for induction, in 11 patients for induction failure, in 13 patients for relapse and in 12 patients for RR-MM. Median cycles per patient was 2 (range 1-4). Bortezomib was given in 66% of cycles. 75% of patients (60/80) were subsequently given high dose melphalan with autograft, of which 8 patients had tandem transplantation (2 of them had auto-allo). 83% of patients were given maintenance. Overall response rate (ORR) following (V)DT-PACE induction treatment was 92% (CR=4%, VGPR=57.3%, PR=30.7%). Patients receiving (V)DT-PACE for induction and relapse achieved higher rates of VGPR and above (76.7% and 54.5%, respectively) than those treated for induction failure or RR-MM (36.4% and 30%, respectively) (P=.009). Following autologous transplantation, ORR increased to 98%, with substantial improvement in CR/sCR rates (sCR=25.4%, CR=11.9%, VGPR=49.1%, PR=11.9%). With a median follow-up of 21 months, progression free survival at 1 and 2 years was 85% and 67% for induction, 76% and 57% for induction failure, 28% and 0% for relapse and 12% and 0% for RR-MM, respectively. Overall survival at 2 years was 85%, 100%, 39% and 19%, respectively. Considering all treatment cycles (n=152), grade 3/4 hematological toxicity rate was 79% and 46% for neutropenia and thrombocytopenia, respectively. The rate of neutropenic fever was 25.7%, with 19/39 of episodes associated with clinically or microbiologically documented infections. Venous thrombosis was infrequent (3.3%). Death attributed to treatment was relatively low (5/80, 6.25%), occurred mainly in the non-induction settings (4/36, 11.1%). Causes of death were arrhythmia (n=3, of which 2 with known systemic amyloidosis), severe infection (n=1) and multi-organ failure (n=1) (Figure 1).

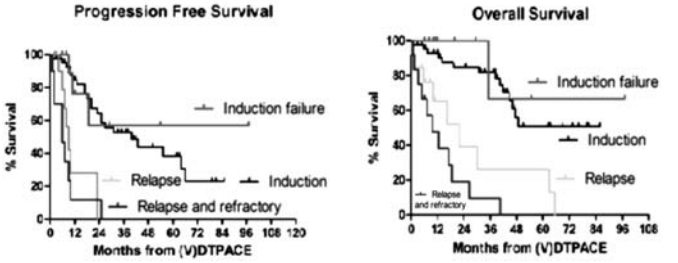

Figure 1.

Summary and Conclusions: TT3-based approach achieved high response rate among MM patients with high-risk disease, with considerable but manageable toxicities. However efficacy was mainly translated into long-term remission in the induction and induction failure settings, while results in relapse and RR-MM are unsatisfactory.

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PHASE I STUDY OF THE COMBINATION OF CARFILZOMIB AND PANOBINOSTAT FOR PATIENTS WITH RELAPSED AND REFRACTORY MYELOMA: A MULTICENTER MMRC CLINICAL TRIAL

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Background: Patients with myeloma ultimately become refractory to current therapies, requiring new approaches to treatment. Carfilzomib is a selective proteasome inhibitor that has demonstrated significant activity in patients with relapsed and refractory myeloma. Panobinostat is a pan-deacetylase inhibitor that can overcome resistance in combination with bortezomib in refractory multiple myeloma patients. Based on preclinical data showing synergistic cytotoxicity of histone deacetylase and proteasome inhibitors, we hypothesized that carfilzomib and panobinostat would be safe and effective for the treatment of relapsed/refractory multiple myeloma. Herein we report the initial findings of the MMRC multicenter phase I study investigtating the combination of panobinostat and carfilzomib in patients with relapsed and refractory multiple myeloma.
Aims: The primary objective is to determine the maximum tolerated dose (MTD) of the combination of panobinostat and carfilzomib using a standard 3+3 design with 5 planned cohorts. An additional 12 patients will be treated at the MTD in an expansion phase to gain further data on safety and efficacy. Secondary objectives are to evaluate response and survival endpoints of enrolled patients.
Methods: Panobinostat is administered orally three times weekly for three of four weeks (range, 15-25 mg).Carfilzomib is administered IV days1,2,8,9,15, and16, ranging from 20/27 mg/m^2 to 20/56 mg/m^2. Doses above 20/27 are administered over 30 minutes. Cycles are repeated every 4 weeks. Dose limiting toxicities (DLT) are determined in the first cycle, and all adverse events are assessed according to CTCAE Version 4. Responses are assessed using IMWG criteria (plus MRs as per the EBMT criteria).
Results: To date, 10 patients in three cohorts have been enrolled, and 9 have completed the first cycle. Median age is 60.6 years (range, 48-73). All patients had refractory and progressive disease. No DLTs were observed in the first 2 cohorts, with 1 patient in cohort 1 being inevaluable due to disease progression. There are currently 2 patients in cohort 3 (panobinostat 20 mg and carfilzomib 20/36 mg/m^2), one of whom had a DLT of grade 4 thrombocytopenia and grade 3 elevated creatinine. Both toxicities resolved after discontinuation of study therapy. Four serious adverse events have occurred, with two (one patient with a grade 5 cardiac arrest and a different patient with elevated creatinine) considered related to study therapy. Patients have completed a medi-

an of 3 cycles (range, <1 to 13). Grade 3 thrombocytopenia occurred in 40% and grade 3 neutropenia in 30% of all subjects. The most common non-hematologic toxicities were nausea, diarrhea, vomiting, hypokalemia and cough, all effectively managed with standard interventions. Preliminary assessment of response revealed an ORR of 30% with 1 sCR and two PRs.
Summary and Conclusions: The combination of carfilzomib and panobinostat is well tolerated with no unexpected toxicities. Preliminary assessment of response data is encouraging, and the study is ongoing with planned dose escalation of carfilzomib to 56mg/m^2 and panobinostat to 20 mg.

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MM-008: A PHASE 1 TRIAL EVALUATING PHARMACOKINETICS AND TOLERABILITY OF POMALIDOMIDE+LOW-DOSE DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) WITH RENAL IMPAIRMENT

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Background: Pomalidomide (POM) recently received US Food and Drug Administration approval for the treatment of patients with RRMM who have received ≥2 prior treatments including lenalidomide and bortezomib. In phase 2 trials, POM+low-dose dexamethasone (LoDEX) has shown significant clinical activity in this patient population (Leleu X, *et al. Blood*. 2013; Richardson *et al. Blood.* 2012). Renal impairment is a common comorbidity for myeloma patients, occurring in over 40% of patients (Eleutherakis-Papaikovou V, *et al. Leuk Lymphoma.* 2007). POM is extensively metabolized, with 2% eliminated renally as the parent drug (Hoffmann M, *et al. Cancer Chemother Pharmacol.* 2013*)*. Thus, renal function may not substantively affect exposure of the parent drug. POM+LoDEX has shown efficacy in RRMM patients with moderate renal impairment (Siegel DS, *et al.* ASH. 2012). However, patients with severe renal impairment have been excluded from previous POM trials. To date, the safety, efficacy, and pharmacokinetics (PK) of POM in patients with renal impairment have not been prospectively evaluated.
Aims: MM-008 is a multicenter, open-label, phase 1 study designed to prospectively assess the PK and safety of POM+LoDEX in patients with RRMM and normal or impaired renal function.
Methods: Patients with RRMM and ≥1 prior therapy were eligible to enroll in this study. Patients with normal renal function (creatinine clearance [CrCl] ≥60 mL/min [cohort A]) or severe renal impairment (CrCl <30 mL/min [cohort B]), but not requiring dialysis, were included. Patients in cohort A received POM 4 mg and patients in cohort B received POM 2 mg or 4 mg on days 1-21 of a 28-day cycle, following a standard 3+3 dose-escalation design. Both cohorts received DEX 40 mg (20 mg for patients aged >75 years) on days1,8,15, and 22. Cohort C will assess patients with severe renal impairment (CrCl <30 mL/min) and requiring dialysis (up to approximately 14 patients planned). Patients were not permitted to enroll in more than 1 cohort. Granulocyte colony-stimulating factor for management of neutropenia was not permitted in cycle1, but could be started on day 1 of the next cycle at the physician's discretion. Treatment was continued until progressive disease or unacceptable toxicity. Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for AEs (V 4.0).
Results: As of February5, 2013, eleven patients have been treated (8 in cohort A; 3 in cohort B). Patients were ages 46-71 years (cohort A) and 57-64 years (cohort B). Five patients are >65 years of age in cohort A (66, 69 [n=3], and 71 years). Seven patients in cohort A have received more than 1 cycle of treatment; 5 have received 3 or more cycles. One patient in cohort B has received more than 3 cycles. All 3 patients in cohort B have completed 1 full cycle of treatment with no dose-limiting toxicities reported. Dose escalation is planned and all patients remain on study. Updated pharmacokinetic and AE data will be presented at the meeting.
Summary and Conclusions: MM-008 is an ongoing trial prospectively evaluating the pharmacokinetics and safety of POM+LoDEX in patients with severe renal impairment. Early tolerability data are encouraging, with dose escalation planned.

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CAUSES OF DEATH 30 AND 180 DAYS AFTER DIAGNOSIS IN NON-HDT TREATED DANISH MULTIPLE MYELOMA PATIENTS: A STUDY FROM THE DANISH MYELOMA STUDY GROUP

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Background: Many elderly patients with multiple myeloma (MM), not eligible for high-dose melphalan with hematopoietic stem cell support (HDT), die shortly after diagnosis.
Aims: To determine the cause of death in non-HDT MM-patients dying within 30 and 180 days of diagnosis in a population based setting.
Methods: The cause of death in non-HDT-treated patients who died within 180 days of diagnosis was identified in the population based nationwide Danish myeloma database (DMMD) established by the Danish Myeloma Study Group. DMMD includes all patients diagnosed with MM since 2005. The cause of death was divided into 1) pneumonia defined by the presence of fever, cough and positive x-rays, 2) septicemia defined by fever and positive blood cultures, 3) other infection defined in case of fever, elevated CRP and infection not identified as pneumonia nor septicemia 4) respiratory failure defined as respiratory distress with no fever. 5) Cardiovascular disease defined as cardiovascular infarction including pulmonary embolism or by the presence of congestive heart disease, 6) intracranial infarction or hemorrhage diagnosed by CTC, MRI or bedside neurological examination defined as stroke and 7) patients on dialysis were classified as having renal failure. All other causes of death were classified as other cause of death.
Results: We found 2071 patients included in DMMD of whom 1497 (72.3%) did not receive HDT. Of these 330 (22.0%) died within 180 days of diagnosis. Medical history was not available in 25 (7.6%) patients. No specific cause of death was found in 17 (5.2%) of the patients. Seventy-six (23.0%) patients died at home, or at a nursing home. Among 212 (64.2%) eligible patients 72 (40.0%) died within 30 days and 140 (60.0%) died within 31-180 days. The following causes of death were found: Infection was the leading cause of death with 96 (45.3%) of the patients dying from infection in total. Thirty-three (45.8%) and 63 (45.0%) patients died from infection within 30 and 31-180 days, respectively. Septicemia was the cause of death in 40 (18.9%) of the patients in total; 17 (23.6%) and 23 (16.4%) patients dying within 30 and 31-180 days. Pneumonia was the cause of death within 30 and 31-180 days in 12 (16.7%) and 25 (17.9%) patients, 37 (17.5%) in total. Nineteen patients (9.0%) died from other infections. Renal failure was the cause of death in 35 (16.5%) patients, cardiovascular causes in 21 (9.9%) patients, respiratory failure in 13 (6.1%) patients and stroke in 9 (4.2%) of the patients. Thirty-eight (18.0%) patients died from other causes. There was no significant difference (Fisher's exact test) in the causes of death within 30 and 31-180 days after diagnosis (P=0.727) (Table 1).

Table 1.

Cause of death	Patients dead 0-30 days after diagnosis (% of patients in group)	Patients dead 31-180 days after diagnosis (% of patients in group)	All patients (% of patients in group)
Pneumonia	12 (16.7)	25 (17.9)	37 (17.5)
Septicemia	17 (23.6)	23 (16.4)	40 (18.9)
Other infections	4 (5.6)	15 (10.7)	19 (9.0)
Renal failure	14 (19.4)	21 (15.0)	35 (16.5)
Cardiovascular	8 (11.1)	13 (9.3)	21 (9.9)
Respiratory failure	5 (6.9)	8 (5.7)	13 (6.1)
Stroke	3 (4.2)	6 (4.3)	9 (4.2)
Other cause of death	9 (12.5)	29 (20.8)	38 (18.0)

Summary and Conclusions: The main causes of death within 180 days of diagnosis in non-HDT-treated MM-patients were infections; with pneumonia and septicemia as the most common specific causes. Renal failure was the second most common cause of death. The failure to produce normal polyclonal IgG, treatment with corticosteroid and myelosuppressive chemotherapy, T cell defects and renal failure are major causes of infections in patients with MM. Early use of prophylactic intravenous IgG and/or prophylactic antibiotic treatment in non-HDT treated patients could possibly prevent some or most of these early deaths.

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PRELIMINARY RESULTS OF A PHASE I/II STUDY OF CARFILZOMIB, LENALIDOMIDE, VORINOSTAT AND DEXAMETHASONE (QUAD) IN RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA (MM)

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Background: The results from Phase I/II studies suggest that carfilzomib as a single agent has clinical activity in relapsed and relapsed/refractory multiple myeloma (MM). Phase I/II data indicate that combining a proteasome inhibitor and corticosteroids with an immunomodulatory agent or a histone-deacetylase (HDAC) inhibitor are synergistic.
Aims: We investigated the tolerability and synergy of the quadruplet using carfilzomib as the proteasome inhibitor in combination with lenalidomide, vorinostat and dexamethasone.
Methods: The primary objectives were to determine the maximum tolerated dose (MTD) and the safety/tolerability of the QUAD combination. Secondary objectives included overall response rate, duration of response, time to progression and time to next therapy. All patients were required to have relapsed or relapsed/refractory disease following at least one line of therapy. Treatment consisted of 28-day cycles of oral lenalidomide days 1-21, oral vorinostat days 1-7 and 15-21, intravenous (IV) carfilzomib days1,2,8,9,15 and 16 and IV or oral dexamethasone 40mg days1,8,15 and 22. A standard 3+3 dose escalation schema was followed based on dose-limiting toxicities (DLTs) occurring in Cycle 1. (See Table 1) Adverse events (AEs) were graded using the NCI-CTCAE v3. Response was assessed by the modified International Myeloma Working Group criteria.
Results: As of February 28, 2013, fifteen patients have been enrolled, with one patient replaced due to inability to complete Cycle 1 due to AE. Four patients failed screening, 3 due to cytopenias and 1 due to QTC interval. The median age was 58 years (range 47-65), 55% were male. The median number of prior regimens was 3 (range 1-9), with a median time from diagnosis of 4 years. All patients had prior stem cell transplant, 10 had prior bortezomib, 10 had prior lenalidomide and 2 had prior vorinostat. Of the 7 patients who received prior VRD, 4 were refractory. Drug-related AEs were experienced by 100% of patients. The most common of these drug-related AEs included anemia (10pts), fatigue (8), thrombocytopenia (8), neutropenia (6), muscle cramping (6) and diarrhea (6). No febrile neutropenia occurred. Nine patients experienced ≥grade 3 AEs including neutropenia (5pts), anemia (3), thrombocytopenia (2), infection (2), electrolyte imbalances (2), hyperglycemia (1), fatigue (1) and constipation (1) and no grade 5 events. Eight SAEs occurred on study, most of which were infection and one was possibly study drug(s) related. Currently in cohort4, the MTD has not yet been determined with no DLTs to date. All 11 patients were evaluable for response with an ORR of 40%. Four patients had a partial response (PR), 2 stable disease (SD), 4 with progressive disease (PD) as best response and one has restaging pending. Of the seven patients treated in cohorts 2 and above, four have had a PR. Five patients have come off study due to PD and one due to patient choice. No patients have discontinued due to toxicity. Two patients have completed 18 cycles of therapy.

Table 1.

Cohort	Carfilzomib (mg/m²)	Lenalidomide (mg)	Vorinostat (mg)	Dexamethasone (mg)
1	15	15	300	40
2	20	15	300	40
3	20	25	300	40
4	20/27*	25	300	40
5	20/27*	25	400	40

* Step-up dosing of carfilzomib 20 mg/m² on Days 1 and 2 of Cycle 1, followed by 27 mg/m² for the remainder of treatment.

Summary and Conclusions: The combination of QUAD is well tolerated in both relapsed and relapsed/refractory MM patients, with no DLTs identified. The overall safety profile is manageable and the response rate of 40% noted in cohort 2 and above is encouraging in this patient population. Accrual is ongoing and additional data will be available at the time of presentation.

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CLINICAL RESPONSE BY BASELINE CHARACTERISTICS IN PATIENTS WITH RELAPSED AND BORTEZOMIB-REFRACTORY MULTIPLE MYELOMA TREATED WITH PANOBINOSTAT, BORTEZOMIB, AND DEXAMETHASONE (PANORAMA 2)

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Background: In PANORAMA2, panobinostat combined with bortezomib and dexamethasone recaptured responses in heavily pretreated patients with bortezomib-refractory multiple myeloma; overall response rate, clinical benefit rate, and progression-free survival were 34.5%, 52.7%, and 5.4 months, respectively, with manageable toxicity (Richardson *et al.* ASH 2012 [abstract 1852]).
Aims: Here, we evaluate clinical response by prior use of bortezomib and dexamethasone, progression on or within 60 days of of a patient's last bortezomib-containing regimen, or high-risk cytogenetics at baseline. Additionally, we evaluate quality of life parameters.
Methods: In the single-arm, phase 2 PANORAMA 2 study, patients with relapsed and bortezomib-refractory multiple myeloma received panobinostat (20 mg, oral)+bortezomib (1.3 mg/m^2, intravenous)+dexamethasone (20 mg, oral). Treatment phase 1 (TP1) consisted of eight 3-week cycles of panobinostat (thrice weekly) and bortezomib (twice weekly) during weeks 1 and2, with oral dexamethasone administered on the days of and after bortezomib dosing. Patients demonstrating clinical benefit entered treatment phase2, which consisted of four 6-week cycles of panobinostat (thrice weekly) and bortezomib (once weekly) during weeks1,2,4, and5, with dexamethasone on the days of and after bortezomib. The primary endpoint was overall response (≥partial response) in TP1. Response was based on European Group of Blood and Marrow Transplantation 1998 criteria. High-risk cytogenetics was defined as del(17p), t(4;14), or t(14;16). Quality of life was measured with the Functional Assessment of Cancer Therapy/Gynecologic Oncology Group Neurotoxicity (FACT/GOG-Ntx) v4.0 scales. All patients provided written informed consent prior to study entry.
Results: Response rate trended higher in patients whose prior bortezomib therapy was not their last line of therapy (Table 1). Although no trend in response rate was noted, progression-free survival appeared longer in patients progressing within 60 days of their last bortezomib-containing regimen than in those progressing on their last bortezomib-containing regimen. In the 14 patients with high-risk cytogenetics, overall response rate (complete response, near complete response, or partial response) was 42.9% and clinical benefit rate (complete response, near complete response, partial response, or minimal response) was 71.4%. The mean FACT/GOG-Ntx subscale did not exhibit a clinically meaningful change from baseline (mean±standard deviation, 114.2±21.1; n=41) to cycle 9 day 1 (day 169; 104.2±15.4; n=16) as determined by 50% standard deviation threshold for minimally important difference. Other quality of life parameters were similarly unchanged.

Table 1.

	N	ORR, % (95% CI)	CBR, % (95% CI)	PFS, months (95% CI)
Disease progression				
On BTZ	40	37.5 (22.7-54.2)	55.0 (38.5-70.7)	4.2 (2.6-5.8)
≤ 60 days of BTZ	15	26.7 (7.8-55.1)	46.7 (21.3-73.4)	7.6 (6.7-9.0)
BTZ in last prior line of therapy				
Yes	27	25.9 (11.1-46.3)	48.1 (28.7-68.1)	4.9 (2.1-7.6)
No	28	42.9 (24.5-62.8)	57.1 (37.2-75.5)	6.0 (3.9-7.6)
Dexamethasone in last BTZ-containing regimen				
Yes	45	26.7 (14.6-41.9)	46.7 (31.7-62.1)	4.9 (2.6-6.7)
No	10	70.0 (34.8-93.3)	80.0 (44.4-97.5)	6.2 (2.6-8.3)
Dexamethasone in last prior line of therapy				
Yes	37	32.4 (18.0-49.8)	54.1 (36.9-70.5)	4.2 (2.6-6.7)
No	18	38.9 (17.3-64.3)	50.0 (26.0-74.0)	6.5 (2.6-9.7)

BTZ, bortezomib; CBR, clinical benefit rate; ORR, overall response rate; PFS, progression-free survival.

Summary and Conclusions: Panobinostat combined with bortezomib and dexamethasone demonstrated activity regardless of baseline demographics in heavily pretreated patients with bortezomib-refractory multiple myeloma.

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PHASE 2A, OPEN-LABEL, MULTI-DOSE STUDY OF ANTI-KAPPA MONOCLONAL ANTIBODY, MDX-1097, IN RELAPSED KAPPA-CHAIN RESTRICTED MULTIPLE MYELOMA WITH STABLE MEASURABLE DISEASE

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Background: MDX-1097 is a monoclonal antibody that binds to a kappa light chain surface antigen (called KMA), on malignant B cells but not normal leucocytes or other cells. A Phase 1 study of single doses of MDX-1097 has been completed in kappa-light-chain restricted multiple myeloma patients. Based on positive safety, pharmacokinetic and efficacy data at a 10mg/kg dose, we now conducted a phase 2a study of repeated dosing of MDX-1097.
Aims: This study aimed to test efficacy and safety of MDX-1097 at 10mg/kg weekly ×8 in relapsed kappa myeloma patients with stable measurable disease.
Methods: We initially enrolled 13 relapsed kappa myeloma patients with stable disease, including patients on maintenance lenalidomide or thalidomide

and low-dose steroids. The study followed a Simon 2-stage minimax design, with ≥1 response needed in the first 13 patients to expand the study. Responses were evaluated by IMWG guidelines (Durie *et al*, 2006). MDX-1097 10mg/kg was given by 90 minute intravenous infusion weekly for 8 weeks. Efficacy and safety data included vital signs, physical examination, ECG, hematology assessments, clinical chemistry, C-reactive protein, β2 microglobulin, immunoglobulin quantification, urinalysis, and creatinine clearance. This study was performed according to ICH-GCP guidelines.

Results: A total of 19 patients completed the study. Repeated MDX-1097 dosing was well tolerated: 4 patients had Grade 1-2 drug-related infusion reactions; 4 patients had Grade 3 AE's (complete heart block, pneumonia, anaemia and pancreatitis), considered unlikely to relate to MDX-1097. There was no evidence of serum sickness, no alteration of renal function, no evidence of increased immunosuppression and no ECG changes. One patient had a VGPR maintained for 12 months post MDX-1097 therapy. A second patient had PR. A third patient with light-chain-only myeloma had PR. On study, 26% of patients continued IMiD(R) maintenance therapy; with no signs that MDX-1097 affected their safety profile.

Summary and Conclusions: Multiple weekly doses of MDX-1097 at 10 mg/kg were safe and well tolerated in patients with relapsed kappa myeloma. Responses to therapy were seen in 3/19 (16%) of patients.

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THE UK LENALIDOMIDE TREATMENT CONTINUATION SCHEME™: TRENDS OF LONG-TERM TREATMENT IN A CLINICAL SETTING

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Background: Patients (pts) with relapsed/refractory multiple myeloma (RRMM) who respond to therapy experience improved survival if treatment with lenalidomide (LEN) plus dexamethasone (DEX) is continued until disease progression. In the United Kingdom, LEN+DEX is reimbursed for the treatment of RRMM pts after ≥2 therapies (current license after ≥1 prior therapy) in accordance with the National Institute for Health and Clinical Excellence (NICE) guidelines. The Treatment Continuation Scheme (TCS) database was part of the NICE-agreed reimbursement scheme. It was designed to track the duration of LEN+DEX treatment in RRMM pts, but not the reasons for dose adjustments or treatment discontinuation.

Aims: To investigate how LEN starting dose and dose modifications during therapy impact treatment duration.

Methods: This anonymised prospective cohort analysis focused on pts with RRMM enrolled in the TCS program between 1 July, 2009 and 30 September, 2010. The cutoff date was 31 October, 2012 allowing at least 2 yrs follow up. Associations between ordered categorical variables (first dose administered, age group, treatment scheme, and number of cycles administered per pt) were measured by Spearman's rank correlation and associations between continuous variables (number of cycles, age, and number of dose changes) were measured by Pearson's correlation. Baseline covariates (age and starting dose) were modeled using multivariable logistic regression with possible interactions considered; $P<0.05$ was considered statistically significant.

Results: A total of1,779 pts from 193 treatment centers were evaluable. The median age was 69 yrs (range 23–91); 35% (624) were aged <65, 40% (702) were 65–74, and 25% (453) of the patients were >75 yrs. The majority of pts (65%; n=1,149) initiated LEN treatment according to the recommended starting dose of 25 mg/day, 15% of pts each started at 15 and 10 mg/day, and 5% at 5 mg/day. Dose modifications were reported in 48% of pts. The median number of dose modifications per pt was 1 (range 0–15). Of pts who started on 25 mg/day and received >1 cycle, almost half (48%) required no dose adjustments. Pts who had at least 1 form of dose adjustment had longer treatment duration compared with pts without a dose adjustment (15.0 *vs* 7.3 cycles, respectively; $P<0.0001$). The median number of cycles administered was 7 (range 1–48); 33.4% pts remained on therapy for ≥12 cycles, 17.6% of pts for ≥24 cycles, and 14.5% pts for ≥26 cycles. Of pts who continued therapy ≥24 cycles, 11.8% required dose modifications directly after cycle1, but the percentage of dose modifications steadily decreased from cycle 6 onwards. There was a positive association between a higher starting dose and longer treatment duration ($P<0.0001$). A significant negative correlation between age and the number of cycles administered was observed: pts aged <75 yrs were 1.51 times more likely to receive ≥24 cycles compared with pts aged ≥75 yrs ($P=0.0093$). In multivariate analyses, age and LEN starting dose were both statistically significant predictors for a treatment duration lasting ≥24 mos (Table 1).

Summary and Conclusions: This large dataset (N=1,779) from a clinical practice setting of pts with RRMM in the United Kingdom shows a positive correlation between the recommended LEN 25 mg/day starting dose and longer treatment duration. Age (<75 yrs) and individual dose adjustments of LEN during therapy were also associated with longer treatment duration.

Table 1. Predictors of treatment duration lasting ≥24 mos.

Factor	Odds ratio (95% CI) [*P* value]	
	Univariate model	Multivariate model
Age	0.983 (0.972–0.995) [0.0068]	0.986 (0.974–0.998) [0.0233]
LEN starting dose		
5 mg vs 25 mg	0.738 (0.402–1.354) [0.3262]	0.746 (0.406–1.371) [0.3451]
10 mg vs 25 mg	0.595 (0.404–0.877) [0.0087]	0.635 (0.429–0.941) [0.0235]
15 mg vs 25 mg	0.844 (0.593–1.20) [0.8962]	0.982 (0.625–1.273) [0.5278]

CI, confidence interval.

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THE EFFICACY AND SAFETY OF POMALIDOMIDE WITH OR WITHOUT LOW-DOSE DEXAMETHASONE IS NOT IMPACTED BY AGE IN PATIENTS WITH ADVANCED RELAPSED AND REFRACTORY MULTIPLE MYELOMA: MM-002 SUBGROUP ANALYSIS

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Background: Pomalidomide (POM) demonstrated clinical efficacy and acceptable tolerability in relapsed and refractory multiple myeloma (RRMM) patients previously treated with lenalidomide (LEN) and bortezomib (BORT), in the randomized, multicenter, open label MM-002 phase 2 trial.

Aims: The impact of age (≤65 *vs* >65 yrs) on efficacy and safety outcomes was assessed in a post-hoc analysis.

Methods: Patients with RRMM who had received ≥2 prior therapies, including LEN and BORT, and were refractory to their last regimen were randomized to either POM+low-dose dexamethasone (POM 4 mg/day, days 1–21 of a 28-day cycle; LoDEX 40 mg/week) or POM alone. At progression, patients receiving POM alone could receive POM+LoDEX at the investigator's discretion. Patients >over 75 yrs received LoDEX, 20 mg/week. All patients received mandatory thromboprophylaxis (daily low-dose aspirin). End points included progression-free survival (PFS), response rate (based on EBMT criteria), response duration, and safety.

Results: A total of 221 patients with a median age of 63 yrs (range 34–88) were randomized to POM+LoDEX (n=113) or POM alone (n=108). Overall, 77 (35%) of patients had LEN as their last prior therapy. The efficacy outcomes and the most common treatment emergent grade 3/4 adverse events (AEs) for the age subgroups according to treatment arm are presented in the Table 1. Median response was durable (7.7–10.6 mos) across the age groups with POM regardless of the addition of LoDEX, and was not impacted by age. Of patients aged ≤65 yrs, 28% (POM+LoDEX) and 32% (POM) required at least one dose reduction; for patients aged >65 yrs these proportions were 29% and 44%, respectively. Median relative dose intensity was 90% in both POM+LoDEX and POM arms in patients aged ≤65 yrs; in older patients it was 90% and 100%, respectively.

Table 1. Efficacy and safety outcomes.

	≤ 65 yrs		> 65 yrs	
	POM + LoDEX (n = 62)	POM (n = 69)	POM + LoDEX (n = 51)	POM (n = 39)
Median age, yrs (range)	59 (34–65)	58 (37–65)	72 (66–88)	74 (66–88)
Efficacy, %				
At least partial response	31	13	37	18
At least minimal response	47	23	43	44
Median duration of response,* mos	10.1	8.3	7.7	10.6
Median PFS, mos (range)	4.7 (3.7–6.7)	1.9 (1.8–2.7)	3.7 (2.1–5.5)	3.3 (2.8–5.5)
Safety	**POM + LoDEX (n = 61)**	**POM (n = 68)**	**POM + LoDEX (n = 51)**	**POM (n = 39)**
Grade 3/4 hematologic AEs, %				
Neutropenia	46	40	35	59
Anemia	26	24	18	26
Thrombocytopenia	18	24	20	21
Grade 3/4 non-hematologic AEs, %				
Pneumonia	16	10	29	21
Urinary tract infection	10	3	8	0

*For patients who achieved at least partial response.

Summary and Conclusions: POM with or without LoDEX was effective and generally well tolerated in heavily pretreated RRMM patients who had already received LEN and BORT, including patients who had progressed on prior LEN. In general, age had no impact on overall response rate, duration of response, or safety. Updated data will be presented at the meeting.

P779

PROMISING ROLES OF AMIFOSTINE AS PROPHYLACTIC AGENTS AGAINST BORTEZOMIB-INDUCED PERIPHERAL NEUROPATHY

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Background: Up to now, as the first proteasome inhibitor to be approved for the treatment of both relapsed/refractory and newly diagnosed multiple myeloma (MM) patients, the bortezomib has emerged as an important therapeutic strategy in the treatment of MM. On account of the BiPN as an adverse effect, the dose and duration of bortezomib treatment were often limited. The incidence of BiPN reached as high as 37%. In addition, there was a lack of the effective evidence concerned with the treatments and prevention measures for BiPN. As an organic thiophosphate, amifostine, was used as a "detoxifying agent" during a cytotoxic treatment procedure. Besides, it was also thought to act as a ROS scavenger, which is an important component for cancer therapy. What's more, it was demonstrated that amifostine could play a potential neuroprotective role on preventing the cisplatin- and paclitaxel-induced neurotoxicityin.
Aims: To examine whether amifostine could protect patients with multiple myeloma (MM) from bortezomib-induced peripheral neuropathy while maintaining the therapeutic efficacy.
Methods: 47previously untreated patients with MM were enrolled and randomly assigned to treatment.All patients receivedbortezomib (1.0 mg/m^2 on days1,4,8, 11),dexamethasone (20-40mg/don days1-4, 8-11) andthalidomide(100mg/d)for four 21-day cycles. Patientswere randomly assigned to receive 400 mg of amifostinebefore bortezomib,then assessed incidence and severity of BiPN after every cycle. *In vitro*, schwann cells(SCs)and myeloma cells(MCs)were pretreated with amifostineat 0,1,2, 4mM for 30min prior to bortezomib exposurefor 5 h at 400nM and 100nM, respectively. After Cell viability and reactive oxygen species (ROS) were examined, aggresome formation and activation of chaperone-mediated autophagy (CMA) in SCswere observed through immunofluorescent analyses and TEM.
Results: Complete response rates did not differ in thepresence or absence of amfostine therapy(34.78% v 37.5%, P>0.05). Incidence of BiPN was lower in patients whoreceived amfostine compared with those who received no amifostine (62.5%v 78.26%, P>0.05), though the difference was not significant. Among the patients with BiPN,NCI grade 3-4 incidence wassignificantly lower in patients who received amifostinethan those who received no amifostine (8.33% v 39.13%, P<0.05).*In vitro*, amifostine pretreatment increased thecell viability while decreased the level of ROS in SCs.In addition, amifostine pretreatment decreased the percentage of SCs forming peripheral myelin protein22 (PMP22) aggregatesandinduced high expression of cytoplasmic chaperoneand receptor of CMA at lysosomal membrane. TEM observedautophagosomes of different stages in SCsinduced by amifostine. Amiforstine did not increased the cell viability and decreased the level of ROS in MCs.
Summary and Conclusions: These results indicate promising roles of amifostine as prophylactic agents against BiPN by reducing ROS generation and aggresome formation through activation of CMA.

P780

MP VS MPT IN FIRST TO FOURTH LINE OF TREATMENT IN MULTIPLE MYELOMA PATIENTS

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Background: Combination of melphalan and prednisolone (MP) was standard treatment of multiple myeloma (MM) for decades. Since the introduction of novel agents the clinical outcome in MM has improved. Several prospective studies with thalidomide, the first novel agent, combined with MP, (MPT) compared to MP have been performed, most of them showing MPT gives a better response rate and median overall survival than MP. A metaanalysis of 6 prospective studies show a rise of median OS and EFS when treating with MPT of 6 months when comparing to MP.
Aims: The aim of the study was to look upon a real life population and see if addition of thalidomide to MP gained real life patients.
Methods: From a material of 1642 patients with symptomatic MM collected from 15 Swedish sites from earliest January 2000, until latest June 2012 we collected all patients treated in first, second, third and fourth line of therapy with MP (n=600, 213, 54 and 21) and MPT (n=170, 66, 23 and 15). MM patients were identified from the Swedish National Cancer Register and medical data were obtained from medical records. Patients were evaluated for response rate, OS and EFS. Multivariate Cox model analysis was made to adjust for Ig-class, age, hypercalcemia haemoglobin and albumin levels at time for MM-diagnose.
Results: The distribution of response rate of nCR/VGPR/PR and NR in the MP population was5,3, 31 and 61% and4,4, 27 and 65% in 1st and 2nd line of therapy respectively. In the MPT population the response rate were significantly better;12,12,46 and 30% in 1st line and9,9, 47 and 34% in 2nd line. Median OS in the MP group after 1st line of therapy was 27 months and in the MPT group 50 months, 95% CI [24;30] and [44;84] respectively (P<0.0001). The relative risk for death in the MPT group *vs.* the MP group was 0.61, 95% CI [0.45;0.84] after adjusting for other prognostic markers. Two years from start of treatment 55% of the patients treated with MP were still alive and hadn't started new treatment *vs.* 70% after MPT. After 2nd line of therapy OS in the MP group was 22 months and in the MPT group 35 months, 95% CI [18;25] and [29;-]. Relative risk for death after MPT *vs* MP was 0.55, 95% CI[0.38;0.83], P<0.01. After 3rd and 4th lines of therapy median OS for MP were 17 and 14 months, 95%CI [13;30] and [4;23] respectively and for MPT 19 and 23, 95% CI[8;32] and [8;-]. The difference after 4th line of treatment was not significant, probably due to the small amount of patients. EFS for patients receiving MP in 1st, 2nd and 3rd lines of treatment was 12.6, 8 and 8 months respectively compared to patients who received MPT where EFS was 22, 18 and 3.2 months respectively, (P<0.001, <0,001 and=0.374 respectively). The reason for the short EFS after 3rd line of treatment in the MPT group is unclear and does not correspond to OS for the same group (Figure 1).

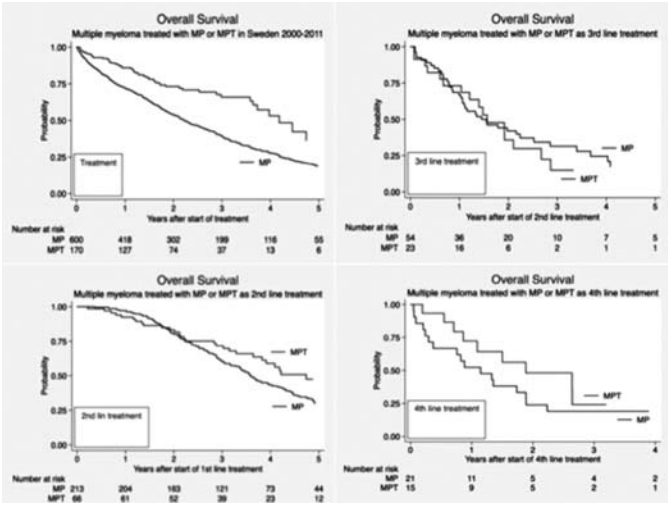

Figure 1.

Summary and Conclusions: Response rates with MPT were significantly better than with MP both in 1st and 2nd line of treatment. Treatment with MPT significantly increased OS and EFS in treatment lines 1 through 3. MPT benefits in our retrospective study of patients in a standard clinical setting were even bigger than in the prospective trials performed.

P781

CAN NOVEL AGENTS OVERCOME THE NEGATIVE PROGNOSTIC IMPACT OF RENAL IMPAIRMENT IN MULTIPLE MYELOMA? - A POPULATION BASED STUDY INCLUDING 1538 PATIENTS

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Background: Renal impairment (RI) is a relatively common feature of multiple myeloma (MM) and it has been shown in several studies that RI at the time of diagnosis correlates to inferior survival.
Aims: To understand the impact of RI on survival in the era of novel agents. The primary endpoint of this retrospective study was overall survival (OS). Time to next treatment (TTNT) was the secondary endpoint.
Methods: The study population included all patients diagnosed with MM since earliest January 2000 until latest June 2011 at 14 Swedish sites. The estimated glomerular filtration rate (eGFR) was calculated using the MDRD-formula and RI was defined as eGFR <60 mL/min/1.73 m^2. Multivariate Cox model analysis was made to adjust for age, calcium, haemoglobin and albumin levels at time for MM-diagnose.
Results: The study population consisted of 1538 patients. Patients with RI at diagnosis (n=680) had a significantly worse median OS of 33 months 95% CI[28;36] compared to those without RI (n=858), with a median OS of 52 months 95% CI[48;56], (P<0.001). High dose treatment (HDT) in 1st line improved medi-

an OS in patients with RI (76 *vs* 26 months, P<0.001). Novel treatment in 1st line significantly improved OS for non-HDT patients with RI (60 *vs* 21 months, P<0.001). This difference was still significant in the multivariate analysis. There was no difference in median OS between non-HDT patients with and without RI that had been treated with novel drugs (60 *vs* 50 months, P=0.86). RI implied a shorter median TTNT after 1st line (13 *vs* 20 months, P<0.001). HDT prolonged TTNT (30 *vs* 11 months, P<0.001). For non-HDT patients with RI novel treament in 1st line also prolonged TTNT from 9 to 19 months, P<0.001 (Figure 1).

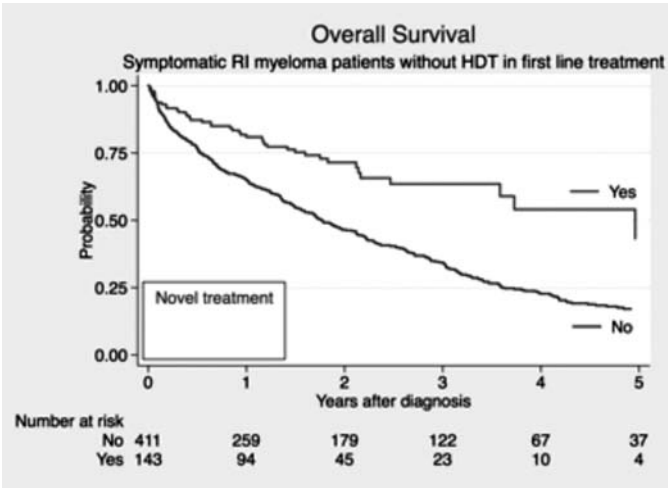

Figure 1.

Summary and Conclusions: RI is still an important prognostic marker in MM. HDT and novel treament regimens can partly overcome the negative impact of RI with improved median OS and prolonged TTNT.

P782

SIGNIFICANCE OF THE ISS AND IMWG RESPONSE CRITERIA IN PATIENTS WITH MULTIPLE MYELOMA WHO RECEIVED ASCT IN THE NOVEL AGENT ERA: A RETROSPECTIVE ANALYSIS OF 1701 JAPANESE PATIENTS

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Background: Although the International Staging System (ISS) and International Myeloma Working Group (IMWG) response criteria for multiple myeloma (MM) are used worldwide, there have been few reports on prognostic assessment of Asian patients using these criteria in the novel agent era.

Aims: The objective of our study was to evaluate the clinical significance of ISS and IMWG response criteria in Japanese patients with MM who were treated with autologous stem cell transplantation (ASCT).

Methods: Study participants included 1701 Japanese patients (967 men and 734 women) with a median age of 57 years (range: 19-73 years) who underwent a single ASCT after high-dose melphalan (200 mg/sqm) (Mel 200) treatment for MM in Japan between October 1995 and December 2011. Data were collected and analyzed retrospectively using the Transplant Registry Unified Management Program (TRUMP) of the Japan Society for Hematopoietic Cell Transplantation. Given that bortezomib, thalidomide and lenalidomide were approved in Japan in December 2006, October 2008 and June 2010, respectively, we categorized patients into two treatment cohorts: pre-novel agent era (1995-2006) and novel agent era (2008-2010).

Results: During the pre-novel agent era (1995-2006) in Japan, 695 patients (386 men and 309 women) with a median age of 56 years (range: 22-70 years) received a single ASCT after Mel 200 treatment between October 1995 and December 2006. The median follow-up time was 4.3 years with a 4-year overall survival (OS) rate of 66%. Median survival rates for ISS I (n=176), II (n=204) and III (n=113) groups were 7.3, 6.4 and 5.3 years, respectively. We could not obtain ISS data for 202 patients. In the ISS I group, OS was significantly prolonged compared to ISS II (P=0.046) and III (P=0.002) groups; however, no significant difference was found between ISS II and III groups (P=0.155). Responses before ASCT were as follows: 64 CR (9.2%), 139 VGPR (20.0%), 374 PR (53.8%), 83 SD (11.9%), 21 PD (3.0%) and 14 non-data (2.1%). Median survival rates for CR, VGPR, PR, SD, and PD groups were 11.3, 5.9, 6.2, 5.4 and 3.3 years, respectively. There were no significant differences in OS between the CR group and other response groups, except for CR *versus* PD (P=0.014) groups. During the novel agent era (2008-2010) in Japan, 1006 patients (581 men and 425 women) with MM received a single ASCT after Mel 200 treatment between January 2008 and December 2010. The median follow-up time was 1.5 years with a 2-year OS rate of 87%. Two-year OS rates for ISS I (n=392), II (n=410) and III (n=204) groups were 90%, 87%, and 82%, respectively. In the ISS I group, OS was significantly prolonged compared to the ISS III group (P=0.03), but no significant differences were found between ISS I and II groups (P=0.59) and between ISS II and III groups (P=0.07) (Figure 1). Responses before ASCT were as follows: 107 CR (10.6%), 316 VGPR (31.4%), 473 PR (47.0%), 80 SD (8.0%), 21 PD (2.1%) and 9 non-data (0.9%). Two-year OS rates for CR, VGPR, PR, SD and PD groups were 90%, 89%, 86%, 83% and 61%, respectively. There were no significant differences in OS between the CR group and other response groups, except for CR *versus* PD groups (P<0.001) (Figure 2). The percentage of CR+VGPR cases (423 of 1006 [42.0%]) before ASCT in the novel agent era increased significantly compared to the pre-novel agent era (203 of 695 [29.2%]) (P<0.0001).

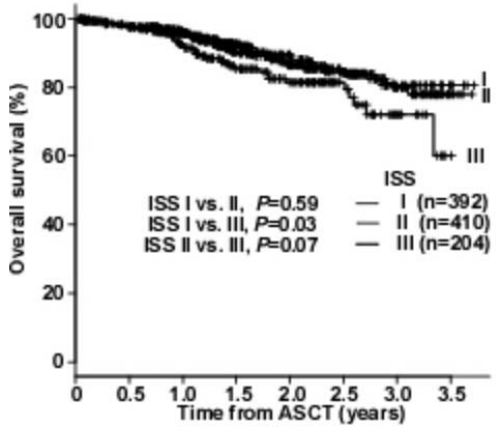

Figure 1.

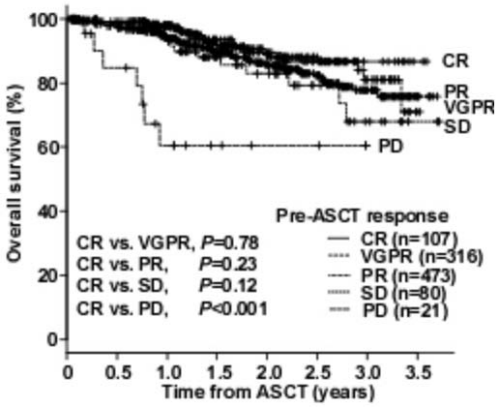

Figure 2.

Summary and Conclusions: In the novel agent era, CR+VGPR cases before ASCT increased. Although the ISS did not clearly stratify the prognosis of Japanese patients with MM who received a single ASCT, this finding should be confirmed in prospective studies.

P783

PREVALENCE AND CLINICAL SIGNIFICANCE OF BRAF V600E MUTATION IN MULTIPLE MYELOMA

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Background: Activating mutations in the BRAF are found in 100% of patients with hairy cell leukemia, 60% with malignant melanoma and 4% with multiple myeloma. Mutation V600E is the most common of approximately 30 BRAF mutations. Clinical characteristics of myeloma patients with BRAF mutations have been published, but the number is still too low to decide whether they have a common phenotype. Here we present 10 cases with BRAF mutation V600E.
Aims: Our objective is to further examine the prevalence and clinical significance of BRAF mutations in multiple myeloma.
Methods: The patient material consists of 153 bone marrow biopsies collected as a part of routine diagnostics at St. Olavs Hospital in Trondheim, Norway in the period 2006-2012. All patients fullfilled criteria for multiple meyloma (>10% plasma cells in BM and/or >30 g/l of M protein). All patients who were still alive at the time of inclusion gave passive consent to analyze the clinical information and biological material. Ethics committee approved the study. Patient samples were examined by real time PCR for the two most common BRAF mutations, V600E and K601N. Clinical disease characteristics from each patient were obtained from clinical records.
Results: In 153 patients we found 10 patients (6,5%) with V600E mutation and no patients with K601N mutation. Median age was 69 (range 52-82) years, 6 male and 4 female, IgG5, IgA 2 (3 missing), kappa8, lambda2, creatinine median 146 (59-900) µmol/l, corrected Ca median2,80 (2,40-3,60), ISS stage II: 3 pts, ISS stage III: 4 pts (3 missing), 8 pts had skeletal disease. Median overall survival was 52 months in BRAF- patients and 26 months in BRAF+ patients. None of the differences were statistically significant.
Summary and Conclusions: The prevalence of BRAF mutation V600E was6,5%. 10 patients with BRAF mutation V600E were more affected by the disease and had shorter survival compared with 143 patients without the mutation indicating that BRAF+ patients may have a more agressive disease.

P784

SESTAMIBI TECHNETIUM-99M BONE MARROW SCAN IS ABLE TO PREDICT OVERALL DISEASE OUTCOME AND MORTALITY COMPARED TO WHOLE BODY MAGNETIC RESONANCE IMAGING IN MULTIPLE MYELOMA

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Background: Bone disease occurs in about 90% of multiple myeloma (MM) patients. There are no data comparing the new diagnostic modalities with whole body Magnetic Resonance Imaging (WB-MRI) and Sestamibi Technetium-99m-MIBI Bone Marrow Scan (MIBI) in MM.
Aims: This study aims to determine whether WB-MRI and MIBI scans in the same myeloma patients produce the same estimate of disease load and location and to study possible association between the bone disease detected by these scans and the effect on disease outcome and survival.
Methods: A prospective comparative study was conducted between WB-MRI and MIBI scans in assessing bone disease and outcome of MM. Sixty two consecutive patients with confirmed MM underwent simultaneous WB-MRI and MIBI scans at the Launceston General Hospital from January 2010 to January 2011, and their survival status was determined in January 2012. The median age was 62 years (range 37-88) with a male to female ratio of 33:29. For the examination of the spines, MRI-T1 weighted turbo spin-echo and turbo spin echo MRI-STIR (Short Tau Inversion Recovery) were performed in the sagittal plane. Within 48 hours all patients underwent MIBI scan. Imaging started at five minutes post injection of MIBI–30mCi (1110MBq) with a wide field of view gamma camera equipped with a low energy high resolution collimator. The whole body scan proceeded at 30cm/minute. When completed, lateral femora images were taken with each view, which took approximately two minutes and, if required, a single positron emission computed tomography (SPECT) was performed. This study is approved by the Tasmanian Human Ethics Committee, Australia. The study was registered prospectively in the Australian and New Zealand Clinical Trials Registry at http://www.ANZCTR.org.au under No: ACTRN12609000761268.
Results: Overall in all bones, the mean MIBI scan result provides a better and earlier prediction of disease progression and mortality than the mean result from the MRI scan versions taken together (MRI_T1 HR for trend 0.51; 95%CI 0.33 to 0.81; P=0.012: and MRI-STIR HR for trend 0.58; 95%CI 0.35 to 0.97; P=0.038) when patients receive standard therapy for myeloma. In vertebrae and long bones, MRI scan detected more disease compared to MIBI scan (P<0.001) but there was less difference in the skull (P=0.09). In the rib-cage, the MIBI scan detected more lytic lesions of the ribs compared to MRI scan (P<0.001). Thirteen of the 62 patients died during the 24 months follow-up. Increased disease detected in all bones by both scans was associated with increased mortality risk (MIBI P=0.001; MRI-STIR P=0.044; but not MRI-T1 P=0.44). In all combined bone groups, the mean MIBI scan results provided a better prediction of mortality than MRI scan over the follow-up period (MRI-T1 *vs* MIBI P=0.019; MRI-STIR *vs* MIBI P=0.047).
Summary and Conclusions: Our study confirms that WB-MRI is more accurate and with a higher sensitivity in detecting myeloma bone lesions.A novel finding shows that MIBI scan obtains an image of all important bone compartments in the body in one single examination, and is less time consuming and more comfortable for the patient than MRI. Furthermore, MIBI scan was able to predict overall disease outcome and mortality better than MRI scan. Further studies to define optimum use of these imaging techniques are warranted.

P785

HETEROGENEITY OF IMWG DEFINED CR AND VGPR ASSESSED BY FREE LIGHT CHAIN ASSAY AND MULTIPARAMETER FLOWCYTOMETRY

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Background: With the development of novel therapeutic agents, complete response (CR) and very good partial-response (VGPR) have become treatment goals in patients with multiple myeloma (MM). Although, the current definition of CR is not fully satisfactory, and other techniques such as serum free light chain (sFLC) assay, immunophenotyping and molecular methods are being investigated. The incorporation of FLC assay in addition to IMWG defined CR criteria defines a more stringent degree of CR (sCR), whereas multiparameter flowcytometry (MFC) may allow the more deeper level of response compared to conventional immunofixation negative response or sCR.
Aims: We retrospectively analyzed the relationship of different level of responses and its clinical relevance on the prognosis after treatment of MM.
Methods: A total of 131 consecutive patients with MM treated from April 2005 to December 2012 at our institute were subjected to this study. *s*FLC was serially measured at least once a week during admission and once a month during the period of outpatient care, and bone marrow examination was performed before and after treatments as clinically indicated. Treatment response was assessed using the IMWG criteria, and the best response to treatment during the course of disease was evaluated by simultaneous serum immunofixation test, sFLC measurements and MFC analysis of bone marrow plasma cells.
Results: Among 131 patients, 32% of patients achieved CR, 27.5% achieved sCR, and 20% achieved immunophenotypic CR. VGPR was obtained 25% of patients and the rest of 42% of patients remained PR or less responses. Survival of patients was correlated with the depth of response in IMWG criteria. Normalization of FLC ratio among patients with CR, VGPR, and PR or less was 86%, 60%, and 9%, respectively. Among 36 CR patients with normal sFLCκ/λ, 26 (72%) were MFC-negative and 10 (28%) were MFC-positive; 4 of 6 CR patients without normal sFLCκ/λ (29%) were MFC-positive. 20 VGPR patients (61%) obtained normal sFLCκ/λ, while only one became MFC-negative. Among 56 patients with less than PR, only 3 obtained normal sFLCκ/λ and none achieved MFC negativity. Among the patients with CR and VGPR, patients achieved sFLC normalization showed significantly better survival compared to those who did not. Patients achieved MFC negativity showed significantly better survival compared to those who did not. Among patients with CR, patients achieved MFC negativity showed better PFS, but other conventional prognostic markers did not give negative impact on PFS, probably due to short follow up and excellent outcome in this group of patients. On multivariate Cox regression analysis for PFS, only MFC negativity was an independent prognostic factor (Hazard-ratio 0.028; 95% CI, 0.004 to 0.21; *P*=0.0005).
Summary and Conclusions: This study confirmed that magnitude of CR and VGPR response defined by IMWG criteria was heterogeneous in terms of sFLCκ/λ normalization and MFC negativity. Although MFC and sFLC analysis frequently gave discrepant results among patients with CR and VGPR, both analyses appeared to give complementary important complementary information for assessing the depth of CR and VGPR category. Achieving immunophenotypic CR translates into superior PFS compared with conventional CR or sCR.

P786

ALPHA 1-ACID GLYCOPROTEIN (AAG) IS A POTENTIAL PATIENT SELECTION BIOMARKER FOR IMPROVED CLINICAL ACTIVITY OF ARRY-520 IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA (MM)

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Background: ARRY-520 is a selective inhibitor of kinesin spindle protein (KSP) that has preferential activity in Mcl-1-dependent tumors and is being developed for patients with relapsed and refractory multiple myeloma (MM). The acute-phase plasma protein alpha 1-acid glycoprotein (AAG) can bind certain drugs, potentially affecting pharmacokinetics (PK), and thus efficacy. AAG levels vary considerably among patients with cancer, including MM.

Aims: To investigate the interaction of ARRY-520 and AAG as it relates to patient outcome following ARRY-520 administration.
Methods: Protein binding, and the effect of [AAG] and [compound] on MM cell line viability were measured *in vitro*. Patient data were obtained from 3 clinical studies of ARRY-520: Ph1 solid tumor study, Ph1/2 AML study, and Ph1/2 study in MM. The MM Phase 2 study consists of two separate cohorts: single agent ARRY-520 and ARRY-520 plus low-dose dexamethasone (dex). [AAG] and the degree of ARRY-520 total protein binding were measured in pre- and post-dose blood samples.
Results: ARRY-520 shows preferential affinity for AAG relative to other common serum proteins, such as HSA. In *in vitro* assays, increasing [AAG] across a clinically relevant range (0.6–3.0 g/L) decreased the unbound concentration of ARRY-520 five-fold and this correlated with increased IC_{50} values for ARRY-520 in MM cell line viability assays. A similar effect on MM cell viability was not seen for several other standard of care MM agents, which do not bind to AAG. Thus, we hypothesize that elevated AAG decreases free concentration of ARRY-520 and leads to a loss of potency. In pre-dose blood samples (n=140), [AAG] ranged from 0.2 to 4.1 g/L. Both [AAG] and measured unbound ARRY-520 correlated with changes in PK: CL and V_d decreased with increasing AAG, consistent with a lower unbound fraction in patients with higher [AAG]. Post-dose blood samples from the MM study indicated that AAG levels did not significantly change with time. Of 72 MM patients evaluated for AAG to date, 26% exhibited pre-dose [AAG] ≥1.1 g/L, and this correlated with a decreased median event-free survival (EFS) (2.3 *vs* 7.8 months; P=0.0024) and no clinical responses (0/19 *vs* 12/53; P=0.028) compared to patients below this cutoff. In the ARRY-520+dex cohort, a 22% ORR (≥PR) in the 1st-stage of this study was observed. The ORR for the subgroup of patients with AAG ≥1.1g/L was 0% (0/6). By contrast, in patients with AAG <1.1g/L ORR was 33% (4/12). High [AAG] also associated with a shorter median overall survival (OS) of 4.5 months *vs.* 20.2 months in patients with AAG ≥1.1g/L. The reported relationship between [AAG] and MM patient prognosis is unclear. In our study, we observed no relationship between pre-study [AAG] and various prognostic markers (*e.g.* b2-microglobulin, HSA, LDH, and ISS score). These results are consistent with AAG not having prognostic value in this disease.
Summary and Conclusions: Preclinical and interim clinical data suggest AAG levels affect the PK and activity of ARRY-520. In preclinical analyses, this effect is specific to ARRY-520 relative to other MM drugs. Patients with [AAG] above a cutoff are predicted to achieve insufficient exposure to gain therapeutic benefit, with a 0% ORR and significantly shorter EFS and OS compared to patients below this cutoff. We hypothesize that selecting patients based on low AAG levels may allow for identification of those patients most likely to benefit from ARRY-520. Additional work is ongoing and will be reported at a later time.

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COMPARISON OF FREELITE AND N LATEX SERUM FREE LIGHT CHAIN ASSAYS AND PREDICTING SURVIVAL

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Background: Availability of serum immunoglobulin-free light chain (FLC) assays Freelite (Binding Site UK) and N Latex (Siemens, Germany) allows for measurement free kappa and lambda immunoglobulin light chains. FLC measurements are an important tool in diagnosing and monitoring patients with multiple myeloma and AL amyloidosis. We report a comparison of the Freelite (a polyclonal assay) and N Latex (a new monoclonal assay) immunoassays in diagnosis and monitoring of patients with systemic AL amyloidosis.
Aims: We assessed the concordance of the assays in detecting the abnormal light chain component, time to first dFLC response, dFLC response at2,4,6 months and outcomes by degree of clonal response.
Methods: Our primary cohort consisted of 94 consecutive AL amyloidosis patients assessed at the UK national amyloidosis centre between January 2011 and April 2012, treated with chemotherapy and available for serial follow up for six months. All patients had serum FLC monitoring at baseline and two monthly thereafter to assess treatment response. The FLC measurements were repeated in duplicate for both the Freelite and N Latex assays at 0,2, 4 and 6 months.
Results: The median age was 64 yrs (range 55-72 yrs) with cardiac involvement in 43% (23% Mayo stage 3) and renal involvement in 76%. Patients had a kappa and lambda clonal light chain in 21% and 79% respectively. 46% had a measurable monoclonal protein (≥1g/L). The follow up was 8.6 months and median overall survival was 24.1 months. The median kappa was 17.3mg/L and 16mg/L, median lambda 48.8mg/L and 52.6mg/L and median dFLC was 107mg/L and 199mg/L by Freelite and by N Latex respectively. There was an abnormal kappa in 41% and lambda in 63% by Freelite assay, and abnormal kappa in 32% and 67% by N Latex assay. An abnormal kappa or lambda was correctly identified in 85%/78% and 82%/83% by Freelite and N latex assays. There were discordant kappa/lambda ratios at presentation with 11/90 abnormal by N Latex and normal by Freelite, and 10/90 abnormal by Freelite but normal by N Latex. The correlation coefficient for kappa was R^2=0.91, lambda was R^2=0.52 and kappa to lambda ratio was R^2=0.87. At 2 months, a complete response (CR) was achieved in 20% and 21%, partial response (PR) 0% and 14% by Freelite and N Latex assays respectively. At 4 months a PR was present in 7% and 16% by the Freelite and N Latex assays (Table 1). Achieving a PR or greater at 4 months post treatment predicted a statistical significant survival advantage by both assays: Freelite (P=0.011) and N Latex (P=0.049).

Table 1.

		Freelite	N Latex
2 months	CR	8	9
	vGPR	4	6
	PR	0	6
	NR	28	22
4 months	CR	13	11
	vGPR	6	6
	PR	3	6
	NR	23	18
6 months	CR	13	13
	vGPR	5	4
	PR	3	5
	NR	10	9

Summary and Conclusions: Both FreeLite and N latex assay can detect abnormal free light chains in patients with systemic AL amyloidosis. In general, there was a good correlation between the assays for detecting the abnormal light chain subtype. However, both assays showed discrepancies in the absolute values of FLC and each assay missed different patients. N Latex assay appears to detect PR earlier than Freelite assay but the clinical significance remains unclear as the p values for improved survival were different for both assays. Although both assays are useful for detection of abnormal FLC measurements in amyloidosis, the values are not interchangeable. Further studies are needed to validate the biological significance of abnormal FLC by the N Latex assay. Joint first authors (S Mahmood and NL Wassef)

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ADVERSE EVENTS AND MANAGEMENT IN MM-003, A PHASE 3 STUDY OF POMALIDOMIDE+LOW-DOSE DEXAMETHASONE (POM+LODEX) VS. HIGH-DOSE DEXAMETHASONE (HIDEX) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background: Optimization of adverse event (AE) management in RRMM patients (pts) is important in order to permit full-dose treatment (Tx) and maximize therapeutic benefit. RRMM pts with advanced disease exposed to multiple prior Tx present with comorbidities and may be predisposed to more frequent AEs. POM was recently approved by the US FDA for use in RRMM following at least 2 prior Tx, including bortezomib (BORT) and lenalidomide (LEN). POM+LoDEX has previously been shown to be effective and well tolerated in this pt population. MM-003 is an open-label, multicenter, phase 3 trial comparing POM+LoDEX *vs.* HiDEX in pts who failed prior LEN and BORT.
Aims: This subanalysis examined the MM-003 safety profile and AE management.
Methods: Pts must have been refractory to their last prior therapy (progressive disease [PD] during or within 60 days) and failed LEN and BORT after ≥2 consecutive cycles of each (alone or in combination). Pts were randomized 2:1 to receive 28-day cycles of POM 4 mg on days 1-21+LoDEX 40 mg (20 mg for those >75 years of age) weekly; or HiDEX 40 mg (20 mg for those >75 years

of age) on days 1-4, 9-12, and 17-20. Tx continued until PD or unacceptable toxicity. Thromboprophylaxis with low-dose aspirin, low-molecular weight heparin, or equivalent was required for all pts receiving POM and those at high risk of thromboembolic events. AEs were graded according to the National Cancer Institute Common Terminology Criteria for AEs (v 4.0). Tx was withheld and started at a lower dose in subsequent cycles for any grade 4 hematologic or ≥grade 3 non-hematologic AE. Dose reduction schemes were predefined. Supportive care in the form of bisphosphonates, antibiotics, hematopoietic growth factors, erythropoietin, and platelet or red blood cell transfusions was allowed. The primary endpoint was progression-free survival (PFS), and safety was a secondary endpoint.

Results: A total of 455 pts were enrolled; 449 pts were included in the safety study population: POM+LoDEX (n=300); HiDEX (n=149). POM+LoDEX significantly improved PFS and overall survival (OS) *vs.* HiDEX. Median follow-up was 4 months. The most common grade 3-4 AEs (POM+LoDEX *vs.* HiDEX) were neutropenia (42% *vs.* 15%), anemia (27% *vs.* 29%), infections (24% *vs.* 23%), and thrombocytopenia (21% *vs.* 24%). With thromboprophylaxis, the incidence of any-grade venous thromboembolism was low (3% *vs.* 2%). Grade 3-4 febrile neutropenia occurred in 7% and 0% of pts, respectively. Any-grade peripheral neuropathy (PN) occurred in 12% and 11%, respectively (new onset: 7% and 7%). Grade 3-4 PN occurred in 1% of pts in each arm. AEs were primarily managed by dose modification and/or supportive care (Table 1). AEs of interest necessitating POM or HiDEX dose reduction included neutropenia (8%; 0%), thrombocytopenia (6%; 0%), anemia (0% for both), infection (1%; 4%), and febrile neutropenia (1%; 0%). Discontinuation due to AEs was infrequent: 7% *vs.* 6% (POM+LoDEX *vs.* HiDEX, respectively). Tx-emergent AEs that led to discontinuation of POM included infections (2%), renal failure (1%), and thrombocytopenia (0.7%). AEs causing discontinuation of DEX in either arm included infection (2%), renal failure (0.9%), and thrombocytopenia (0.4%). Updated data will be presented at the meeting.

Table 1.

	POM + LoDEX (n = 300)	HiDEX (n = 149)
Dose management		
Median average dose, mg (range)	POM: 4.0 (2.6-4.0) LoDEX: 40 (8-40)	40 (20-40)
≥ 1 dose reduction (%)	POM: 24 LoDEX: 17	26
Dose reduction for neutropenia (%)	POM: 8 LoDEX: 0	0
Dose reduction for febrile neutropenia (%)	POM: 1 LoDEX: 0.3	0
Median time to first dose reduction, mo (range)	POM: 1.0 (0.3-7.6) LoDEX: 1.9 (0.3-10.3)	1.1 (0.9-10.2)
Supportive care (%)		
Granulocyte colony-stimulating factor	38	9
Red blood cell transfusion	43	48
Platelet transfusion	17	19
Antibiotics	72	67

Summary and Conclusions: In this study, POM+LoDEX improved PFS and OS with manageable and acceptable tolerability in advanced RRMM. The majority of pts did not have a dose reduction and there were few discontinuations due to AE. POM+LoDEX should be considered a new Tx option for these pts.

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VTD CONSOLIDATION, WITHOUT BISPHOSPHONATES, REDUCES BONE RESORPTION AND IS ASSOCIATED WITH A VERY LOW INCIDENCE OF SKELETAL-RELATED EVENTS IN PATIENTS WITH MULTIPLE MYELOMA POST-ASCT

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Background: Bortezomib monotherapy is associated with increased osteoblastic activity and reduced osteoclast function in relapsed/refractory myeloma (MM). The co-administration of zoledronic acid in all reported studies to-date may suggest a synergistic stimulation on bone turnover by the two agents but has not allowed the independent evaluation of bortezomib on bone metabolism.
Aims: The prospective evaluation of the combination of bortezomib, thalidomide and dexamethasone (VTD) consolidation on bone metabolism of MM patients who underwent high-dose melphalan followed by ASCT in the absence of bisphosphonates (BPs).
Methods: MM patients in first remission post-ASCT were included. Patients did not receive any BP during or post-ASCT as well as throughout the period of VTD consolidation. VTD started on day 100 post-ASCT: V was administered at a dose of 1.0 mg/m^2 on days1,4,8,11; T was given at a dose of 100 mg/day, po, on days 1-21 and D at a dose of 40 mg/day on days 1–4 of a 21-day cycle. Patients received 4 cycles of VTD (first block), were followed without treatment for 100 days and then received another 4 cycles of VTD (2nd block). Patients were assessed for skeletal-related events (SREs) throughout the period of the study (12 months). The following bone markers were measured before and after each block of VTD (4 measurements for each patient): i) osteoclast regulators [sRANKL and osteoprotegerin (OPG)], ii) osteoblast inhibitor dickkopf-1 (Dkk-1), iii) bone resorption markers (CTX and TRACP-5b) and iv) bone formation markers [bone-specific alkaline phosphatase (bALP) and osteocalcin (OC)].
Results: Forty-two consecutive patients were treated: 36 completed both VTD blocks while six patients completed only the first block (one due to PD and five due to toxicity). Just before VTD administration, 16 patients were in CR (9 in sCR), 16 in vgPR and 10 in PR. Although most of these patients were rated as vgPR or better, they had increased serum levels of sRANKL (P=0.037), Dkk-1 (P=0.001), CTX (P=0.002), TRACP-5b (P<0.001), compared to 18 healthy controls of similar age and gender, indicating sustained osteoclast activity despite minimal tumor load. Levels of Dkk-1 and sRANKL/OPG ratio positively correlated with bone resorption markers (P<0.01). The first block of VTD resulted in a significant reduction of sRANKL (P=0.001), sRANKL/OPG ratio (P=0.02), CTX (P=0.05), but also of bALP (P=0.002) and OC (P=0.001), while Dkk-1 showed no alterations. After the first block of VTD, 33.3% of patients improved their status of response; however alterations of the studied molecules were irrespective of further response or not improvement. Before the administration of the 2nd block of VTD, CTX was reduced compared to values after the first block of VTD (P=0.015) and was further reduced after the completion of the study (P=0.01). Dkk-1, OC and sRANKL/OPG ratio had no further alterations, while bALP was increased before (P=0.012) and reduced after the 2nd block of VTD (P=0.05), but remained stable compared to pre-VTD values. Overall, CTX and OC serum levels were reduced after the two blocks of VTD and 20/42 (47.6%) patients improved their status of response. During the study period, only one patient with PD developed a SRE (*i.e.* radiation to bone). The median follow-up after ASCT was 48 months (13-73 months) and 24 of 42 patients have progressed. The median TTP after ASCT was 34 months (CI 95% 27.7-40.2). The median time to next treatment was 40 months (CI 95% 25.7-54.2).
Summary and Conclusions: VTD consolidation post-ASCT reduces bone resorption and is associated with a very low incidence of SREs despite the absence of BPs. However, bortezomib was not able to produce a significant anabolic effect on the bones when combined with TD even in these patients with low myeloma burden.

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IMPACT OF RENAL IMPAIRMENT ON THE EFFICACY AND SAFETY OF MELPHALAN-PREDNISONE-LENALIDOMIDE (LEN) INDUCTION FOLLOWED BY LEN MAINTENANCE IN NEWLY DIAGNOSED MULTIPLE MYELOMA: MM-015 POST-HOC ANALYSIS

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Background: MM-015 is a phase 3 pivotal trial in transplant-ineligible patients with newly diagnosed multiple myeloma (NDMM), which found that induction with melphalan-prednisone-LEN followed by LEN maintenance (MPR-R) significantly prolonged progression-free survival (PFS; 31 mos) compared with MPR (14 mos) or MP (13 mos) (P<0.001 for both comparisons).
Aims: In this analysis, we retrospectively compare the efficacy and safety of MPR-R, MPR, and MP in the subset of MM-015 patients with moderate renal impairment (RI; defined as creatinine clearance [CrCl] <60 mL/min).
Methods: Treatment regimens have been presented previously (Palumbo A, *et al.* N Engl J Med. 2012;366:1759-69). NDMM patients were randomized to MPR-R (MPR induction followed by LEN maintenance [10 mg, D1–21/28-day cycle] until disease progression or unacceptable adverse events [AEs], or MPR or MP without maintenance therapy. CrCl was estimated using the Cockcroft-Gault formula and, for this analysis, patients were divided into two groups: CrCl <60 mL/min and CrCl ≥60 mL/min. Patients with severe RI (serum creatinine >2.5 mg/dL [>221 µmol/L]) were excluded from the trial. Dose adjustments were not recommended for patients with RI.
Results: CrCl <60 mL/min was observed in 51% of MPR-R patients, 45% of MPR patients, and 49% of MP patients. Among patients with CrCl <60 mL/min, median PFS was significantly higher with MPR-R (26 mos [95% confidence interval (CI): 14–48]) than MPR (13 mos [95% CI: 12–15]) or MP (14 mos [95% CI: 12–16]; P<0.001 for both). CrCl <60 mL/min was not a significant prognostic factor for PFS in a Cox proportional model (P=0.69). The most common grade 4 AEs were hematologic, occurring predominantly during induction. The proportion of patients with moderate RI who died during the study was similar across treatment groups: 10% (MPR-R), 7% (MPR), and 8% (MP) (Table 1); ≤1% of deaths in the RI population were associated with RI or disease progression. Updated data will be presented at the meeting.

Table 1. Safety outcomes according to baseline renal function.

	MPR-R		MPR		MP	
CrCl, mL/min	**≥ 60 (n = 71)**	**< 60 (n = 77)**	**≥ 60 (n = 82)**	**< 60 (n = 69)**	**≥ 60 (n = 77)**	**< 60 (n = 75)**
Median CrCl, mL/min (IQR)	84 (70–95)	47 (38–55)	76 (68–85)	46 (37–54)	79 (68–92)	47 (34–55)
Gr 4 hematologic AEs during induction, %						
Neutropenia	32	35	27	38	3	12
Thrombocytopenia	7	16	6	20	1	7
Anemia	1	4	1	4	1	1
Leukopenia	4	4	2	9	1	1
Febrile neutropenia	0	3	0	3	0	0
Gr 3–4 non-hematologic AEs occurring in ≥ 5% of patients during induction, %						
Pneumonia	1	1	1	9	5	1
Fatigue	1	9	0	4	0	5
Asthenia	0	4	1	6	1	0
Hypokalemia	3	4	2	6	0	1
Bone pain	1	4	4	4	5	3
Rash	4	5	2	9	3	1
Patients who died during the study, %	1	10	0	7	1	8

IQR, interquartile range.

Summary and Conclusions: In patients with moderate RI, PFS was significantly improved with continuous LEN treatment with MPR-R compared with MPR or MP, and with an acceptable safety profile, which is consistent with the overall trial results. However, CrCl and AEs should be closely monitored in this patient population.

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TREATMENT WITH BORTEZOMIB-BASED REGIMENS IMPROVES OVERALL RESPONSE AND PREDICTS FOR SURVIVAL IN PATIENTS WITH PRIMARY OR SECONDARY PLASMA CELL LEUKEMIA: ANALYSIS OF THE GREEK MYELOMA STUDY GROUP

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Background: Plasma cell leukemia (PCL) is a rare and aggressive plasma cell disorder. The reported median survival of both primary (pPCL) and secondary (sPCL), before the introduction of novel agents, was about 8 and 2 months, respectively. Bortezomib-based regimens (BBR) are highly effective for the treatment of Myeloma. However, there is limited information about their efficacy and safety in PCL.
Aims: To explore the efficacy and safety of BBR in PCL treatment and investigate possible prognostic factors for survival.
Methods: We retrospectively collected data of pPCL and sPCL patients treated and followed in 9 centers of the Greek Myeloma Study Group between 2000 and 2013.
Results: We evaluated 42 consecutive PCL patients (pPCL:25/sPCL:17) out of 2089 myeloma patients treated at the same period in the 9 centers. Their characteristics were: M/F: 29/13, median age: 61 years (range 41-85 years), ISS1:4, ISS2: 10 ISS3: 28, IgG: 23, IgA:3, light chain:13, non-secretory: 3. At PCL diagnosis, median values (range) of laboratory characteristics were as follows: Hb 9.3 g/dL (6-11.7 g/dL), WBC 9.2×10^9/L ($2.9\text{-}90.1\times10^9$/L), ANC 3.1×10^9/L ($1\text{-}14\times10^9$/L), PLTs 103×10^9/L ($6\text{-}26\times10^9$/L), β2M 7.4 mg/L (3.6-27 mg/L), LDH 243 U/L (115-5205 U/L), serum creatinine 1.3 mg/dL (0.5-10.5 mg/dL), serum albumin 3.3 g/dL (1.9-5.3 g/dL), serum Ca 9.3 mg/dL (7.8-13.8 mg/dL). Serum Ca, PLTs and ANC were significantly higher in pPCL (P<0.05); LDH was higher in sPCL (P<0.05); age and PS did not differ between pPCL and sPCL (P>0.05). Immunophenotype was available in 32 patients; CD56 was negative in 75%. FISH data were available in 25 patients; 64% had high risk cytogenetics. Seventeen patients were primary refractory, 24 patients responded to treatment and 1 patient had stable disease. Seventeen patients progressed after initial response; 16 patients received treatment for progression (10: novel agents, 6: conventional therapy). Thirty-four patients died and 8 are still alive (7 without progression and one with PD). Treatment with BBR was administered in 29/42 patients; 6/25 patients with pPCL underwent ASCT. In patients treated with BBR, grade 3/4 toxicity was observed in 34%, 31%, 31%, 17%, 3.4%, 7% and 31% of patients for neutropenia, anemia, thrombocytopenia, GI complications, renal toxicity, neurotoxicity and infections, respectively. Objective response (≥PR) was achieved in 57% of patients (CR/vgPR:10%/12%). Objective response was 80% in pPCL and 23.6% in sPCL (P=0.001). Median time to response was 2 months(1-8). With a median follow-up of 51 months (2-80), time to progression (TTP) and overall survival (OS) from PCL diagnosis for pPCL and sPCL were 13 *vs.* 5 months (P=0.01) and 14 *vs.* 2 (P<0.001), respectively. The median TTP for patients received BBR *vs.* those received conventional treatment did not significantly differ, whereas OS from PCL diagnosis was statistically different (13 *vs.* 2 months, respectively, P=0.007). Median OS from PCL diagnosis of patients with pPCL and sPCL treated with BBR was 18 and 7 months, respectively. In the multivariate analysis PLTs, treatment with BBR and response type predicted for OS from PCL diagnosis (P<0.05).
Summary and Conclusions: Treatment of PCL with BBR is effective, induces high response rates and prolongs OS. The median OS of pPCL and sPCL patients of this study is considered as one of the highest reported in the literature. BBR, type of response and PLT counts were the most powerful prognostic factors for survival. Grade 3/4 toxicity with BBR was manageable.

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EUROPEAN POST-APPROVAL SAFETY STUDY (PASS) OF RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM): UPDATED DATA ON SAFETY AND SPM INCIDENCE IN PATIENTS TREATED WITH LENALIDOMIDE, THALIDOMIDE, AND BORTEZOMIB

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Background: EU PASS is an observational non-interventional study designed

to investigate the safety of lenalidomide (LEN) and other agents in the treatment of RRMM in a real-world setting.
Aims: To assess the incidence of adverse events (AEs) of special interest, including neutropenia, thrombocytopenia, venous thromboembolism (VTE), peripheral neuropathy (PN), opportunistic infections, and second primary malignancies (SPMs) in RRMM patients (pts) treated with LEN and other antimyeloma therapies according to current clinical practice.
Methods: RRMM pts who had received ≥1 prior therapy were enrolled at investigator's discretion into a LEN cohort (LEN+dexamethasone, the approved combination for the treatment of RRMM) or a background cohort (all other treatments, including novel agents). Thromboprophylaxis was per local standard practice. AEs were graded according to NCI-CTCAE (v3). SPMs were defined using MedDRA terms under Neoplasms SOC. Assessments for SPMs were to be conducted up to 36 mos after treatment discontinuation.
Results: As of November 2012,3,285 pts across 268 institutions in 17 European countries were enrolled. Of those, 66% received LEN (n=2,164), 26% received bortezomib (BORT; n=842), 3% received thalidomide (THAL; n=114), 5% received other therapies (116) or had missing data at the time of the analysis. In total, 129 pts from the background cohort initiated LEN during the course of follow-up as per physician's decision. Baseline characteristics were similar across the cohorts. Median age was 70 yrs (range 25–95) and 54% were males. Most pts (65%) had good performance status (ECOG score 0–1); however, 17.5% had an ECOG score of 2–4. Median number of prior therapies was 2 (range 0–6); 24% had 1 prior treatment, 54% had 2 prior therapies, and 21% had ≥3 prior therapies. The median duration on study treatment was 6.4 mos (range 0–39.6) for LEN after prior THAL, 6.1 mos (0–42.5) for LEN after BORT, 3.7 mos (0–32.5) for BORT after THAL, and 3.7 mos (0–14.9) for BORT after BORT. At a median follow-up of 6.2 mos (range 0–42.5), 25%, 2%, and 15% of pts in LEN, BORT, and THAL cohorts, respectively, had a treatment duration of >12 mos. Overall, 47% of pts (n=1,534) had grade 3–4 AEs. Grade 3–4 neutropenia occurred in 16%, 4%, and 5% of pts in the LEN, BORT, and THAL cohorts, respectively, and grade 3–4 thrombocytopenia in 9%, 8%, and 4%. The overall incidence of PN was 33% (6% grade 3–4) in the BORT cohort, 14% (2% grade 3–4) in the LEN, and 30% (3% grade 3–4) in the THAL cohort. Grade 3–4 VTE developed in 3%, 1%, and 2% of pts in the LEN, BORT, and THAL cohorts, respectively. The overall incidence of SPMs was 1% (Table 1). Incidence rate for invasive SPMs was 1.58/100 pt-yrs (py), of which 1.11/100 py was for solid SPMs and 0.47/100 py was for haematologic SPMs; results were similar for all treatments. Overall treatment discontinuation rate was 79% LEN, 88% BORT, and 90% THAL, with a similar discontinuation rate due to AEs in each cohort. Incidence of death from any cause was similar across all treatments (6% LEN, 4% BORT, and 4% THAL).

Table 1. SPM summary.

SPM characterization	LEN (n = 2,164)	THAL (n = 114)	BORT (n = 842)	Overall (N = 3,236)
All invasive SPMs, n (%)	22 (1)	1 (1)	7 (1)	30 (1)
Incidence per 100 pt-yrs (95% CI)	1.47 (0.97–2.23)	1.67 (0.23–11.83)	2.4 (1.15–5.04)	1.58 (1.11–2.26)
Haematologic tumours, n (%)	7 (0.3)	0	2 (0.2)	9 (0.3)
Incidence per 100 pt yrs (95% CI)	0.46 (0.22–0.97)	–	0.68 (0.17–2.73)	0.47 (0.25–0.91)
Solid tumours, n (%)	15 (0.7)	1 (1)	5 (0.6)	21 (0.6)
Incidence per 100 pt-yrs (95% CI)	1 (0.6–1.65)	1.67 (0.23–11.83)	1.72 (0.71–4.13)	1.11 (0.72–1.7)

CI, confidence interval.

Summary and Conclusions: Results of this ongoing, non-interventional, observational study in RRMM show that AEs were similar across cohorts except for higher rates of neutropenia and lower rates of PN with LEN compared with THAL or BORT. Incidence of significant VTE was lower than observed in previous series. The incidence of SPM across all cohorts was 1%. Despite the current short, overall follow-up, treatment duration with LEN, THAL, or BORT appears unaffected by prior treatment received.

P793

HEAVY LIGHT CHAIN PAIR SUPPRESSION BUT NOT SUPPRESSION OF THE NON-INVOLVED ISOTYPES CORRELATES WITH POOR PROGNOSIS IN IGG AND IGA MULTIPLE MYELOMA

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Background: The heavy light chain (HLC) assay allows measurement of both the involved and non-involved pair of the affected isotype, *i.e.* in IgA kappa myeloma the precise quantification of IgA kappa and of IgA lambda. This information allows for a) the calculation of a ratio between the involved and non-involved pair of the affected isotype (HLC ratio) in analogy to the FLC ratio, and b) the evaluation of a possible suppression of the non-involved pair of the affected isotype (HLC pair suppression), a phenomenon which was not assessable before.
Aims: Here, we studied whether HLC pair suppression and suppression of the non-involved isotype in patients with IgA and IgG myeloma correlated with survival. In addition, we evaluated possible associations of other risk factors with survival.
Methods: 156 patients with multiple myeloma, measurable disease, and started on first line chemotherapy were enrolled. Median age: 66 (32-94) years, male/female: 82/74, ISS stage I: 59, II: 63, and III: 34. Patients received different treatment regimens and were followed for a median of 46.1 months. Age, gender, ISS stage, LDH, Hb, creatinine, albumin, ß2-microglobulin, calcium, response to therapy, FLCratio, HLCratio, and HLC pair suppression were studied and correlated with survival. Kaplan-Meier survival curves were compared using the log rank test; univariate and multivariate analysis were performed using Cox proportional regression analysis (SPSS, version 18).
Results: Severe HLC pair suppression (>50% below lower level of normal) correlated significantly with OS (median: 31.0 *vs.* 60.5 months, P<0.040) (Figure 1A) while no significant correlation was noted between suppression of the non-involved isotype and survival (P=0.233). HLC pair suppression was not correlated with suppression of the non-involved isotypes (non-involved IgA and IgM in patients with IgG M-component: r=0.307, and r=0.071, respectively; non-involved IgG and IgM in patients with IgA MM: r=0.224 und IgM r=0.247). As previously reported, survival was significantly lower (39.6 months) in patients with highly abnormal HLC ratio (<0.022 or >45) compared to those with less abnormal HLC ratio (0.022-45)(71.9 months, P<0.04). Other risk factors found to be independently associated with survival were LDH (P<0.00001), creatinine (P<0.012), ß2-microglobulin (P<0.021), response ³VGPR (P<0.006), and the combination of stage and HLC pair suppression (P<0.002). A four tiered model combining stage and HLC pair suppression showed significantly different survival curves: A) stage I+II with <50% HLC pair suppression; median: 72 months, B) Stage I+II with >50% HLC pair suppression, median: 62 months C) stage III with <50% HLC pair suppression, median: 62 months D) Stage III with >50% HLC pair suppression, median: 28 months, P<0.0001 (Figure1B).

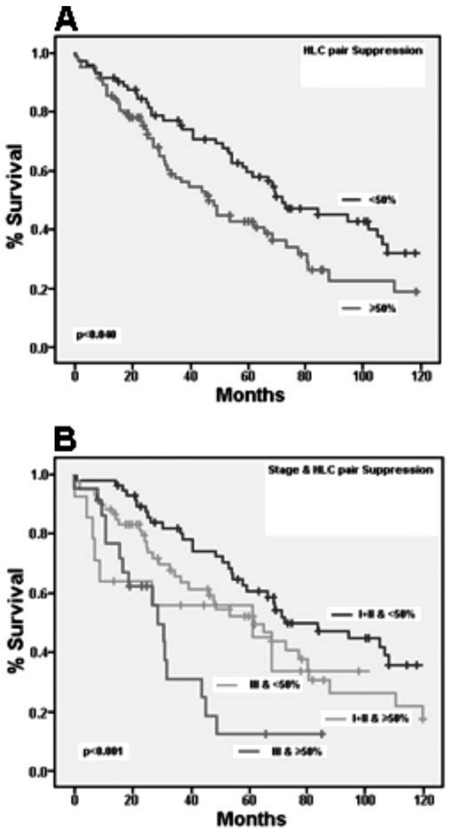

Figure 1.

Summary and Conclusions: HLC pair suppression is a significant risk factor for survival, while for suppression of the non-involved isotype no relationship with survival was noted. These data may be interpreted as an attempt of the host's defence system to control the malignant clone with specific suppression of the involved isotype. This constriction seems to be heavy chain but not light

chain specific. In addition, a prognostic model employing both HLC pair suppression and stage has been constructed which revealed significant distinction between individual risk groups.

P794

CIRCULATING SOLUBLE RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-KAPPA B LIGAND (SRANKL) AND C-C MOTIF LIGAND-3 (CCL-3) LEVELS CORRELATE WITH SURVIVAL IN PATIENTS WITH WALDENSTROM'S MACROGLOBULINEMIA

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Background: Waldenstrom's macroglobulinemia (WM) is a rare lymphoplasmacytoid lymphoma characterized by the production of IgM monoclonal immunoglobulin. Interplay between the malignant cells with the microenvironment is crucial for the biology of the disease. CCL-3 is a chemo-attractant cytokine for macrophages and mast cells, while RANKL is a TNF superfamily member, which is mainly produced by activated T-lymphocytes. We have previously shown that WM cells produce CCL-3 (Terpos *et al.*, Clin Lymphoma Myeloma Leuk 2011;11:115-7), while serum sRANKL was elevated in WM patients (Terpos *et al.*, Br J Haematol 2006;133:301-4). However, there is no information for the prognostic value of these molecules in WM.
Aims: To evaluate the role of RANKL and CCL-3 in survival of WM patients.
Methods: We measured the circulating levels of sRANKL, its decoy receptor osteoprotegerin (OPG) and CCL-3 in the serum of 55 patients with symptomatic WM before the administration of any kind of therapy, in 5 patients with asymptomatic WM (AWM), in 12 with IgM-MGUS and in 30 healthy subjects of similar age and gender who served as controls. Circulating sRANKL, OPG and CCL-3 were measured using an ELISA method (Biomedica, Vienna, Austria for sRANKL and OPG; R&D Systems, Minneapolis, MN, USA for CCL-3).
Results: The serum levels of sRANKL (median-range: 0.357 pmol/l, 0-1.524 pmol/l *vs.* 0.126 pmol/l, 0-0.152 pmol/l; P<0.001) and CCL-3 (64.8 pg/mL, 10.6-528 pg/mL *vs.* 13 pg/mL, 1.4-54 pg/mL; P<0.001) were markedly elevated in WM patients compared to controls. On the contrary, there was no difference regarding OPG levels between patients and controls. Circulating CCL-3 was also increased in WM patients compared to patients with IgM-MGUS (15.4 pg/mL, 0-54.7 pg/mL) and AWM (21.4 pg/mL, 13.4-65.9 pg/mL), while the sRANKL levels were elevated in IgM-MGUS and AWM patients compared to controls. In symptomatic WM patients, both CCL-3 and sRANKL circulating levels correlated with serum beta2-microglobulin (P=0.02 and P=0.031, respectively), while only CCL-3 positively correlated with MVD (P=0.041) and ISSWM stage (the values for low, intermediate and high-ISSWM were 28.3 pg/mL (1.4-188.6 pg/mL), 77 pg/mL (16.2-528 pg/mL) and 76.7 pg/mL (20.8-177.6 pg/mL), respectively; P=0.017). Regarding immunochemistry in trephine biopsies, in all WM cases, the whole number of the neoplastic cells, including CD20(+)/CD138(-)/MUM-1(-) small B-lymphocytes, plasmacytoid lymphocytes and rare immunoblasts as well as CD20(-)/CD138(+)/MUM-1(+) plasma cells revealed strong cytoplasmic positivity for CCL-3. The median follow-up of symptomatic WM patients was 35 months. The median overall survival (OS) has not been reached yet, while the probability for 3-year OS was 76%. The median progression-free survival (PFS) was 57 months and the 3-year probability of PFS was 56%. High circulating sRANKL values (above the median) predicted for shorter median OS (16 months *vs.* not reached, P=0.001; Figure 1) and had borderline significance for shorter PFS (0.086). High serum levels of CCL-3 (above the median value) predicted for shorter median PFS (27 months *vs.* not reached, P=0.048) and showed borderline association with shorter median OS (67 months *vs.* not reached, P=0.09).

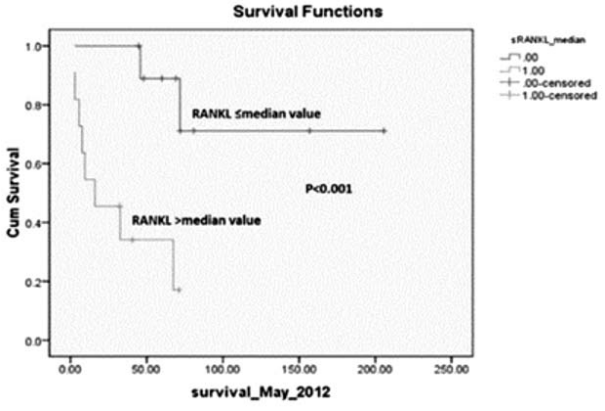

Figure 1.

Summary and Conclusions: We conclude that WM cells produce CCL-3 and enhance the production of RANKL in the bone microenvironment. The correlation of circulating sRANKL and CCL-3 with survival reveals the importance of these cytokines in disease biology and highlights the significance of the interactions between WM and stromal cells for the development of the disease. These data also give the rationale for the use of anti-RANKL and anti-CCL3 drugs in patients with WM.

P795

RESULTS OF THE DUTCH COMMUNITY BASED TRIAL "VALEO" WITH BORTEZOMIB TREATMENT IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS

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Background: Bortezomib (btz) became available in the European Union in 2004. Data available from the use in daily clinical practice is limited.
Aims: In the phase-IV-trial VALEO (clinical trials.gov identifier NCT00440765) the efficacy and safety of btz alone or in combination with other agents was examined in real-life daily practice in 342 relapsed/refractory multiple myeloma (MM) patients (pts) in the Netherlands.
Methods: Informed consent was obtained. Subjects were enrolled in one of two arms: arm A (2nd line, who received not more than 1 previous line of therapy) or arm B (≥3rd line, who received at least 2 lines of therapy). Btz was started in a dose of 1.3 mg/m^2 intravenously on day1,4, 8 and 11. Treatment emergent Adverse Events (TEAEs) were assessed from start of btz until 30 days after treatment.
Results: Here we report the intent to treat (ITT) results prior to database lock (at conference final data will be presented). Number of pts in arm A: n=106; in arm B: n=233. Three pts were treated in newly diagnosed MM (protocol violation, however included in the ITT analysis). Median age A: 66 years (yrs), B: 68 yrs. Male A: 57%, B: 56%. MM type IgG/IgA/non-secretory/other A: 66%/16%/11%/7%, B: 58%/27%/6%/10%. Salmon&Durie stage IIIIA/IIIB A: 66%/25% and B: 70%/17%. Median Karnofsky Performance Status A: 90%, B: 80%. High risk (t(4;14), t(14;16), t(14,20), del17p13, gain 1q, del1p, plasma cell leukemia) A: 28%, B: 31%. Standard risk (t(6;14), t(11;14), gain5q, hyperdiploid, del13 without t(4;14) and/or del17p) A: 81%, B: 79%. K13 A 9% and B: 12%. Median duration from diagnosis of MM was A: 1.8 yrs, B: 3.8 yrs. Median number of cycles administered A:5, B: 4. Best ≥partial response (PR) was A: 61%, B: 46%; ≥very good PR A: 19%, B: 16%; (near) complete response A: 12%, B: 11%. Pts with relapse or progression A: 89%, B: 89% (others censored). Median time to progression was A: 229 days, 95% confidence interval (CI) 189-277, B: 191 days 95% CI 166-214. Pts progression or dying A: 93%, B: 96% (others censored). Median progression free survival time was A: 215 days, 95% CI 184-265; B: 171 days 95% CI 153-206 days (Figure 1). Btz dose was adjusted in 3% of treatment emergent adverse events (TEAEs) (both in group A and B), temporarily discontinued in A: 9%, B: 10% of TEAEs, btz was stopped in A 3%, B: 5% of TEAEs. Frequently reported TEAEs (≥20%) were: thrombocytopenia, fatigue, neuropathy, anaemia, nausea, diarrhoea, constipation and malaise. TEAEs grade 1 were A: 55%, B: 47%; grade 2 were A: 29%, B: 32%; grade ≥3 were A: 16%, B: 20%. 51% of pts experienced a serious TEAE of which 31 pts died (7 causally related: 4 sepsis, 2 pulmonary, 1 cardiac).

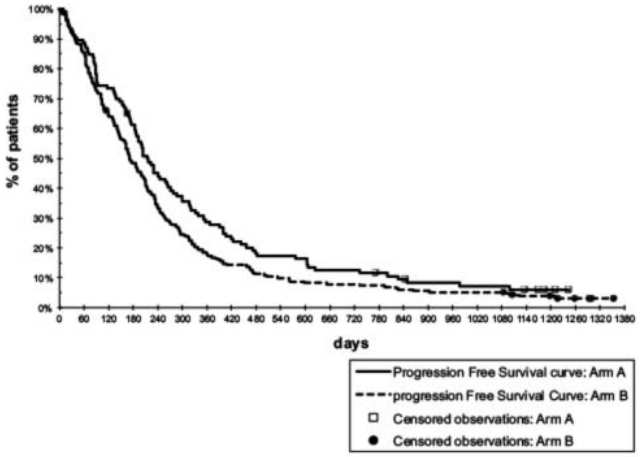

Figure 1. Progression-free survival time (days).

Summary and Conclusions: Treatment with btz in a community-based setting in the Netherlands results in an expected efficacy with 61% PR or higher in second line of treatment and 46% or higher in third line of treatment. Btz was generally well tolerated with acceptable and manageable TEAEs in this type of pts.

P796

ABNORMAL MEDULLARY LESIONS IN APPENDICULAR SKELETONS DETECTED BY WHOLE BODY LOW-DOSE MULTIDETECTOR CT IS ASSOCIATED WITH HIGHER TUMOR BURDEN AND POORER PROGNOSIS IN PATIENTS WITH MYELOMA

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Background: Imaging is playing an increasing role in the diagnosis, staging and treatment response monitoring of patients with multiple myeloma (MM). Whole body Low-dose multidetector CT (WBLD MD-CT) can provide information on the degree of myeloma cell infiltration, especially in the appendicular skeleton (AS).
Aims: This study was performed to determine the incidence and prognostic implications of abnormal medullary lesions in the appendicular skeleton detected by WBLD MD-CT in patients with monoclonal gammopathy of undetermined significance (MGUS), and asymptomatic and symptomatic MM
Methods: Between January 2008 and January 2013, WBLD MD-CT was performed in 89 patients with MM and MGUS as an initial evaluation of lytic bone lesion and medullary and extra-medullary MM lesions. WBLD MD-CT was performed in a non-enhanced manner. Medullary CT density of the humerus and femur were measured using circular regions of interest (ROI), and the results were documented in Hounsfield Units (HU). The highest HU value was selected as representative for each bone, and the mean was calculated. The relationships between abnormal medullary lesions and clinical variables and outcome were studied. The prognostic implications of mean CT value in patients with symptomatic MM were also examined.
Results: Eighty-nine consecutive patients (44 male and 45 female, median age 71yr, symptomatic MM 73, MGUS/asymptomatic 16) were analyzed. Among patients with symptomatic MM, IgG, IgA, light chain only and non-secretary subtypes were 36, 18, 18 and1, respectively. Patients with staging1, 2 and 3 were7, 8 and 59 in Durie—Salmon staging system (DS), and were11, 26 and 37 in International Staging System (ISS), respectively. Medullary abnormalities were found in 61 of 73 patients (83.6%) and 4 in 16 patients (25%) in patients with symptomatic MM and patients with asymptomatic MM/MGUS, respectively. Patients with symptomatic MM had significantly higher mean HU than patients with MGUS/asymptomatic MM (-5.64 *vs.* -69.99, P<0.001). Patients with DS stage 3 showed significantly high HU values compared to those with DS stage 1 and 2 (-35.30 *vs.* 1.11, P<0.001) whereas the mean HU value was not significantly different between ISS 3 and 1 or 2 (P=0.186). Patients with mean HU value >0 had significantly shorter median OS (40.6 months *vs.* not reached, P=0.040) and tended to have shorter PFS (9.8 *vs.* 15.7 months, P=0.106) compared to those with mean HU value <0. In univariate analyses (sex, age□76 yo, high risk cytogenetics, DS stage3, ISS3, serum Cr□2.0, over 25% of abnormal medullary lesions in AS, and CT value of >0), Cr >=2.0 and CT value >0 HU were associated with poorer survival outcome. On multivariate analysis, serum Cr remained significant (P<0.01), and CT value >0 HU was marginally significant (P=0.059) on poor OS.
Summary and Conclusions: This study indicated that medullary abnormalities in the AS detected by WBLD MD-CT might be associated with high tumor burden of MM. In addition, its presence after treatmetn appeared to relate poor possible prognostic outcome.

P797

HIGH RISK CYTOGENETICS, ELEVATED LDH AND ISS-3 IDENTIFY A SUBGROUP OF MYELOMA PATIENTS WITH VERY POOR PROGNOSIS

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Background: Several disease features have been associated with prognosis in multiple myeloma and are associated with a high-risk of progression and death. International Staging System (ISS) identifies 3 risk groups with significantly different outcome and specific cytogenetic abnormalities also may identify patients at high risk for progression or death due to myeloma. The combination of high risk ISS stage (ISS-3) and high risk cytogenetic abnormalities may improve prognostication in patients with MM. Moreau *et al.* (abstract 598, ASH 2012) proposed a prognostic score based on the presence of ISS-3, elevated LDH and high risk cytogenetics; this score identified patients at high risk for death due to myeloma within 2 years for initiation of therapy, in a cohort of intensively treated patients.
Aims: The aim of our analysis was to identify patients who are at high risk for early death in a population of unselected patients with MM and validate in an independent unselected cohort of patients the score proposed by Moreau *et al.*
Methods: We analyzed consecutive unselected patients with MM with available FISH studies for the presence of high risk cytogenetic abnormalities.
Results: 246 consecutive unselected patients, who started therapy after 1/1/2005, were included in the analysis; 95% of the patients received first line therapy with novel agents (thalidomide, lenalidomide, bortezomib). Median PFS is 26 months and median survival is 51 months, however, 5% of the patients died within the first 2 months from treatment initiation while 1- and 2-year mortality rate was 16% and 24% respectively. ISS-1, 2 & 3 disease was present in 22%, 38% and 40% respectively. High risk cytogenetics (del17p, t(4;14), t(14;16), add1q21 or non-hyperdiploid karyotype) were present in 45.5% of our patients. High risk cytogenetics were more common in patients with advanced ISS (30%, 45% and 56% for ISS1,2 & 3 respectively, P=0.008). Elevated LDH (≥300 IU/L, ULN 225 IU/L) was correlated with high risk cytogenetics (P=0.034) and ISS-3 disease (P=0.032). Shorter PFS was associated with ISS-3 (18 *vs* 33 months for ISS 1-2, P<0.001), high risk cytogenetics (20 *vs* 34 months for standard risk, P<0.001) and elevated LDH≥300 IU/L (13 *vs* 28 months for <300 IU/L, P=0.002). Shorter OS was also associated with ISS-3 (41 *vs* 70 months for ISS 1-2, P<0.001), high risk cytogenetics (45 *vs* >70 months for standard risk, P=0.007) and elevated LDH≥300 IU/L (28 *vs* 60 months for <300 IU/L, P=0.05). The combination of these 3 variables produced a scoring system consisting of 4 categories with 0-3 of the risk features. For patients with a score of 0 (all risk factors absent, 35% of the patients) the median PFS was 3.5 years and the OS was 67% at 5 years. For patients with a score of 1 (one of the risk factors present, 40% of the patients) the median PFS was 2 years and the median OS was 4 years. For patients with a score of 2 (two of the risk factors present, 21% of the patients) the median PFS was 1.5 years and the median OS was 3.3 years. Finally, for patients with all risk factors present (a score of 3), which included only 4% of the patients, the median PFS was 12 months and the median OS was just 2 years.
Summary and Conclusions: The use of a simple scoring system which is based on readily available parameters (ISS-3 and LDH≥300 IU/L) in combination with cytogenetics allows the identification of a small group of patients with very high-risk disease and a very dismal prognosis. Patients with all high risk features present should be encouraged to participate in clinical trials due to their poor prognosis with the currently available therapies

P798

BONE BIOMARKERS ARE USEFUL IN MONITORING MYELOMA BONE DISEASE AND AS EARLY PREDICTOR FOR RELAPSE DISEASE IN MULTIPLE MYELOMA

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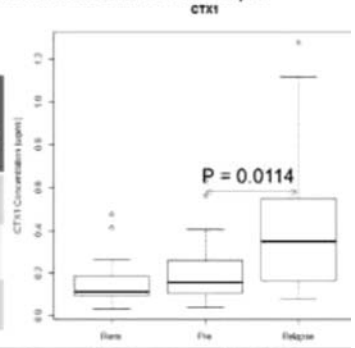
Background: Multiple myeloma is a plasma cell disorder and bone disease is a well-known devastating complication. The uncoupling effect of osteoblast and osteoclast activity is the major element in development of myeloma bone disease (MBD). It causes significant impact to the quality of life and morbidity in multiple myeloma. Imaging techniques are used as the current standard method for detection of bony lesions. They have limitations as they cannot provide a real-time assessment of bone turnover. Early detection of relapse disease is crucial to allow preventative therapeutic intervention could significantly impact on quality of life.
Aims: Bone biomarkers such as C-terminal telopeptide of type 1 collagen (CTX-1) and procollagen type 1 N-propeptide (P1NP) can be used as an early predictor marker for relapse cases with myeloma bone disease and to monitor the myeloma bone disease at diagnosis and remission.
Methods: CTX-1 and P1NP were measured by chemiluminescent immunoassay on fasting plasma samples from 115 patients including newly diagnosed multiple myeloma (n=27), remission (n=30), relapses (n=22) and control (n=27). These were measured at regular intervals over a 27 month study period. Relapse disease was identified by conventional biomarkers like paraprotein and serum free light chains, and confirmed by imaging and bone marrow biopsy. In a subset of patients with disease relapse, the Mann-Whitney test was used to compare bone markers pre-relapse and at relapse.
Results: CTX-1 levels were significantly higher in newly diagnosed multiple myeloma compared to remission and control groups (P<0.0001). In relapse group, CTX-1 rose significantly at the time of pre-relapse to relapse state (P=0.0007). A rise of ≥2.0 fold rise in the level of CTX-1 from remission to relapse disease was noted. The median time between the pre-relapse sample and relapse disease was 3 months (range 1-14 months). Most of them had new bone lesions at relapse. This proves that it has potential as an early predictor of disease relapse/progressive bone disease. A case showed CTX-1 was the only biochemical parameter to rise significantly on relapse as compared to the other conventional biomarkers (ie. paraprotein and serum free light chain) As for P1NP, the rise in P1NP from pre-relapse to relapse was not significant (P=0.0840) (Figure 1).

Duration to monitor CTX-1 from either Remission or Pre-relapse stage to Relapse stage is:

- Range = 1-14 months
- Median = 3 months

	Mann-Whitney Test (p value)	Student T-test (p value)
Remission vs Pre-Relapse	0.2313	0.1818
Remission vs Relapse	0.0007	0.0026
Pre-Relapse vs Relapse	0.0161	0.0114



CTX-1 level	Remission	Pre-relapse	Relapse	Fold rise
Mean (µg/L)	0.156786	0.22209091	0.424386	2.123839
SD (µg/L)	0.109635	0.16738732	0.315917	1.3702

Figure 1. CTX-1 levels in relapse category.

Summary and Conclusions: Osteoclast biomarker serum CTX-1 correlates accurately with the disease burden in newly diagnosed multiple myeloma patients as compared to the rest of the groups. It is a more sensitive early predictor of disease relapse/progressive disease than established biomarkers. It is a more robust marker than P1NP. The rise in P1NP goes against the theory that there is an uncoupling of bone turnover in myeloma bone disease and requires further study. CTX-1 is more cost effective and accessible than imaging and should be used routinely when monitoring bone disease activity in multiple myeloma patients, facilitating early intervention when relapse occurs.

P799

EX VIVO PHARMACOLOGICAL EVALUATION OF 19 DRUGS IN AN AVERAGE OF 50 MULTIPLE MYELOMA PATIENTS USING WHOLE BONE MARROW SAMPLES ANALYZED BY AUTOMATED FLOW CYTOMETRY

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Background: We are pioneering a high throughput flow cytometry to measure the chemical biology space of drugs used to treat Multiple Myeloma (MM) in patient samples in collaboration with PETHEMA.
Aims: To examine the *ex vivo* pharmacology of MM drugs against the malignant cell population in bone marrow samples from MM patients.
Methods: Bone-marrow samples from patients diagnosed with MM were sent to Vivia from 18 Spanish hospitals. Drugs were incubated in 8 concentrations for 48 hours using the intact sample without isolating leukocytes. Afterwards leukocytes were isolated and analyzed by our ExviTech© platform. Drug activity is measured as cell depletion, labeling blasts with monoclonal antibodies and AnnexinV-FITC. Standard dose response fitting generate efficacy (Emax) and potency (EC50) parameters for each drug listed in the Table 1. Inter-patient variability for each drug is measured as IPV=STDDEV*100/MEAN).
Results: The average pharmacological profiles of 19 different MM drugs evaluated in 10-106 samples are shown in Table 1. Drugs are separated into conventional (top lines) and novel (lower grey shaded lines) drugs. 2nd column shows the number of samples tested per drug. Dex and Pre have only 10-11 samples because their initial testing at 48 h was insufficient and were evaluated at 96 h incubation. Standard pharmacological parameters (Emax, columns 3-5) and potency (EC50, columns 6-8) are shown in terms of their mean, standard deviation and interpatient variability (IPV). Drugs are further grouped by mechanism of action (columns 9-10). Among conventional drugs, Bortezomib is the best depleting drug eliminating all cells (mean 2.3±5) with highest potency (lowest EC50 0.03 uM). All conventional drugs except corticoids (Dex, Pre) show maximum efficacy depleting all MM cells (mean-STDDEV). Dex is 390-fold more potent than Pre, albeit Pre is more used in MM. Cell cycle arrest drugs are ordered by their mean potency. Doxorubicin is the most potent though not often used. Vincristine is also potent with the largest interpatient variability, suggesting very sensitive patients could benefit at low doses with less neurotoxicity. Bendamustine is less potent with lowest interpatient variability suggesting a lesser therapeutic potential. All novel drugs (lower shaded lines) have maximal efficacy eliminating all MM cells (mean-STDDEV). The most potent new drugs by far are the epigenetic drugs Panobinostat and Vorinostat, Panobinostat being 48-fold more potent. Among the targeted therapies Tanespimycin has highest potency combined with high interpatient variability, and this approach could serve as companion diagnostic.

Table 1.

COMPOUND NAME	N	EFFICACY (EMAX %) MEAN	SD	IPV(%)	POTENCY (EC50 µM) MEAN	SD	IPV(%)	MOA
BORTEZOMIB	106	2.30	5.14	223.35	0.03	0.03	99.32	Proteas. Inhib.
DOXORUBICIN	35	4.67	12.78	273.76	0.93	0.85	91.24	Cell cycle arrest
VINCRISTIN	47	13.91	17.40	125.07	3.21	13.74	427.88	
MELPHALAN	44	14.95	23.33	156.03	14.07	15.43	109.66	
CYCLOPHOSPHAMIDE	59	10.93	19.43	177.69	54.00	55.87	103.46	
ETOPOSIDE	25	11.32	17.03	150.37	95.30	125.00	131.16	
BENDAMUSTINE	90	1.43	5.57	390.20	118.89	65.11	54.77	
DEXAMETHASONE-96h	11	37.40	16.10	43.05	0.04	0.04	97.50	Glucocorticoid
PREDNISOLONE-96h	10	34.26	22.00	64.21	15.65	47.21	301.66	
PANOBINOSTAT	49	5.64	11.46	203.26	0.05	0.06	119.96	Epigenetic
VORINOSTAT	51	6.30	9.92	157.42	2.38	2.19	92.17	
TANESPIMYCIN	39	7.34	12.07	164.41	12.47	24.28	194.70	Molecular Target
TEMSIROLIMUS	36	0.55	2.11	384.49	40.35	36.98	91.67	
RAPAMYCIN	31	6.16	13.65	221.81	51.58	45.93	89.06	
EVEROLIMUS	38	4.80	14.23	296.72	38.56	30.42	78.91	
TIPIFARNIB	31	3.06	7.83	256.21	35.33	29.92	84.70	
PERIFOSINE	25	11.81	20.68	175.06	38.18	64.10	167.91	

Summary and Conclusions: We have developed an automated system that, in a fast and accurate way, is able to determine the *ex vivo* sensitivity of multiple samples to many different drugs. This approach could be used as a companion diagnostic to identify subsets of patients for which new treatments such as panobinostat or Tanespimycin could be effective. It could also be used as a Personalized Medicine test to help guide therapy if it can be correlated with clinical outcome. Correlation of this *ex vivo* sensitivity with the clinical efficacy is currently being performed in a study under the supervision of the PETHEMA/GEM groups.

P800

THE IMPACT OF 8 GY ONE FRACTION RADIOTHERAPY ON PAIN RELIEF AND RECALCIFICATION IN MULTIPLE MYELOMA PATIENTS WITH PAINFUL BONE DESTRUCTIONS.

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Background: Multiple myeloma (MM) induces osteolysis and disturbs the balance between bone formation and bone resorption. Radiotherapy, surgery and analgesics are required in order to overcome pain, to evoke recalcification and to improve the quality of life of MM patients.Pain relief is obtained in 75–100% and recalcification is achieved in 40–50% of irradiated bone destructions. The randomized trials showed the same effect of single (SF) and multiple fractions (MF) in pain relief and recalcification for patients with painful bone metastases from solid tumors. The role of different palliative radiotherapeutic regimens for MM is not well established due to the lack of clinical trials. There are only few retrospective studies regarding dose-response relationship with analgesia and recalcification.
Aims: To evaluate the impact of 8 Gy one fraction regimen in the treatment of MM on pain relief, analgesics consumption and recalcification.
Methods: From 2010 until 2012, 46 patients (27 women and 19 men, median age: 69 years, 52–88 years) with MM and painful bone destructions were treated by 8 Gy one fraction regimen.Pain was evaluated by using visual analogue scale (VAS) with the scale endpoints from 0 (no pain at all) to 10 (worst imaginable pain) and recalcification was measured with radiographs. Pain score and analgesics usage was measured prior and4, 12 and 24 weeks after radiotherapy.
Results: The pain before radiotherapy was mild (VAS 1-4) in 4 patients (9%), moderate (VAS 5-7) in 15 patients (32%) and severe (VAS 8-10) in 27 patients (59%). The decrease of pain after radiotherapy of 8 Gy one fraction was observed in 36/46 patients (78.3%): 20 patients (43.5%) were found to be

completely responsive and 16 patients (34.8%) were partially responsive according tothe international consensus on palliative radiotherapy criteria. Six patients (13%) were using non-opioid drugs prior to radiotherapy and all of them have ceased analgesic intake for six months after termination of treatment. 38 patients (83%) used opioids drugs. The use of opioids analgesics was reduced in 26/38 patients (68.4%), while a complete cessation of opioids analgesics was observed in 12/38 patients (31.6%) stopped it totally. Significant parameters in pain relief were: age <65 years (Chi square test P=0.034) and IgG type paraprotein (Chi square test P=0.037). Recalcification was observed in 22 patients (55%): a complete response was observed in 14 patients (35%) and a partial response in 8 patients (20%). Pathological fractures in the irradiated field were observed in 6 patients. The most significant parameter regarding recalcification was: age <65 years (Chi square test P=0.022). The hematological and non-hematological toxicity was evaluated on a five point scale according to the toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC) in the first 4 weeks after radiotherapy. The side effects after treatment were uncommon, usually of first grade and reversible.

Summary and Conclusions: One 8 Gy fraction regimen is effective for pain relief, analgesics reduction and on recalcification for MM patients with painful bone destructions.

P801

RESULTS OF LENALIDOMIDE TREATMENT FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA IN A REAL WORLD SETTING: FEASIBILITY AND FACTORS CONTRIBUTING TO LONG TREATMENT DURATION

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Background: Lenalidomide in combination with dexamethason (LEN-DEX) is approved in relapsed, refractory Multiple Myeloma (RRMM) for continuous treatment until disease progression (PD) or unacceptable toxicity. Although there is evidence that continuing treatment beyond best response is associated with improved survival (Dimopoulos *et al.*, 2009), data assessing feasibility, benefits and potential risks of long-term treatment in a non-study RRMM cohort are limited.

Aims: We therefore analyzed data from a German non-interventional multicentre trial to prospectively assess factors influencing treatment duration in a real world setting.

Methods: Selection criteria were diagnosis of RRMM with at least one prior treatment regimen and decision (with patient´s informed consent) to treat relapse/progression with LEN-DEX. Patients (pts) starting treatment between February 2008 and December 31, 2010 were eligible for this analysis. Pts with a treatment period of at least 18 months (mos) were defined as long treatment cohort (LTC), and pts treated for a maximum of 3 mos (91 days) form the short treatment cohort (STC). Data cutoff was November 29, 2012. Statistical analysis included uni– and multivariate models on factors potentially influencing treatment duration. Median overall survival (OS) for LTC and STC was calculated using the Kaplan Meier approach.

Results: Until December 31st2010, 436 pts started treatment, of which 156 (35.8%) were treated for a maximum of three mos and 47 (10.8%) were treated at least 18 mos. Both cohorts showed no significant difference in age, ECOG performance status, time since diagnosis and number of pretreatments received, preexisting neuropathy, and number of VTE in history. On the other hand, they differed in prior treatment with autologous stem cell transplant (ASCT), type of paraprotein and preexisting cytopenia (Table 1). Reasons for treatment discontinuation in STC were related to adverse events (AEs) in 56 pts (35.9%), death in 36 pts (23.1%), and disease progression in 16 pts (10.3%), and in 48 pts (30.8%) treatment was stopped due to pts wish or other causes. The observed toxicities reflect the well known safety profile with mainly hematological AEs (≥grade 3 in 30% of pts), however febrile neutropenia occurred in only 1% of pts. The most frequently reported non hematological AEs of any grade were infections (37.4%), fatigue (21.2%), diarrhea (13.3%), muscle cramps and dizziness (both 11.8%). In LTC therapy is ongoing in 20 out of 47 pts (42.6%) and only 2 out of 27 pts in LTC discontinued treatment due to toxicity. Median OS was significantly higher in LTC. Median OS of LTC was 51 mos, whereas OS of STC was 14.3 mos. If STC pts with PD or death were excluded, the OS still remains significant (21.1 mos *vs* 51 mos, P<0.0001). In uni-variate analysis the following factors positively influenced treatment duration; previous treatment with autologous transplant, IgG type of paraprotein, absence of light chain disease, achievement of an objective response (best response PR or better), and use of anticoagulation (ASA, LMWH). The following two parameters remain the only significant factors in a multivariate model; use of anticoagulation and achievement of an objective response.

Table 1.

	LTC, n=47	STC, n=156	p - value
Age (median, range)	68 (41-85)	71 (37-87)	0.0539
ECOG 0 -1 / ≥ 2[a]	75.8 / 24.4	57.4 / 42.6	0.0685
Time since diagnosis (mos)	34 (4-213)	34.5 (1-360)	0.2762
Number of prior therapies	2 (1-8)	2 (1-9)	0.5734
Prior ASCT (%)	42.6	20.5	0.0041*
Prior Bortezomib (%)	72.3	60.9	0.2248
Prior Thalidomide (%)	21.3	16.0	0.5090
Prior Lenalidomide (%)	6.4	8.3	>0.999
Preexisting neuropathy, (%)	34	28.8	0.7153
VTE in history (%)	17	16.7	>0.999
Type of paraprotein			
IgG/ IgA/ light chain (%)	76.6/ 19.1/ 2.1	57.7/ 25.0/ 16.0	0.0110*
Preexisting neutropenia (%)	4.3	2.6	0.6242
Preexisting thrombocytop. (%)	0	8.3	0.0417*
Preexisting anemia (%)	0	10.9	0.0142*
Creatinine increased (%)	0	5.1	0.2016

[a]Missing data in 30 and 26% of pts in LTC or STC resp., * Statistically significant at 5%

Summary and Conclusions: Here we showed that long-term treatment with LEN-DEX for RRMM is feasible and consistent with previous reports in real world settings. The identification of anticoagulation as a statistically significant factor contributing to long-term treatment underscores the importance of concomitant supportive measures. Outcome of pts with long term treatment is superior to those treated only for a short term period. This is noteworthy, since treatment in a considerable proportion of pts was stopped early for reasons other than progression, death or toxicity. These data thus corroborate previous evidence that LEN-DEX treatment in RRMM should be continued beyond best response.

P802

THE PRESENCE OF IMMUNOPHENOTYPIC ALTERATIONS IN BONE MARROW CELLS OTHER THAN PLASMA CELLS FROM MULTIPLE MYELOMA PATIENTS PREDICTS FOR MYELODYSPLASIA-ASSOCIATED CYTOGENETIC ABNORMALITIES

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Background: The treatment of multiple myeloma (MM) has experienced an extraordinary improvement in the last years. However, the progress achieved has been accompanied by an increasing concern for therapy-related secondary primary malignancies (SPMs). High-dose melphalan (HDM) and more recently lenalidomide have been associated with higher risk of SPMs development, particularly myelodisplastic syndromes (MDS) and/or acute myeloid leukemia. Consequently, given that the benefits of HDM and lenalidomide for MM patients' survival outweigh the potential risk of SPMs it is of notable relevance to identify patients at higher risk of developing SPMs, preferably by using routinely available techniques.

Aims: Here, we investigated if the presence of MDS-associated phenotypic alterations (MDS-FC) predicted for MDS-associated cytogenetic abnormalities in a total of 70 MM patients.

Methods: The presence of MDS-FC was previously investigated in 70 MM patients and was detectable by flow cytometry (FC) in 23 (33%). In these latter cases, FACS purified CD34+ hematopoietic stem cells (HSC), neutrophils, monocytes and erythroblasts were analyzed by FISH (in male patients, n=12), for the detection of -5/del(5q), -7/del(7q), del(20q), trisomy8, and nulisomy Y. Conversely, in female patients (n=11) these cell populations were screened for clonality using the human androgen receptor X-chromosome inactivation test (HUMARA).

Results: From all patients, four male patients (33%) showed cytogenetic abnormalities; 3 cases with del(5q31) and 1 with –Y, both abnormalities being detected in all 4 cell populations. Six of the 11 (55%) female patients showed a clonal HUMARA test. The presence *vs.* absence of MDS-FC alterations predicted for a clonal HUMARA test in the correspondent cell compartment, namely HSC (100% *vs* 11%; P=.005) and neutrophils (67% *vs* 0%; P=.05), with a similar trend being also found in erythroblasts (80% *vs.* 29%; P=.08).

Summary and Conclusions: Our results show that immunophenotypic dysplastic features are present in approximately one-third of MM patients at diagnosis and that in half of them these alterations are associated with clonality at the genetic level.

P803

OUTCOME OF PATIENTS WITH MULTIPLE MYELOMA (MM) RELAPSING AFTER FRONT-LINE AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT): IMPACT OF REDUCED INTENSITY ALLOGENEIC TRANSPLANTATION (RICALLO).

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Background: Both RICALLO and new drugs have been used for salvage therapy for MM relapsing after ASCT. But the optimal treatment remains to be determined.
Aims: The aim of this single centre study was to assess the outcome of pts with MM following the relapse after ASCT, according to whether or not a RICALLO was performed.
Methods: Records of the patients were reviewed and the criteria for entering the study were: Symptomatic MM treated frontline with a program including single or double ASCT, relapse at any time following ASCT, response (CR, VGPR or PR) to a second line treatment. RICALLO was proposed to pts with no significant co-morbidities, having a suitable donor (either sibling or MUD) and who gave their consent after precise information on the risk of the RICALLO.
Results: One hundred and thirty-eight pts treated between 01/2000 and 12/2012 fulfilling the inclusion criteria were identified. 44 pts received a RICALLO (Allo group) while in second or third response with a median of 7.4 months (2-48) after relapse. The RIC consisted of fludarabine plus either 2Gy ICT or busulfan and ATG (according to ongoing available protocols in the centre). The graft was PBSC from sibling in 17 pts and from MUD in 27 pts. 94 pts (CT group) received therapies according to ongoing protocols or available standard of cares. The main characteristics of the patients in each group: median age (58 y.o for Allo and 59 y.o for CT group), MM prognostic factors at diagnosis, type of 1st line therapy (VAD or bortezomib containing regimens), single or double ASCT, time to relapse after ASCT and 2d line treatment were similar between the 2 groups. The initial treatment for relapse consisted of VD (42 pts), RD (37 pts), TD (43 pts), VTD (12 pts) or MPV, MPT, Bendamustin or ASCT (1 pt for each). The 3y OS from the time of relapse for the entire cohort was: 58% (CI95%, 54-62). It was of 58% (CI95% 50-66) and of 58% (CI95% 52-64) for the Allo group and the CT group respectively (P=ns). The causes of death were, relapse in 14 and 41 pts, or treatment toxicity in 13 and 0 pts in the Allo group and CT group respectively. The 3y EFS from the time of relapse was 43% (CI95%, 38-51) and 26 (CI95%, 21-31) for the Allo and the CT group respectively (P=ns) (Figure 1).

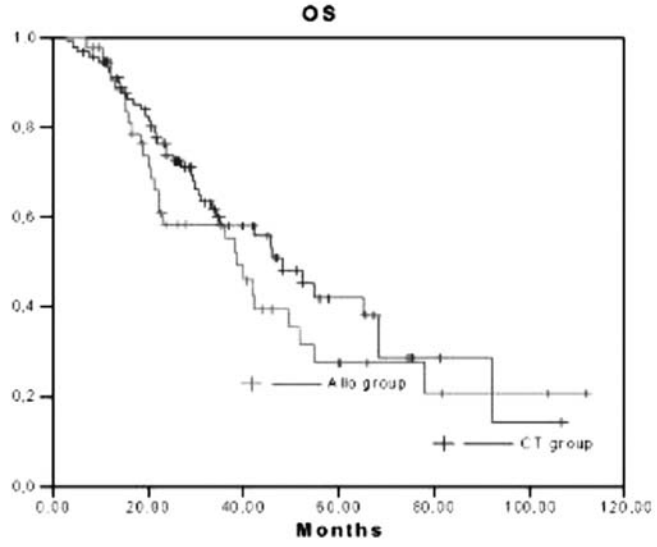

Figure 1.

Summary and Conclusions: We did not observe any difference in survival between Allo-SCT and CT in patients relapsing after front-line ASCT. Prospective study is needed to determine the optimal treatment in this domain.

P804

FLOW CYTOMETRIC ANALYSIS AND MINIMAL RESIDUAL DISEASE IN MULTIPLE MYELOMA: A COMPARISON BETWEEN FLOW CYTOMETRIC IMMUNOPHENOTYPING AND CYCLOSCOPE-MG.

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Background: Multiple myeloma (MM) is a plasma cell neoplasm which resides in the bone marrow (BM) and is critically dependent upon the BM microenvironment for its survival. Recent studies show that MM is consistently preceded by a precursor state, MGUS; however, we lack reliable markers to predict progression from MGUS to MM. In patients with MM, the depth of response is an important prognostic factor.
Aims: The aim of this study is to analyze and compare the clinical utility of Minimal Residual Disease studies in Multiple Myeloma with two differentes flow cytometric techniques: Flow Cytometric Immunophenotyping and Cycloscope-MG.
Methods: These studies included 45 patients: 15 patients with active MM, 10 with non-active MM, 10 with MGUS and 10 controls that were evaluated by flow cytometric immunophenotyping and Cycloscope-MG.The panel of monoclonal antibodies we used in our studies included the minimal test antigens for classifying plasma cells: CD38, CD138, CD45; CD19 and CD56 in order to distinguish myeloma plasma cells (CD19-, CD56+) from normal plasma cells (CD19+, CD56-), and CD117, CD40 and CD27 as recommended markers. Cycloscope-MM kit included a mixture of primary antibodies for the detection of antigens present in human plasma cells (CD38 and CD138); a secondary antibody (FITC labelled IgG goat anti mouse IgG F); an erythrocyte lysing solution and a DNA labelling buffer containing detergent, propidium iodide and RNase for DNA staining.
Results: Previous studies have demonstrated the use of CD38, CD138, CD45, CD 19 and CD56. Furthermore, CD117, CD27 and CD40 have been used in our studies to differentiate between neoplastic and normal plasma cells. Cycloscope-MG showed the aneuploidy of myeloma plasma cells for the detection of minimal residual disease (MRD).The results between morphological analysis, immunophenotypic analysis and Cycloscope analysis were compared in the different groups under study. Significant changes were found in the marrow infiltration of myeloma plasma cells, and, mainly, in the aberrant expression of phenotype and in the expression of aneuploid cells peack. Patients with active MM showed both the characteristic aberrant immunophenotype (CD38+CD138+CD19-CD56 +) and a visible peak in G0/G1 detectable in Cycloscope. The aberrant phenotype and the peak of aneuploid cells, were absent in patients used as control. The comparison between the three methods used for the evaluation of bone marrow infiltration in patients with MM (morfologia, FACS, aneuploidy) confirms the general overestimation of infiltration if the only microscopy is performed. Therefore, it is necessary, for a correct evaluation of patients, to use at least another one technique (molecular, flow cytometric, aneuploidia evaluation) (Figure 1).

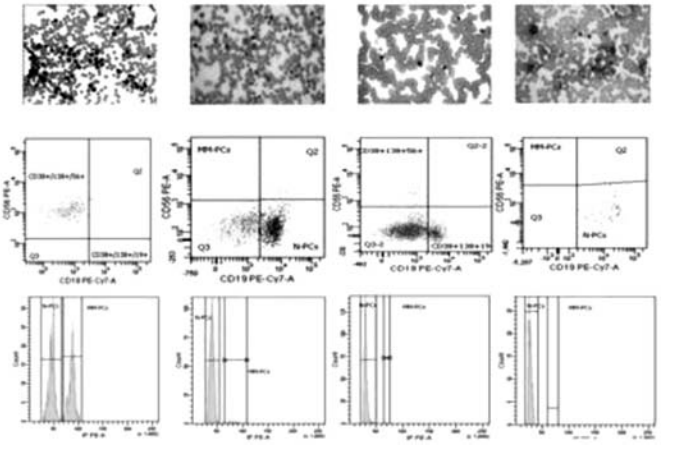

Figure 1.

Summary and Conclusions: Flow Cytometric Immunophenotyping is directly compared with Cycloscope-MG. Our results show that both methodologies may help to discriminate the risk categories of MM patients, based on the level of residual aberrant plasma cells. The Cycloscope-MG™ evaluation provided the possibility to establish a cut-off value that distinguishes patients with active disease from patients in post treatment remission. In fact, patients with active MM had a percentage of aberrant plasma cells (ie plasma cells in the aneuploid Cycloscope-MG™) at medullary level greater than or equal to 2%.Patients with non-active MM showed a percentage of aberrant plasma cells below this cutoff value. Regarding patients with MGUS, the percentage of aberrant medullary plasma cells was always less than 0.5%. Flow cytometric Immunophenotyping is directly compared with Cycloscope-MG™. Our results show that both methodologies may help to discriminate the risk categories of MM patients, based on the level of residual aberrant plasma cells. These studies have shown that residual disease above a level of 2% is clinically relevant in MM.

P805

NUMBER OF ADVERSE CYTOGENETIC LESIONS DETECTED BY FISH IS ASSOCIATED WITH PROGNOSIS IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE-BASED REGIMENS

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Background: Cytogenetic abnormalities are considered the major prognostic factors in multiple myeloma (MM). The most important chromosomal aberrations associated with unfavourable prognosis are translocations involving immunoglobulin heavy chain gene located in chromosome 14q32, like t(4,14)(p16;q32), t(14;16)(q32;q23) or t(14;20)(q32;q12), amp(1q21), del(17p13) and del(13q14).
Aims: In this study we assessed the prognostic value of t(4,14)(p16;q32), amp(1q21), del(17p13) and del(13q14) as well as the combination of these lesion in newly diagnosed MM patients treated with thalidomide-based regimens.
Methods: The study group consisted of 128 patients treated with cyclophosphamide, thalidomide and dexamethasone(CTD, n=96) or melphalan, prednisone and thalidomide (MPT, n=32). Responding patients from CTD group were given high-dose melphalan with autologous stem cell support (HDT/ASCT, n=65). Bone marrow aspirates obtained from patients at diagnosis were analyzed for the presence of t(4,14)(p16;q32), amp(1q21), del(17p13) and del(13q14) using fluorescence *in situ* hybridization (FISH). The cut-off level for all abnormalities was 20% according to the recommendations of the European Myeloma Network.
Results: FISH analysis detected t(4,14)(p16;q32) in 20%, amp(1q21) in 45%, del(17p13) in 17% and del(13q14) in 44% of patients; 30% had 1 lesion, 24% 2 lesions and 17% 3 or more. On univariate analysis the presence of all analyzed abnormalities was associated with shorter progression-free survival (PFS) and overall survival (OS): for t(4,14)(p16;q32) PFS was 7.0 *versus* 18.9 months (P=0.003) and OS 23.6 *versus* 44.3 months (P=0.026); for amp(1q21) PFS 8.0 *versus* 29.0 months (P<0.001) and OS 24.0 *versus* 55.3 months (P<0.001); for del(17p13) PFS 5.5 *versus* 17.0 months (P=0.048) and OS 12.0 *versus* 45.0 months (P=0.016); for del(13q14) PFS 8.0 *versus* 28.0 months (P=0.003) and OS 23.8 *versus* 52.9 months (P<0.001). Multivariate analysis was performed for six covariates: age, HDT/ASCT and four examined genetic lesions. It confirmed amp(1q21), del(17p13) and del(13q14) as being independently associated with shorter PFS (P<0.001, P<0.001 and P=0.042 accordingly) and OS (P<0.001, P<0.001 and P=0.007 accordingly) and HDT/ASCT with longer PFS (P<0.001) and OS (P<0.001). The number of genetic abnormalities detected in patients significantly influenced both PFS and OS. The median PFS was 38.9 months in patients with no lesions, 18.4 months in patients with 1 lesion, 11.5 months in patients with 2 lesions and 5.0 months in patients with 3 or more lesion (P<0.001). The median OS was 79.6 months, 39.6 months, 25.0 months and 12.0 months accordingly (P<0.001). Adverse prognosis associated with multiple genetic changes was also seen in the subgroup of patients undergoing HDT/ASCT (median PFS 46.3 months, 31.8 months, 12.0 months and 5.0 months accordingly, P<0.001; median OS 98.6 months, 39.7 months, 26.9 months and 12.0 months accordingly, P<0.001).
Summary and Conclusions: The results of the study showed that the presence of amp(1q21), del(17p13) and del(13q14) is associated with significantly shortened PFS and OS in newly diagnosed MM patients treated with thalidomide-based regimens. Accumulation of adverse cytogenetic abnormalities resulted in further reduction of survival and the number of coexisting genetic lesions defined risk groups with different PFS and OS. Impact of cytogenetic changes on survival was also seen in patients undergoing HDT/ASCT suggesting that this procedure is not able to change poor prognosis associated with high-risk genetic abnormalities.

P806

EVALUATION OF TNF SUPERFAMILY MOLECULES IN MULTIPLE MYELOMA PATIENTS: CORRELATION WITH BIOLOGICAL AND CLINICAL FEATURES.

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Background: B-cell activating factor (BAFF), a proliferation-inducing ligand (APRIL) and apoptosis inducing ligand (TRAIL), all members of the tumor necrosis factor (TNF) family, represent three of the main survival factors for immature, naive and activated B cells. What is more, BAFF and APRIL, can directly activate the NF - κB pathway, have been identified as two of the main survival factors for healthy plasma cells and multiple myeloma (MM) cells.
Aims: The purpose of the present study was to evaluate serum levels of BAFF, APRIL and TRAIL in healthy volunteers and in MM patients to determine whether there was any correlation between ligands and some prognostic biological parameters of MM patients, and to explore their clinical significance in predicting the disease activity of MM.
Methods: 52 patients with newly diagnosed MM were included in the study. Median age of patients at the time of sample collection was 65, and the range was 59-70. At the time of diagnosis, all patients were divided into three groups based on International Staging System, ISS (12 patients in stage I, 16 patients in stage II, and 24 in stage III). Patients' initial treatment was thalidomide–dexamethasone and cyclophosphamide (CTD), prior to monotherapy of thalidomide 100 mg until progression. Response was assessed after six cycles of chemotherapy with the same methods as for diagnosis, according to the EBMT/IBMTR/ABMTR criteria. The control group consisted of 20 healthy volunteers, age- and sex- matched. Quantitative assessments of cytokines were performed by commercially available ELISA assays. Results were expressed as means SD. The non-parametric Kruskal–Wallis test and the one-way analysis of variance (ANOVA) were assessed to test for different stages. The Student's t-test was used for pairwise comparison of subgroups. Comparisons between the MM and the control groups were made using the non-parametric Mann–Whitney test. The Spearman's order correlation coefficient was applied to determine correlations between the measured parameters. Survival analysis was performed by means of the Kaplan–Meier method. Comparisons of survival curves were performed using the Wilcoxon test. P-values below 0.05 were considered to be statistically significant
Results: Pre-treatment myeloma patients had significantly higher serum concentration compared to healthy volunteers: BAFF (1211.5±1768.8 pg/mL *vs* 309.2±216.1 pg/mL, P<0.0001), APRIL (2.56±0.44 ng/mL *vs* 1.59±0.41 ng/mL, P=0.04), TRAIL (149.41±40.25 pg/mL *vs* 80.28±16.24 pg/mL, P=0.005). MM patients with advanced disease stage III had higher serum levels of all the studied parameters compared to stage I: for TRAIL, P=0.02, APRIL, P=0.04, BAFF, P=0.00002, respectively. Furthermore, the study showed statistical decrease of concentrations of all studied parameters after anti-angiogenic regimen chemotherapy for TRAIL (136.05±73.2 pg/mL, P=0.03), APRIL (1.74±1.05 ng/mL, P=0.01) and for BAFF (826.11±1780.7 pg/mL, P=0.007). Moreover, the concentrations were found to be lower in the subgroup of patients with (CR+VGPR+PR) compared to the SD: for BAFF (652.57±533.2 pg/mL *vs* 1322.3±1592.2 pg/mL, P=0.01 and for APRIL (1.04±0.03 ng/mL *vs* 2.14±1.02 ng/mL, P=0.02, but not for TRAIL (151.39±7.0 pg/mL *vs* 123.27±76.4 pg/mL, P=0.23). In addition, the concentration of APRIL was found to correlate significantly and positively with the concentration of BAFF (P=0.02), IL-6 (P=0.004), LDH, (P=0.003) and negatively with TRAIL (P=0.01). The study also showed a positive correlation between the concentration of BAFF and IL-6 (P=0.009), and β2m and LDH, (P<0.05), but not with concentrations of BAFF and TRAIL (P=0.61). We did find, however, a meaningful link between the concentration of TRAIL and TNF (P=0.03), but not the concentration of IL-6 (P=0.98). Additionally, we observed that pre-treatment MM patients with serum BAFF values higher than the median (847.98 pg/mL) and with serum APRIL values higher than the median (2.26 ng/mL) had significantly shorter progression free survival (PFS) than patients with lower values: for BAFF, P=0.001 and for APRIL, P=0.02. There were no statistically significant differences between PFS values in the subgroups of MM patients with regard to the median values (123.58 pg/mL) of TRAIL, P=0.2.
Summary and Conclusions: In conclusion, our results have demonstrated that serum concentrations of BAFF and APRIL (but not TRAIL) could be a useful biomarker of MM disease activity and progression. Pre-treatment concentrations of BAFF and APRIL could also serve as a prognostic factor of PFS. Both ligands may therefore be a novel therapeutic target in MM.

P807

TOXICITY PROFILE DURING TREATMENT WITH BENDAMUSTINE-BORTEZOMIB-DEXAMETHASONE IN PATIENTS WITH RELAPSED/ REFRACTORY MULTIPLE MYELOMA

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Background: Treatment with bendamustine-bortezomib-dexamethasone (BBD) exerts significant activity in patients with relapsed/refractory multiple myeloma even in those pretreated with bortezomib or lenalidomide or both, but may be associated with clinically relevant side effects.
Aims: Here we analyze the side effect profile with specific emphasis on infections, cytopenias and neuropathy in patients enrolled in a trial with BBD for relapsed/refractory multiple myeloma.
Methods: Seventy-nine patients with relapsed/refractory MM have been

enrolled. Median age: 64 years (range 40-86), male/female: 37/42, ISS stage I/II/III: 27, 31, and 21, respectively. ECOG status 0-I/≥II: 75, and 4 patients, respectively. Previous treatment lines: 1-2: 50, 3-4: 23, >4: 6 patients, respectively. Treatment regimen: bendamustine 70 mg/m^2 day 1+4, bortezomib 1.3 mg/m^2 days1,4, 8 and11, dexamethasone 20 mg on days1,4, 8 and11, repeated every 4 weeks. Planned number of treatment cycles was8, with discontinuation after 4 cycles in case of no response. Toxicity grading was performed using the CTC v 3.0 scale. The FACT-GOG/NTX instrument was used for patients self-assessment of neuropathic side effects.

Results: G3/4 infections were noted in 16 (20%) patients and 2 patients died due to infection/sepsis (G5). Low baseline ANC (<2.800/mL), a higher number of cycles (>4 cycles), and age >65 years showed a weak, but statistically non-significant correlation with G3/4 infections. G3/4 thrombopenia was recorded in 28 (35%) patients; 18 of them presented with more than one episode (median:3, range 2-8). Severe thrombopenia resulted in treatment discontinuation in2, and in delay of therapy in 7 patients, respectively. Lower baseline platelet levels (<182.000/μL) were associated with higher risk for G3/4 thrombopenia (P<0.021). Peripheral neuropathy assessed by the clinical care team was reported in 44 (56%) patients. G3/4 neuropathy was observed in 5 patients only. The occurrence of neuropathy increased, albeit not statistically significant, with increasing number of treatment cycles. Self-assessment of neuropathy by patients revealed a much higher PNP incidence with G1/2 PNP reported by 37 (47%) and G3/4 PNP reported by 38 (48%) patients. Pretreatment with bortezomib or thalidomide, or both bortezomib and thalidomide was not associated with higher incidence of G3/4 PNP.

Summary and Conclusions: Low baseline ANC and were associated with a higher risk for G3/4 neutropenia (P<0.012) and low baseline platelets counts were associated with G3/4 thrombopenia (P<0.021). Lower baseline ANC levels correlated with shorter PFS (P<0.01). Higher age, longer therapy and low baseline ANC tended to correlate with G3/4 infections. Furthermore, incidence of G3/4 neuropathy was low, but increased slightly with increased treatment duration. In stark contrast to evaluations by care givers, a much higher incidence of PNP was revealed when patients graded the incidence and severity of PNP by using a self-assessement instrument. Physicians should be aware of the substantial underrating of PNP by health professionals.

P808

VALUE OF WHOLE-BODY DIFFUSION-WEIGHTED MAGNETIC RESONANCE IMAGING IN THE STUDY OF MONOCLONAL GAMMOPATHIES AND CORRELATION WITH BONE-RELATED CYTOKINES

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Background: MM is related with overproduction of several cytokines that are responsible for imbalance between osteoclast and osteoblast activity. Thus, some cytokines increase bone resorption by increased osteoclast activity, one of which is the *Macrophage inflammatory protein1-alpha (MIP1-alpha).* Another group inhibits bone formation through a reduction in osteoblast activity, we should highlight the Wnt pathway inhibitors such as *Dickkopf-1(DKK-1)* and *Sclerostin*. The use of new imaging techniques is increasingly necessary for the correct study of Multiple Myeloma. One of more promising techniques is the whole body magnetic resonance (WB-MRI) including diffusion/perfusion sequences (DWI), This "functional" imaging test analyze brownian motion of water molecules in all areas allowing high sensitivity and specificity to evaluate early bone marrow involvement. Thus, this exam allows quantitative assessment of bone marrow involvement, through the so-called Apparent Diffusion Coefficient (ADC). Previous descriptions indicate that ADC is greater as higher is the bone marrow infiltration.

Aims: To evaluate the usefulness of DWI in patients diagnosed with Monoclonal Gammopathy (MG). To correlate the ADC value to circulating levels of cytokines related with bone resorption and formation.

Methods: Prospective study was approved by the local IRW. All patients gave their written informed consent. 39 patients(20 Monoclonal Gammopathy of Unknown Significance (MGUS),5 smoldering Multiple Myeloma (sMM),13 symptomatic Multiple Myeloma (MM) were enrolled between May/2011-Oct/2012. The patients were divided on the basis of bone involvement by WB-MRI according infiltration pattern: focal, diffuse and mixed. The ADC values were evaluated in DWI in six different anatomical areas, with b800 (diffusion weighted sequence) in cervical spine, dorsal spine, lumbar spine, iliac crest, femoral head and humeral head; and with b200 in iliac crest and femoral head. *Sclerostin, Dkk-1 and MIP-1*α were measured by double-sandwich enzimoimmunoassay (EIA). *Statistical analysis:* Non-parametric test for comparison of two independents samples (U de Mann-Whitney). Correlations between two variables of the same sample (Spearman's test).

Results: No MGUS patients showed bone marrow involvement in MRI. 2 of the 5 patients diagnosed with sMM had bone marrow involvement in MRI, one of them showed focal pattern of infiltration and another one showed diffuse pattern. 9 of the 13 patients diagnosed with MM had bone marrow involvement, 3 showed focal pattern of infiltration and 6 showed mixed pattern.5 patients (45.5%), with focal bone marrow involvement had affected more than ten anatomical areas. The ADC value in all areas (Table 1) was higher in MM patients than MGUS patients, but there was significant difference only in ADC values of cervical spine (b800) and of femoral head (b200). When we correlate the bone-related cytokines with ADC of all patients with MG, only significant association between sclerostin and ADC in humeral head (P<0.05). In those cases in which there was focal involvement in ribs, the ADC of these lesions showed significant correlation with MIP-1 alpha (P<0.05).

Table 1.

		b=800						b=200	
	n	Median ADC Cerviacal Spine	Median ADC Dorsal Spine	Median ADC Lumbar Spine	Median ADC Iliac Crest	Median ADC Femoral Head	Median ADC Humeral Head	Median ADC Iliac Crest	Median ADC Femoral Head
Multiple myeloma symptomatic	13	837	700	559.5	720.5	472	466	2016.5	1432.5
MGUS	20	585.5	603	416.5	576	376.5	375	1346	1101
p		0.002	0.577	0.220	0.077	0.201	0.195	0.051	0.02

Summary and Conclusions: DWI and ADC value can be useful in the evaluation of bone marrow involvement in MG patients. In these patients there is correlation between levels of bone-related cytokines in MM and parameters of this new "functional" imaging study.

We must increase the number of cases and expand cytokines and bone remodeling markers studied to obtain more significant difference and to reinforce these conclusions.

P809

THE COMBINATION OF BORTEZOMIB AND DEXAMETHASONE (VD) ALONG WITH ZOLEDRONIC ACID INCREASES BONE MINERAL DENSITY (BMD) IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: RESULTS OF A PHASE II STUDY

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Background: Retrospective studies have reported a bone anabolic effect of bortezomib (V) in multiple myeloma (MM), but there is lack of prospective data in this field.

Aims: We scheduled a prospective, non-comparative, open-label, phase II study to evaluate the effects of VD in combination with zoledronic acid (ZOL) on bone disease of relapsed/refractory MM patients. The primary objective was the effect of 4 VD cycles on BMD. Secondary objectives were: i) the evaluation of BMD after 8 VD cycles and 18 months post-VD or at study termination (PD or discontinuation); ii) the evaluation of bone pain, SREs, osteolytic lesions and bone markers [bone alkaline phosphatase (bALP), osteocalcin (OC), C-telopeptide of bone collagen (CTX)] after 4 and 8 VD cycles and 18 months post-VD or at study termination.

Methods: Patients with relapsed/refractory MM, after 1-3 prior lines of therapy, received VD for up to 8 cycles in combination with ZOL (4mg, iv, monthly). V was administered at the standard dose of 1.3 mg/m^2, iv, on days1,4,8,11 of a 21-day cycle, while D was given at a dose of 12 mg/m^2 p.o., on days 1-2, 4-5, 8-9 and 11-12. DXA of the lumbar spine (L1-L4) and the femoral neck (FN) was performed at baseline, on day 21 of cycles 4 and 8 and then every 3 months for up to 18 months post-VD or at PD. Skeletal survey using conventional radiography was performed at baseline, on day 21 of the 8th cycle and then every 6 months for up to 18 months post-VD or at PD.

Results: In total, 17 patients (median age: 70y, range: 56-86y) were enrolled; 14 had received2, one 3 and one 5 lines of previous therapies. At baseline, 2 patients had no osteolytic lesions, one had only one lytic lesion, while 14 patients had multiple lytic lesions (5 had >10 lesions) or multiple fractures (6 patients). Twelve patients completed the 8 VD cycles, while 2 patients received 6 cycles due to peripheral neuropathy, one received 5 cycles and then progressed and two patients died after receiving one and two VD cycles, respectively (due to heart attack and sepsis). Overall, we evaluated 15 patients who completed 4 cycles of therapy; 12 (70%) achieved a PR and two had stable disease. Regarding the primary objective of the study, there was a dramatic improvement of BMD after 4 cycles of VD: median T-score for L1-L4 increased from +0.53 to +0.90 (P=0.013), for the lowest T-value of L1-L4 from -1.29 to -0.34 (P<0.01) and for FN from -2.25

to -1.80 (P=0.027). This improvement was continued for the 8 cycles of VD: median T-score for L1-L4 was 1.56 (P<0.01), for the lowest value of L1-L4 0.97 (P<0.01) and for FN -2.0 (P<0.01). Patients reduced the VAS pain score dramatically after 4 and 8 cycles of therapy (median, range: from 5 (3-10) at baseline to 1.5 (1-5) and 0.3 (0-3) after 4 and 8 cycles, respectively; P<0.01), while they showed no new lytic lesions and no new SREs during the 8 cycles of therapy. Patients showed an increase of both bone formation markers bALP and OC after 4 (P<0.01) and 8 cycles of therapy (P<0.01) along with a reduction of CTX (P<0.01 for both comparisons). During follow-up period of 18 months, 12 patients relapsed (median TTP: 7.5 months), 7 patients showed new lytic lesions but no patient developed a new SRE. Both markers of bone formation reduced at progression, while CTX remained suppressed.
Summary and Conclusions: Our study supports the beneficial effect of bortezomib on BMD when it is given in combination with D and ZOL. This combination reduces pain and bone resorption, while it increases bone formation. Bone formation reduced again at disease progression.

P810

LONG TERM THERAPY WITH LENALIDOMIDE DOES NOT SIGNIFICANTLY AFFECT THE CELLULAR COMPOSITION OF THE BONE MARROW
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Background: Maintenance therapy is an attractive option for MM patients and three recent studies have shown that Lenalidomide (Len) maintenance significantly improves PFS and, in one of them, also OS;[1-3] however concerns have been raised regarding the possibility that long term therapy might increase the risk of second primary malignancies (SPMs).[1-3] Len has a known myelosuppressive effect, but its mechanism of action on the bone marrow (BM) still needs to be fully elucidated.
Aims: To evaluate the impact of Len on the BM we have performed a flow cytometry analysis of BM of MM patients.
Methods: Three, 8 colour, panels (Pacific Blue, Pacific Orange, FITC, PE, PerCP-Cy 5.5, PE-Cy7, APC, APC-H7) were used to investigate relative percentages of the different BM populations (CD3, CD45, MPO7, CD79a, CD34, CD19, CD7, HLADR), B cells (CD20, CD45, TdT, CD10, CD34, CD19, CD123, CD38) and myeloid cells (CD16, CD45, HLADR, CD13, CD34, CD117, CD11b, CD10).[4]
Results: Thirteen samples from 12 patients who have received Len therapy have been analyzed; 5 samples from MM patients who never received Len were used as controls. Whilst no differences could be seen in terms of percentages of T cells and myeloid cells, a decrease in the number of CD19+ B cells was observed in Len treated patients. The decrease of B lymphocytes in patients receiving Len was due to a reduction of the CD19+ population as a whole and not of specific subpopulations. The lowest values were observed in patients on Len for >1 year, and a concomitant relative increase in the CD19- lymphoid population was also observed. No significant difference was observed in the myeloid population either in the percentage of the CD13+ myeloid cells or in the relative percentages of the different fractions (myeloid precursors, myelocytes, metamyelocytes, neutrophils). However, patients receiving Len showed a trend towards a higher percentage of immature myeloid and B cell forms (Table 1).

Table 1.

	Time point	N pts					
BM populations			Myeloid cells		T cells		B cells
	No Len	5	45.6%		9.4%		5.2%
	Len<12 months	7	50.3%		16.1%		4.6%
	Len>12 months	6	47.8%		12.7%		1.8%
Tot		18					
B cells			CD19+	Haemato-gones	Pre B	Naïve B	Mature B	CD19-
	No Len	5	26.5%	0.9%	24.2%	50.1%	22.4%	63.8%
	Len<12 months	6	12.5%	1.4%	40.2%	33.8%	10.5%	78.1%
	Len>12 months	6	5.1%	2.1%	31.7%	29.6%	12.2%	94.8%
Tot		17						
Myeloid cells			CD13+	Myeloid precursors	MC	MMC	PMN	
	No Len	5	48.9%	0.4%	28.4%	33.0%	21.0%	
	Len<12 months	6	55.3%	6.9%	22.9%	26.9%	16.6%	
	Len>12 months	6	54.9%	0.7%	28.3%	26.5%	26.1%	
Tot		17						

Summary and Conclusions: We were unable to identify any cellular indication that Len treated patients are more likely to develop hematologic SPMs, such as a significantly higher percentage of immature forms or the co-expression of aberrant markers. We saw no indication of immune stimulation and furthermore we were able to show that patients on Len have a lower percentage of B cells, that is proportional to the time on Len therapy.

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P811

A RETROSPECTIVE REVIEW OF THE INCIDENCE OF CONTRAST INDUCED NEPHROPATHY IN THE MYELOMA SETTING
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Background: Multiple myeloma is a malignant plasma cell disorder accounting for approximately 10% of haematological malignancies. Renal impairment is a common complication of myeloma. Myeloma patients frequently require contrast enhanced procedures given their age (average age at diagnosis is 69), co-morbidities, and their higher than normal risk of venous thrombo-embolism and second malignancies. Contrast induced nephropathy (CIN) is a reversible form of acute renal failure. The patho-physiology is not well understood but contrast is thought to lead to the precipitation of light chains leading to renal tubular obstruction. The incidence in myeloma patients is thought to be 4-8 times that of the general population. There are few dedicated studies on the subject and these focus on computed tomography (CT) only.
Aims: To investigate the incidence of CIN in myeloma patients undergoing contrast enhanced radiological procedures and to identify risk factors that predispose myeloma patients to CIN.
Methods: The medical charts of patients with a diagnosis of myeloma attending a tertiary referral centre in Ireland between Jan 1st 2007 and Dec 31st 2012 were retrospectively reviewed to ascertain the incidence of CIN, defined as an increase in creatinine >25% or an absolute increase of 44umol/L within 7 days of a contrast enhanced procedure. Patient demographics, staging and myeloma subtypes were analyzed to see if there were clear risk factors for developing CIN. Ethical approval was obtained from the ethics committee governing the institution.
Results: 217 patients with myeloma were identified. 112 patients had undergone a total of 240 contrast-enhanced procedures. 18 patients were excluded as they were on dialysis, the procedures were performed prior to myeloma diagnosis or they did not have appropriate blood tests performed. This left 94 patients and 165 procedures available for analysis. The male to female ratio was 3:2. IgG, IgA and Light chain were the most common subtypes accounting for 47%, 24% and 20% respectively. 21% were ISS stage I, 40% stage II and 18% were stage III (stage was unknown in 20%). Overall, there were 3997 months of follow-up with a median follow up of 35 months per patient (Range 1- 147). Patients had on average of 1.8 scans (Range 1-7). 86% of procedures were CTs. The remainder were venograms (8%), coronary angiograms (4%), lower limb angiograms (1%) and intravenous pyelograms (1%). 36% of pre-procedure creatinines were elevated (>104 umol/L). 2% of pre-procedure calcium levels were elevated (>2.62 mmol/L). 18/165 (11%) met the criteria for CIN. 61% (11/18) of creatinines returned to baseline within one month. Non-parametric tests were carried out to see if there was any association between baseline creatinine, baseline calcium, myeloma subtype, type of procedure and development of CIN but none were found to be significant.
Summary and Conclusions: Contrast induced nephropathy occurred in 11% of this population. CIN is important to consider before performing a contrast-enhanced procedure on a patient with myeloma as a deterioration of their renal function may significantly worsen their prognosis. The risk of CIN should not be an absolute contraindication to scanning in the appropriate clinical setting, rather a reminder to the clinician to minimize concomitant nephro-toxic agents and to give special attention to fluid balance at the time of the procedure. This is the largest series to date investigating this issue.

P812

EFFECTS OF BORTEZOMIB ON RENAL IMPAIRMENT MULTIPLE MYELOMA PATIENTS
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Background: Bortezomib was widely used on Chinese multiple myeloma (MM) patients, but its effects on renal function insufficiency MM patients were real reported.
Aims: To retrospectively analyze the clinical features of myeloma patients with renal impairment who were treatment by bortezomib-based regimens in 3 Chinese top myeloma centers.
Methods: 958 newly diagnosed symptomatic MM patients were involved in this study. Those patients were diagnosed during the year 2005-2011 in the 3 centers. Clinical characteristics of the 958 symptomatic MM patients analyzed, and divided to bortezomib-based or non-based group. The response was defined according to IMWG criteria.
Results: The median age of patients was 59 years (range, 23-88), with 583 males and 375 females (1.55:1). The most common monoclonal protein was IgG subtype (43.7%). 230 patients (24.0%) presented with renal impairment (serum creatinine >176.8 μmol/L), while the renal impairment rate in subtype IgD, λ and κ was 50%, 40.3% and 36.6% respectively. With a median follow-up of 17.3 months, the estimated median PFS and OS time were 28.3 and 61.0months, respectively. Patients who received bortezomib-based regimens demonstrate a longer estimated median PFS and OS time compared with those who received non-bortezomib-based regimens (34.4 months *vs.* 24.9 months; P<0.001; not reached *vs.* 50.7 months; P=0.005). The estimated median OS time of patients with renal impairment was shorter than that of patients with normal renal function (41.7months *vs.* 63.4 months, P<0.001), but the estimated median PFS time of these two groups didn't show statistic difference (26.7 months *vs.* 28.4 months, P>0.05). Response rate was analyzed in 680 patients who had available data. 528 patients (77.6%) achieved either a complete response (CR; 21.9%), a very good partial response (VGPR; 17.8%) or a partial response (PR; 37.9%). Patients who received bortezomib-based regimens demonstrated an improved ORR compared with those who received non-bortezomib-based therapy (84.2% *vs.* 58.3%; P<0.001). The overall response rate (ORR) in patients with renal impairment was 75.8%, with 16.1%, 13.4% and 46.3% of patients achieving CR, VGPR and PR, respectively. In this population, patients who received bortezomib-based regimens showed a better ORR compared with non-bortezomib group (84% *vs.* 58.3%).
Summary and Conclusions: The median age of Chinese MM patients is younger then the other counteies. Bortezomib-based regimens were able to improve OS and ORR in MM patients with or without renal impairment.

P813

THE MM-021 CHINA REGISTRATION TRIAL: A PHASE 2 STUDY OF LENALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE IN CHINESE RELAPSED/REFRACTORY MYELOMA PATIENTS

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Background: In China there is an unmet clinical need for the effective treatment of patients (pts) with relapsed/refractory multiple myeloma (RRMM), and multiple myeloma (MM) pts who cannot tolerate bortezomib and/or thalidomide. Previous studies have shown that lenalidomide (LEN)+low-dose dexamethasone (DEX; LoDEX) may have a better safety profile compared with LEN+high-dose DEX in newly diagnosed MM pts. It is hypothesized that LEN+LoDEX may also provide benefits in pts with RRMM. The MM-021 China Registration Trial is one of the largest studies in Chinese pts with RRMM.
Aims: The trial assessed the safety, efficacy, and pharmacokinetic (PK) profile of LEN+LoDEX in Chinese pts with RRMM.
Methods: In this phase2, multicenter, single arm, open-label study, 199 pts received LEN (25 mg/day on days 1–21) and LoDEX (40 mg on days1,8,15, and 22; or 20 mg in pts aged >75 years [yrs]) in each 28-day cycle until progression. The primary end-point was overall response rate (ORR; defined as at least partial response; based on IRAC review) and the secondary end-points included duration of response (DOR), progression-free survival (PFS), overall survival (OS), safety, and PKs. All pts provided informed consent.
Results: As of September 26, 2012, 187 pts were evaluable for efficacy and 199 pts were evaluable for safety. Median age was 60 yrs (range 35–81), 86% of pts had advanced MM (Durie-Salmon stage 3), and 57% had received ≥4 prior therapies. After a median treatment of 8 months (mos) (range 1–23), the ORR was 48% (N=187) with complete response in 4% of pts, which was consistent regardless of renal function (Table 1). Median time to first response was 2 mos (range 1–10), DOR was 9 mos (range 0.4–19), PFS was 8.3 mos (95% CI: 6.5–9.8), and 1-yr OS rate was 72%. Most common grade 3–4 adverse events (N=199) were anemia (26%), neutropenia (25%), thrombocytopenia (15%), and pneumonia (13%). Febrile neutropenia and deeP-vein thrombosis were experienced by 1 pt (0.5%) each. LEN was rapidly absorbed and eliminated (T_{max}: 0.93 [range 0.5–1.0] h; $t_{1/2}$ 3.34 [41.9] h) with no evidence of accumulation. Co-administration with DEX did not affect LEN multiple-dose PKs.
Summary and Conclusions: LEN+LoDEX was associated with a relatively high response rate and was generally well tolerated in this heavily pretreated population of Chinese RRMM pts. The PK profile was similar to that observed in studies of Caucasian and Japanese pts. LEN+LoDEX has the potential to address the unmet clinical need for effective treatment of Chinese pts with RRMM.

Table 1. Response rates in Pts receiving LEN+LoDEX.

	n (%)
Overall Response (N = 187)	89 (48)
Prior therapies (N = 199)	
1–3	86 (43)
≥ 4	113 (57)
Renal impairment (N = 199)	
None-to-mild (CrCl ≥ 60 mL/min)	131 (66)
Moderate (CrCl ≥ 30 to < 60 mL/min)	54 (27)
Severe (CrCl < 30 mL/min)	14 (7)
Patients with IgD (N = 199)	10 (5)

CrCl, creatinine clearance; IgD, immunoglobulin D.

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P814

SENSITIVE MONITORING OF V617F POINT MUTATIONS OF THE JAK2 GENE USING ULTRA-DEEP AMPLICON RESEQUENCING

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Background: Detection of minimal residual disease has become one of the most important fields of diagnostics in hematology. Whereas translocations can be sensitively monitored using Real-Time qunatitative PCR (QPCR), detection of point mutations using QPCR is much more challenging, because of unspecific background amplification. Next-Generation sequencing is a novel procedure, which not only allows genome wide analysis of genetic alterations, but also for the first time enables to study point mutations with much higher sensitivity compared to Sanger sequencing, because by increasing the number of reads per amplicon up to several 100.000 (ultra-deep sequencing; UDS) in principle enables detection of even very small clonal populations of mutant alleles. However, PCR using conventional *Taq*-DNA-polymerases is known to be error prone, thereby limiting this theoretical value to a usable sensitivity of about 0.5-1%.
Aims: To overcome this limitation and to make NGS-based single base mutation analysis with sufficient sensitivity practically available, we studied the G1849T mutations of the JAK2-gene leading to V617F as a prototypical single base pair point mutation, and we here describe an optimized procedure for the sensitive and quantitative detection of the V617F single base pair point mutations in JAK2 using NGS-based UDS.
Methods: All analyses were performed on an IonTorrent PGM semiconductor based device. Different chiP-sizes were tested, enabling read numbers between 800.000 and 7.000.000/chip. Cell dilutions of V617F-positive HEL-cells as well as normal peripheral blood cells were studied using several reaction conditions (amount of template, cycle numbers, different proof-reading and non-proof-reading enzymes). To explore the potential clinical applicability of this method, we analyzed 152 serial follow-up samples of 15 patients with JAK2-mutated diseases (OMF; n=13, sAML; n=2) undergoing allogeneic stem cell transplantation (SCT).
Results: Among the different parameters tested, especially the use of a high accuracy, proofreading enzyme significantly decreased the background of unspecific mutations and allowed to increase the sensitivity by a factor of 5-10 fold. Using cell dilutions of the HEL cell line as well as DNA from a patient with PV and homozygous JAQ2 V617F-mutation, we were able to reliably detect and quantify one mutant V617F-positive cell in a background of 10.000 cells when applying a coverage of 100.000 reads per amplicon. This sensitivity could be further improved by an increase of the read-depth. To study the clinical applicability, we analyzed follow-up samples of patients after SCT. A total of 20.000.000 reads was performed, median read depth per sample was 130.000 (range 73.000-207.000). Median follow-up was 1237 days (range 52-2697 days), 3 patients developed clinical relapse, (med. 1111 days post Tx; range 382-1292 days). UDS for JAK2 V617F detected an increase of mutant alleles in median 203 days before clinical relapse (range 176-296 days). All patients below the detection threshold of 0.01% remained in clinical remission.
Summary and Conclusions: Taken together, our results indicate that UDS for a single-base pair point mutation using next generation sequencing on a semiconductor based device is feasible with excellent sensitivity and reproducibility. Using our optimized conditions, we were able to monitor patients after SCT and show that early detection of relapse is possible. UDS thus may become an important tool to monitor MRD post treatment.

P815

TITLE: SURVEY FOR THE VALIDATION OF MODIFIED MPN-SYMPTOM ASSESSMENT FORM FOR PATIENTS WITH MAST CELL DISORDERS

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Background: Patients with Mast Cell Disorders (MCD) represent a rare, clinically heterogeneous population.Patients can be completely asymptomatic patients to those with debilitating mediator symptoms. To date there is no single validated tool available to measure the impact on the quality of life/efficacy of treatments used.
Aims: To develop a validated QOL tool for MCD patients.
Methods: This is a collaborative study between Stanford University(USA)and Guys & St Thomas' Hospital (UK). The MPN-SAF TSS 10 form is a validated QOL tool standard used in clinical practice (Emmanuel *et al.*). We modified the MPN-SAF TSS by adding 12 MCD specific questions. Each question was given a rating score of 0-10. Specific questions were asked about skin symptoms and anaphylaxis. A free text question regarding whether the survey addressed all symptoms for MCD and /comments was added. We included the validated Fatigue Inventory (Cleeland: MD Anderson).Ethics approval was granted to carry out a validation survey of this anonymised electronic questionnaire. The survey was presented to the UK and USA SM patient support groups in October and November 2012. The questionnaire takes 15-20 minutes to complete.
Results: Data collection: October 2012– January 2013. Total number of completed responses=258 (UK 78;SA 171;Other 9).M:F ratio 1:5.8. Median age of responder 48.7 yrs (range 18-87). Patient declared diagnosis: Cutaneous Mastocytosis (13%), Indolent SM (34%), Smouldering SM (3%) ASM (4%), SM-AHNMD (4%), Mast Cell Activation Syndrome (26%), don't know (7%) and other (9%). Skin Symptoms: 62% (159/258) patients had skin symptoms. Treatment modalities used: topical steroids, Epsom salts, PUVA, ice, laser treatment, surgical removal, cauterisation, antifungal and antibiotic creams and anti- histamines. Anaphylaxis: 44% (114/258) patients reported anaphylactic reactions; 50% (57/114) had been hospitalised as a result in the 6 months proceeding the time of questionnaire, 43% (49/114) of these patients needed to use epipens over the proceeding 12 months (median number of injections 2–range 1-10). Treatment modalities reported where complex and multiple with the majority of patients requiring between 4–12 combination drugs (H2, H1 antagonists, mast cell stabilisers, Leucotriene inhibitors, antiepileptics, sedatives and neuroleptics to manage symptoms.
Summary and Conclusions: This is the largest cohort of Mast Cell Patients completing a single validation survey. The data confirms the significant burden of MCD on their quality of life. This preliminary data on TSS and MCD symptom scores is being analysed and suggestions by the patients from this survey have been incorporated in an updated version. Further detailed analysis is also being carried out to compare the TSS scores with related MPN patients.

P816

LONG TERM FOLLOW-UP OF TREATMENT WITH IMATINIB IN EOSINOPHILIA-ASSOCIATED MYELOID/LYMPHOID NEOPLASMS WITH PDGFR REARRANGEMENTS IN ADVANCED PHASE

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Background: On treatment with imatinib, the vast majority of eosinophilia-associated myeloid/lymphoid neoplasms (MLN-eo) with *PDGFR* rearrangements in chronic phase achieve rapid and durable complete hematologic (CHR) and also complete molecular remissions (CMR).
Aims: We here report on long-term follow-up of 17 patients with advanced disease which was defined as i) myeloid blast phase [MBP, >20% blasts in peripheral blood (PB) and/or bone marrow (BM)], ii) extramedullary tissue infiltration by myeloid blasts (chloroma) or iii) lymph node (LN) infiltration by lymphoblasts of T-cell origin (lymphoid BP, LBP).
Results: 13 patients were *FIP1L1-PDGFRA* (FP) positive (MBP, n=8; chloroma, n=2; LBP, n=3). In 3 patients, *FP* could be identified contemporaneously in myeloid cells obtained from BM and CD3-positive lymphoblasts obtained from LN. 4 patients (MBP, n=2; LBP, n=2) were positive for a rearrangement of *PDGFRB* (partner genes: *ETV6*, n=1; *DTD1*, n=1; *SART3*, n=1; unknown partner gene, n=1). All patients were male (median age, 46 years, range 37-66). Significant eosinophilia >1.5×10^9/l was present in 12 of 13 (92%) *FP*-positive patients and 2 of 4 (50%) patients with *PDGFRB* fusion genes. The most common clinical features included splenomegaly (17/17) and organ involvement of skin (4/17), lung (4/17) and heart (3/17). Without primary knowledge of the underlying fusion gene, 9 patients received intensive chemotherapy. Despite clear signs of remission, *e.g.* clearance of blasts, eosinophilia persisted in all 9 patients. The underlying fusion genes (*FP*, n=6; *X-PDGFRB*, n=3) were subsequently identified and imatinib was initiated in 7 of 9 patients. At diagnosis of MBP, *FP* was identified in 8 patients and imatinib was initiated directly. Overall for the 15 imatinib-treated patients (starting dose 400mg/d, n=6; 100mg/d, n=9), CHR was achieved in every case after a median of one month (0.1-15). CMR was detected in all 12 *FP* positive patients after a median of 5.4 months (2.9-32.0). One patient died due to a cerebral hemorrhage while in CMR for 8.6 months. The remaining 11 patients are in sustained CMR for a median of 65 months (7-103). The 3 patients with a *PDGFRB* fusion gene are in sustained CHR for median 56 months (24-56). Two patients (*FIP1L1-PDGFRA*, n=1; *ETV6-PDGFRB* plus complex karyotype, n=1) received an allogeneic stem cell transplantation (SCT) within 6 months (related donor, n=1; unrelated donor, n=1) after diagnosis of MBP but both patients relapsed within the first 3 months. Following relapse, the *FP* positive patient was treated with imatinib and is in sustained CMR up to 236 days after SCT. The *ETV6-PDGFRB* positive patient relapsed 4 weeks after SCT with leptomeningeal

involvement. On imatinib, he achieved CHR and CMR in PB and BM but subsequently died due to progressive leptomeningeal involvement.
Summary and Conclusions: In conclusion, our data highlight several new aspects for diagnosis and treatment of MLN-eo with *PDGFR* fusion genes: i) mimicking *de novo* AML, blast phase of MLN-eo with an underlying imatinib-sensitive *PDGFR* fusion gene is frequently not identified, ii) in the absence of *CBF* fusion genes, patients with eosinophilia and increased numbers of blasts should be actively screened for *PDGFR* fusion genes by cytogenetics, FISH and/or PCR and iii) our excellent long-term data suggest that monotherapy with imatinib should be initiated early because durable remissions can not be achieved through intensive chemotherapy or allogeneic SCT.

P817

JAK2V617F MONITORING IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA: CLINICAL USEFULNESS FOR PREDICTING MYELOFIBROTIC TRANSFORMATION

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Background: The JAK2 V617F allele burden, assessed at a single time-point either at diagnosis or during follow-up, has been related with the risk of thrombosis and disease transformation in polycythemia vera (PV) and essential thrombocythemia (ET). However, the JAK2 V617F allele burden does not remain stable in all cases, since it can be modulated by cytoreductive therapy or by the natural clonal evolution of the disease.
Aims: To evaluate the clinical usefulness of monitoring the JAK2 V617F allele burden for predicting disease transformation in patients with PV and ET.
Methods: JAK2V617F monitoring was performed by quantitative allele specific PCR in the peripheral blood granulocytes from 346 patients (PV=162, ET=184). Median time from diagnosis to first JAK2V617F quantification was 10 months, with 196 patients (80%) starting on molecular monitoring in the first year after diagnosis. Median molecular follow-up was 2.3 years (range: 0.5- 12). In each patient, the evolutionary pattern of the JAK2V617F allele burden was stratified as following: stable <25%, stable 25% to 49%, stable 50% to 74%, stable ≥75%, progressive increase and unexplained decrease. Patients receiving cytoreductive therapy were assigned to their corresponding category after achieving a steady state (usually after 6-12 months of therapy). The probability of disease transformation (myelofibrosis and acute leukemia) according to the evolution of the JAK2V617F allele burden was calculated by the Kaplan-Meier method.
Results: Patients were stratified according to their JAK2V617F evolutionary patterns as follows: stable <25% (n=162, PV 45, ET 117), stable 25% to 49% (n=97, PV42, ET 55), stable 50% to 74% (n=25, PV 23, ET 2), stable ≥75% (n=26, PV 25, ET1), progressive increase (n=25, PV 17, ET 8) and unexplained decrease (n=11, PV10, ET 1). Twenty-six patients evolved into myelofibrosis (18 post-PV, 8 post-ET) at a median of 9 years after diagnosis. The pattern of the JAK2V617F allele burden in these 26 patients was stable <50% (n=3), stable ≥50% (n=7), progressive increase (n=12) and unexplained decreased (n=4). The probability of myelofibrosis at 10 years from diagnosis was 0% in PV patients with a stable JAK2V617F mutational burden below 50% in comparison with 21% in the combined group of PV patients showing a JAK2V617F stable ≥50%, a progressive increase or an unexplained decrease of the JAK2V617F (P=0.001). In ET, the probability of myelofibrosis at 10 years was 4.5% in patients with stable JAK2V617F below 50% in comparison with 31% in cases with stable JAK2V617F >50%, progressive increase or unexplained decrease of the JAK2V617F (P<0.001). Five patients with ET fulfilled PV criteria during follow-up, with 3 of them having a documented progressive increase in the JAK2V617F mutational load prior to PV transformation. Acute leukemia developed in 9 patients. No correlation was observed between the JAK2V167F evolutionary pattern in the chronic phase and leukemic transformation.
Summary and Conclusions: Quantitative JAK2V617F monitoring is useful in the clinical follow-up of patients with PV and ET for predicting myelofibrotic transformation.

P818

CLINICOPATHOLOGICAL CHARACTERISTICS OF ADULT PATIENTS WITH ABNORMAL MAST CELLS WHO DO NOT MEET WHO DIAGNOSTIC CRITERIA FOR CUTANEOUS OR SYSTEMIC MASTOCYTOSIS

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Background: Current diagnostic criteria for systemic mastocytosis (SM) require meeting one major (aggregates of ≥15 mast cells [MC]) and one minor, or at least three minor criteria (>25% MC are spindled, presence of *KIT*D816V mutation, MC expression of CD25 or CD2, and serum tryptase level >20 ng/mL) in extra-cutaneous organs. Routine screening of patients suspected of SM has revealed a subgroup with sub-diagnostic involvement (ie. satisfy only 1-2 minor criteria). The latter group has hitherto not been systematically studied.
Aims: To describe the clinicopathological characteristics of patients with sub-diagnostic systemic involvement with abnormal MC, and to compare the findings to patients with indolent SM.
Methods: We retrospectively studied consecutive patients suspected of SM on the basis of characteristic symptoms, who were found to satisfy only 1 or 2 minor criteria for SM. Since transient elevation of tryptase level >20 ng/mL can be seen after activation of normal MC, we did not count this as a minor criterion for this study. Patients with characteristic skin involvement by mastocytosis were excluded. Bone marrow (BM) histology was centrally reviewed (DC, CAH); MC number and morphology was studied by tryptase/CD117 stain and MC CD25/CD2 expression by immunohistochemistry and/or flow cytometry. Presence of *KIT*D816V was studied on BM aspirate by allele-specific PCR (≤0.01% sensitivity).
Results: Twenty one patients (62% female; median age at diagnosis 48 years) were included in the study. Per definition, none of the patients exhibited BM MC aggregates; the MC were distributed singly and the overall BM involvement was minimal (≤1%) in all cases. Fifteen patients (71%) satisfied one SM minor criterion only (CD25/CD2 expression=9; *KIT*D816V=6). The remaining 6 patients satisfied 2 minor criteria (*KIT*D816V plus CD25/CD2 expression=4; >25% spindled MC plus either *KIT*D816V or CD25/CD2 expression=1 each). The median time from symptom onset was 60.1 months (range 2-363.6). Five patients (24%) reported prior anaphylaxis episode(s); prevalence of symptoms was as follows: dermatological (hives, flushing, pruritus) 86%, cardiovascular (palpitations, chest pain, syncope) 76%, gastrointestinal (nausea, vomiting, diarrhea, abdominal cramps) 57%, and neurological (headache, cognitive difficulties, paresthesias, vertigo) 48%. The median hemoglobin level was 14.5 g/dL (range 10.2-16.6), leukocyte count 7.1×10^9/L (range 4.3-11.1) and platelet count 261×10^9/L (119-400). The baseline serum tryptase level was 10.6 ng/mL (range 2.7-23.4). A tryptase level above normal range (≥11.5 ng/mL) and >20 ng/mL was noted on at least one occasion in 61% and 37% of patients, respectively. Abnormal MC were identified most frequently by MC immunophenotyping (*n*=17; 82% positive), followed by *KIT*D816V (*n*=18; 61% positive). MC morphology was relatively insensitive, with only 2 patients (10%) exhibiting >25% spindled cells in the current cohort; no patient was identified on the basis of MC morphology alone.
Summary and Conclusions: The current data reveal a high symptom burden despite minimal BM involvement with abnormal mast cells in patients not meeting the threshold for systemic mastocytosis. Mast cell immunophenotyping and *KIT*D816V mutation analysis are key screening tests since they identified most cases. In contrast, baseline serum tryptase was normal in 58% cases and >20 ng/mL in only 11%. Similarly, assessment of mast cell morphology was of limited utility in this setting. These data if confirmed independently may influence future diagnostic criteria for systemic mastocytosis.

P819

HOW MANY CHILDREN WITH THROMBOCYTOSIS OF "UNDEFINED ORIGIN" HAVE A MYELOPROLIFERATIVE NEOPLASM?

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Background: Chronic Myeloproliferative Neoplasms (MPN) are clonal diseases of middle-advanced age while they are extremely rare in pediatric patients.*JAK2*V617F mutation is the main molecular marker of MPN, occurring in the great majority of patients: 95% of patients with polycythemia vera (PV) and 50-60% of those with essential thrombocythemia (ET)andmyelofibrosis (PMF).The rare cases of children with MPN seem to have different biological characteristics in comparison to adult MPN, but few data are published on this subject.
Aims: In this study, we report our experience in a large cohort of pediatric patients with high platelet count in whom *JAK2*V617F and *MPL*mutations have been searched.
Methods: We report 86 children with sustained thrombocytosis (>600×10^9/L) over at least 6 months, in the absence of recognizable reactive or secondary cause, followed in one of the Centers linked to the Italian Pediatric Hemato-Oncology Association. There was no family history of MPN. The genotyping of the *JAK2*V617F mutation was performed by allele-specific PCR and the mutant allele-burden was measured by quantitative real time-polymerase chain reaction assay.*MPL*mutations were searched by direct sequencing. Clonality was studied on females according to HUMARA method.Differences in the distribution of continuous variables between categories were analyzed by the Mann-Whitney test.

Results: *JAK2*V617F mutation was found in 11 (13.4%) patients (allele burden 26.26±9.78%).Mutations in the *MPL* gene were searched in 57 patients with thrombocytosis and one (1.7%) had a somatic mutation (W515L). Clonality was available in 21 females and 6 of them (26.5%) resulted monoclonal (2 carrying JAK2 mutation). On a whole 18.6% of patients had a clonal disease, while other 74 patients had a thrombocytosis of undefined origin. In the Table 1 the hematological data of the patients are summarized.

Table 1.

	Clonal		Non Clonal		
	Median	Percentile range, 5th to 95th	Median	Percentile range, 5th to 95th	P
Plts (x 10^9/L)	1027	481 – 2370	1078	447,7 – 3156,35	n.s.
WBC (x 10^9/L)	7,89	5,5 – 11,8	9,15	5,3125 – 22,33	n.s.
Ht (%)	39,45	28,4 – 45	33	12,21 – 41,17	n.s.
Hb (g/dL)	13,6	9,3 – 14,4	12,3	7,1 – 14,3	n.s.

Summary and Conclusions: Our data confirm that clonal ET is rarely found even in children with sustained, prolonged thrombocytosis without any recognizable cause. No significant hematological difference was observed between patients with a sure MPN and those with an undefined thrombocytosis. *JAK2*V617F mutation is uncommon and other mutations such as *MPL*W515L are anecdotal. If clonal ET in pediatrics would represent 50-60% of all ET as it is in adults, then additional 40% of children would be found within our series, their number adding at the most to 10 children. Therefore, all other studied patients could be "undefined thrombocytosis" and their diagnostic process and follow-up need to be further established.

P820

IMPACT OF CYTOREDUCTIVE THERAPY ON THE OUTCOMES OF PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS AND HEPATO-SPLANCHNIC VEIN THROMBOSIS

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Background: Myeloproliferative neoplasms (MPN) are the commonest cause of splanchnic vein thrombosis (SVT), their prevalence being approximately 30% in portal vein thrombosis (PVT) and 40% in Budd-Chiari syndrome (BCS). There is no consensus regarding the use of cytoreductive therapy in patients (pts) with MPN and splanchnic vein thrombosis (SVT), these pts often presenting with normal blood counts.
Aims: Search for prognostic factors predicting the risk of achieving major vascular or liver-related events after the diagnosis of SVT, and assess the impact of cytoreductive therapy on the occurrence of such major events.
Methods: Retrospective observational study of SVT pts jointly followed in 4 hematology and hepatology reference centers for MPN and SVT. Inclusion criteria were: a diagnosis of BCS or PVT according to previously published criteria; evidence for an underlying MPN defined by the presence of the JAK2V617F mutation and/or clear bone marrow histopathological features; exclusion of associated malignancy. The composite endpoint of the study included major vascular events (thrombosis including new or extension of SVT and thrombosis in other territories, hemorrhage), liver-related complications (refractory ascites; hepatic encephalopathy; hepato-renal syndrome; liver transplantation) and death. Time to event was measured from the date of SVT diagnosis to the date of first major event. Estimated probabilities of EFS were calculated using the Kaplan-Meier method, and the two-tailed log-rank test evaluated differences between survival distributions
Results: 109 pts were included with a median follow-up of 4.4 years (range: 1 month - 27.4 years), including 46 BCS pts (median follow-up: 5.2 years) and 63 PVT pts (median follow-up: 3.6 years). Among the BCS pts, 33 (72%) experienced at least one major event. Median event free survival (EFS) from diagnosis of SVT was 9.4 months (IC95: 4.1-36.4). Cytoreductive therapy (P<0.0001), platelet count above 250×10^9/L at time of BCS (P=0.099) and gender (P=0.110) were the 3 variables associated with a higher risk of major event after BCS in univariate analysis. Absence of cytoreductive therapy after BCS diagnosis was the only independent prognostic factor associated with an increased risk of subsequent major event (P<0.0001).Median EFS was 59.8 months (IC95: 35.9-NA). Initiation of cytoreductive therapy after the diagnosis of PVT was significantly associated with a lower risk of subsequent major event (P=0.011) in univariate analysis, as the presence of esophageal varices of grade <2 (P=0.117). In multivariate analysis, only cytoreductive therapy remained an independent prognostic factor (P=0.048).
Summary and Conclusions: This study provides the first evidence for a beneficial role of cytoreductive therapy in SVT patients with underlying MPN, in particular for reducing the risk of severe liver-related complications and significantly improving EFS in both BCS and PVT, regardless of MPN characteristics. Confirmation in a larger study will require international collaboration due to the rarity of this type of thromboses.

P821

CLINICAL FEATURES OF PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS COMPLICATED BY PORTAL HYPERTENSION

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Background: Portal hypertension (PHTN) is a rare complication of Philadelphia-negative myeloproliferative neoplasms (MPNs) that can result in significant morbidity. There is a paucity of published data in this area, with most of the current literature taking the form of anecdotal case reports/series, the largest of which has 13 patients. PHTN is currently reported in 7-18% of patients with MPNs.
Aims: The main objective of this study is to understand the clinical features and natural history of patients who have been diagnosed with Philadelphia-negative MPNs and PHTN at our participating institutions.
Methods: This study was approved by the ethics committees at all of the participating institutions. Twenty-nine patients with the diagnosis of both MPN and PHTN were identified from databases at Princess Margaret Hospital and Sunnybrook Health Sciences Centre in Toronto, Canada. The MPNs included polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), post-PV myelofibriosis (PPV-MF) and post-ET myelofibrosis (PET-MF). Patients were confirmed to have the diagnosis of PV, ET and PMF based on WHO criteria, and PPV-MF and PET-MF based on IWG-MRT criteria. PHTN was defined as the presence of ascites and/or varices in the absence of a known cause. A detailed chart review was performed, and the data was abstracted into a case report form designed for this study.
Results: Among the 29 patients (52% male) in the study group, the etiology of the MPNs consisted of 11 patients with PMF (38%), 9 PV (31%), 7 PPV-MF (24%), 1 ET and 1 PET-MF. The median follow up of survivors was 81 months (range 12–168 months). JAK2 V617F (JAK2) mutation status was available on 28 patients, of which 22 patients (79%) had mutated JAK2. JAK2 was positive in 15 of 16 patients with the diagnosis of PV and PPV-MF (94%). After excluding PV/PPV-MF patients, JAK2 was positive in 58% of patients with PMF, ET and PET-MF. Among the 19 myelofibrosis patients (including PPV-MF and PET-MF), DIPSS score at PHTN diagnosis was: low risk, 4; intermediate-1, 8; intermediate-2, 3; and high risk, 4. At the diagnosis of PHTN, 10 patients presented with ascites, 11 patients with varices, and 8 patients had both. Of the19 patients with varices, 32% presented with gastrointestinal bleeding, whereas the others were found on screening. Among the 29 study patients, 17 patients (59%) had a history of splanchnic vein thrombosis (SVT). The incidence of SVT was significantly higher in patients with PV/PPV-MF compared to PMF (81% *vs.* 27%, P=0.01). Of the 19 patients with myelofibrosis, 9 have died, whereas only 1 patient with PV/ET has died. The causes of death include 4 with blastic transformation, 3 secondary to PHTN, 2 with sepsis, and 1 secondary to hepatocellular carcinoma.
Summary and Conclusions: In summary, PHTN appears to be most prevalent in patients with PV/PPV-MF compared to other classical MPNs with ET being the least common. The etiology of PHTN in PV patients appears to be secondary to a thrombotic event in the splanchnic circulation, whereas non-thrombotic etiology appears most prevalent in PMF patients. These results are useful in the planning of screening strategies for portal hypertension in MPN patients, and provide some clues towards understanding the mechanisms of PHTN in patients with MPNs. Updated data on 55 patients will be presented at the EHA meeting by inclusion of cases from Mayo Clinic, Scottsdale and Medical College of Wisconsin.

P822

EOSINOPHILIA IN ROUTINE BLOOD SAMPLES AND THE SUBSEQUENT RISK OF HEMATOLOGICAL MALIGNANCIES AND DEATH

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Background: Blood eosinophilia may represent an early paraclinical sign of hematological malignant disease. No reports exist on the predictive value of eosinophilia for hematological malignancies. Such a study may prove helpful for physicians to interpret eosinophilia and aid them to make early diagnosis and targeted treatment of underlying morbidities.
Aims: The aim of the present study was to investigate eosinophilia in routine blood samples as a potential risk marker for the development of hematological malignant disease and death using a comprehensive primary care cohort.
Methods: From the Copenhagen Primary Care Differential Count (*CopDiff*) database, we identified 359,950 individuals with at least one differential cell count (DIFF) from 2000-2007. From these, one DIFF was randomly chosen from each individual. The individuals were categorized as no ($<0.5 \times 10^9$/l), mild ($\geq 0.5–1.0 \times 10^9$/l) or severe eosinophilia ($\geq 1.0 \times 10^9$/l). From the Danish Cancer Registry and the Danish Civil Registration System we ascertained hematological malignancies and death within three years following the DIFF. From the Danish National Patient Register we computed Charlson´s comorbidity Index (CCI) within three years before the DIFF. Using multivariate logistic regression odds ratios (ORs) were calculated and adjusted for previous eosinophilia, sex, age, year, month, CRP, previous cancer and CCI. *P*-values less than 0.0271 were considered to be significant as this controls the false discovery rate at 5% using the method of Benjamini-Hochberg.
Results: In the total cohort of 359.950 individuals from a primary care setting there was an equal sex-distribution and a mean age of 48.3 years. 14.406 individuals (4%) exhibited eosinophilia. The incidence of hematological cancer was 89/100.000 person-years. ORs for developing Hodgkin´s disease (HD) was significantly increased in patients exhibiting severe eosinophilia, OR=9.09 (2.77-29.84), P=0.0003, but not in cases with mild eosinophilia and no association was found with non-Hodgkin lymphoma. The association with classical myeloproliferative neoplasms (cMPNs) also showed an increasing risk with eosinophilia with OR=1.65 (1.04-2.61) P=0.0322 and OR=3.87 (1.67-8.96) P=0.0016) for mild and severe, respectively. Eosinophilia was also associated with chronic lymphatic leukaemia (CLL). OR=2.57 (1.50-4.43), P=0.0006 and OR=5.00 (1.57-15.94), P=0.0065 for mild and severe eosinophilia, respectively. Finally, eosinophilia was associated with all-cause death with OR of 1.16 (1.09-1.24), P<0.0001 and 1.60 (1.35-1.91), P<0.0001, for mild and severe eosinophilia, respectively.
Summary and Conclusions: In a large cohort resistant to surveillance bias, and with an incidence of hematological malignancies which is compatible to the reported national incidence in Denmark, we confirm known associations between eosinophilia and HD and cMPNs. In addition we demonstrate for the first time that eosinophilia *per se* is a risk marker for both CLL and death. Therefore, a subclinical hematological malignancy should be considered in case of unexplained eosinophilia. The question whether the eosinophil is an effector cell in the evolution of CLL remains open, and this is a study question that deserves consideration in future preclinical and prospective clinical trials.

P823

CHRONIC MYELOPROLIFERATIVE NEOPLASMS REVEAL INCREASED RISK OF OSTEOPOROTIC FRACTURES

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Background: The classical chronic myeloproliferative neoplasms (CMPNs) are chronic hematological malignancies that originate at the level of the pluripotent stem cell. ET and PV are often viewed as part of the same biological continuum, rather than separate diagnostic entities (1). We have previously shown that CMPN patients are at higher risk of femoral fractures than the general population but the risk of other osteoporotic fracture types is unknown (2). We hypothesize that bone modelling rate gets disturbed due to a chronic inflammation analog to patients with mastocytosis. Corresponding to the biological continuum, the classic CMPNs reflect a stepwise increase of the inflammation from ET to PV (3).
Aims: With this study we assess the risk of osteoporotic fractures at several anatomical sites including distal antebrachium, humerus and proximal femur.
Methods: We conducted a population-based cohort study of the risk of osteoporosis among patients with ET and PV using data from the Danish health care system. Patients were identified from Danish National Registry of Patients (DNRP), and linked to the Danish Civil Registration System (CRS) in the study period 1 January 1995 to 31 December 2010. Each Danish resident has a unique, permanent 10-digit civil registry number allowing unambiguous individual-level linkage among all Danish registries. Patients with a first-ever CMPN diagnosis in the DNRP were identified by means of their ICD-8 diagnosis code until 1994 and ICD-10 diagnosis code thereafter. By this means, we established two distinct cohorts of ET and PV patients. For each CMPN patient, 50 general population comparison cohort members without CMPN were identified in the CRS matched on age, sex, and calendar year, creating two separate comparison cohorts. Follow-up started 1-year from the date of diagnosis for CMPN patients and until first fracture of prox. femur, humerus or distal antebrachium. The comparison cohort members were assigned the same index date as their index CMPN case. Patients and comparison cohort members with a previous diagnosis of osteoporosis or osteoporotic fractures were excluded. Cox regression was used to estimate hazard ratios (HRs) as a measure of relative risk of fracture for each CMPN cohort compared to the comparison cohort, adjusted for comorbidity and diagnoses related to alcohol abuse.
Results: We identified1,644 ET patients and 70,685 matched comparison cohort members, as well as2,189 PV patients and 70,685 matched comparison cohort members. Figure1 shows the relative fracture rates for prox. femur, humerus and distal antebrachium.
Fracture rates of:
- Proximal femur: HR_{ET}=1.16 (95%CI: 0.9-1.5) and HR_{PV}=1.53 (95%CI: 1.3-1.9).
- Humerus: HR_{ET}=1.05 (95%CI: 0.7-1.6) and HR_{PV}=1.33 (95%CI: 1.0-1.8).
- Distal antebrachium: HR_{ET}=1.14 (95%CI: 0.9-1.5) and HR_{PV}=1.31 (95%CI: 1.0-1.7).

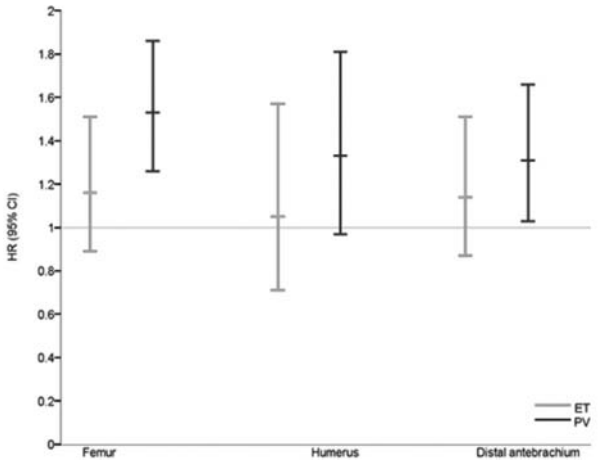

Figure 1. HR and corresponding 95% CI for femur, humerus and distal antebrachium.

Summary and Conclusions: CMPN patients are at higher risk of osteoporotic fractures in femur, humerus and distal antebrachium than the general population. Fracture rates increase in a steP-wise pattern from ET to PV.
Adjustment for comorbidity and diagnoses related to alcohol abuse increases fracture ratio, and does therefore not explain the increased fracture rate in CMPN patients.

P824

DETERMINANTS AND FREQUENCY OF THROMBOTIC AND BLEEDING COMPLICATIONS IN AN ITALIAN COHORT OF 88 PATIENTS WITH PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS (MPN): THE ROLE OF ADAMTS-13 AND VWF ACTIVITIES

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Background: Patients with Ph-negative Myeloproliferative Neoplasms (MPN) such as Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF) can be exposed during the course of these MPN to thrombotic and bleeding complications, with increased morbidity and mortality. Age, previous history of thrombosis, increased White Blood Cell (WBC) and Jak2 allele burden have been proposed as risk factors for Venous (VTE) and Arterial (ATE) thromboses while bleeding has been previously associated with abnormalities of the von Willebrand factor (VWF).
Aims: to investigate any significant role of ADAMTS-13 and VWF activities in the thrombotic and bleeding complications observed in a small but well characterized cohort of MPN patients.

Methods: 88 consecutive patients were diagnosed at the Hematology and Transfusion Medicine Division, L.SACCO University Hospital of Milan, according to WHO criteria. Patients signed an informed consent to participate in this clinical study with a protocol approved by local IRB and they showed the following features: [MPN type (%), mean age (range), gender M/F and Jak2 positivity (%)]: PV[n=42 (48%), 68 (36-86), 18/24; 85.7%]; ET [n=34 (38%), 66 (30-93), 10/24, 61.7%]; PMF [n=12 (14%), 67 (37-88), 7/5, 58%]. Thrombotic and bleeding episodes were recorded and managed from the time of diagnosis and associated with the use of aspirin (ASA) and of other MPN therapies. Among additional lab parameters, plasmatic ADAMTS-13 and VWF activities were also measured at enrolment as endothelial/platelet marker. These activities were assayed with Technozym ADAMTS-13 activity (Technoclone GmbH, Austria), Innovance VWF-GPIb activity (Siemens AG, Germany) and HemosIL-VWF antigen (Instrumentation Laboratory, USA). Multimeric analyses were also tested using very sensitive intermediate SDS-agarose gel electrophoresis. Statistical analyses were performed by SPSS-17.2
Results: 59/88 (67%) patients did not show any thrombotic or bleeding complications during the 6-year follow-up. In these cases mean (range) values of VWF:GPIb and VWF:Ag were 104 (29-202) and 133 (52-288) U/dL while ADAMTS-13 was 102 (63-143). 20/88 (23%) cases showed at least one thrombotic event (13ATE/7VTE): AMI (6), STROKE (6), TIA (2), PE (1), DVT (7). Patients with thromboses showed relatively higher values VWF:GPIb and lower ADAMTS-13 and this was confirmed in multivariate analysis especially for ET [VWF:GPIb=135 (61-237) U/dL, P=0.004 and ADAMTS-13=89(62-134), P=0.009]. Major bleeding episodes mainly mucosal (5 gastrointestinal, 3 post-surgery, 1 severe menorrhagia) requiring blood transfusions or hysterectomy were observed in 9/88 (10%) patients. At the multivariate analysis, major bleedings were significantly associated with lower VWF:GPIb [68 (25-111) U/dL, P=0.022), lower VWF:Ag [93 (35-146) U/dL, P=0.016] and to the ASA intake (P=0.006). Most of these bleeders showed also a relative loss of the highest molecular weight multimers.
Summary and Conclusions: Based on these observations, we confirm that thrombotic events in MPN may certainly have multiple risk factors: however, lower ADAMTS-13 and higher VWF activities might play a role as additional risk factors especially in ET. Conversely, lower levels of VWF with loss of the largest multimers are important risk factors for bleeding in MPN especially in patients treated with ASA.

P825

INTERNATIONAL PROGNOSTIC SCORE FOR ESSENTIAL THROMBOCYTHEMIA (IPSET) AND IPSET-THROMBOSIS–ONE CENTRE ANALYSIS
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Background: In order to homogenize the approach to patients (pts) with essential thrombocythemia (ET), it was recently elaborated the IPSET model, that takes into account only 3 parameters: age ≥60 years-old (2), leukocytosis ≥11×10^9/L (1) and prior thrombosis (PT) (1). The sum of points obtained stratifies pts into 3 risk categories: low (0), intermediate (1-2) and high (3-4). Later, another model was proposed: the IPSET-thrombosis (IPSET-T), taking into account 4 parameters: age ≥60years-old (1), PT (2), cardiovascular risk factors (CRF) (1) and JAK2V617F mutation (2). The sum of points obtained also stratifies pts into 3 risk categories: low (<2), intermediate (2) and high (>2).
Aims: To apply the IPSET and IPSET-T models to 145 pts diagnosed with ET between 2000-2010.
Results: The median age at diagnosis was 62years-old [20;88] (53.1% had ≥60years-old), with a predominance of females (60.7%) and a median follow-up of 53.5months [2;154]. Nineteen (17.6%) pts had PT and 46 (42.6%) had CRF [mostly diabetes (30.6%), hypertension (11.1%) and hypercholesterolemia (17.6%)]; it was observed anemia (hemoglobin <12g/dL-women; <13g/dL-men) in 20pts; and leukocytosis in 34. Out of 84pts submitted to the analysis, the JAK2V617F mutation was present in 49 (45.4%); this showed a correlation with PT (P=0.002), age ≥60years-old (P=0.016), anemia (P=0.004) and leukocytosis (P=0.011). IPSET stratification was possible in 92pts; IPSET-T in 84pts; and both in 68pts (due to missing data). There was an even distribution between the 3 IPSET risk groups [low (32.6%), intermediate (39.1%) and high (28.3%)] but not so much for the IPSET-T [low (39.3%), intermediate (17.9%) and high (42.9%)]. Almost all (94.4%) pts in the high risk IPSET group were JAK2V617F-positive (P=0.003); and 83.3% of the pts without leukocytosis were in the low risk IPSET-T group (P=0.01). Therefore, the 2 models were strongly correlated: 94.4% of the pts with high IPSET risk were also high risk according to the IPSET-T model (P<0.0001). 80% of the patients with anemia were in the intermediate/high IPSET risk group (P=0.023), but 66.7% of them were in the low risk IPSET-T group (P=0.043).Only 2pts had a thrombotic event after diagnosis: 1 had intermediate risk IPSET and both of them had high risk IPSET-T; both were JAK2V617F-positive. Fifteen (10.3%) patients died, 1 by progression to acute leukemia (intermediate IPSET risk; high IPSET-T risk), 2 due to secondary malignancies (breast cancer) and the remaining by infection/ complications of their other co-morbidities. The 100-month overall survival (OS) was lower for pts with ≥60years-old (63.4% *vs.* 98.1%; P=0.001) and anemia (50.5% *vs.* 89.7%; P=0.003). There was a tendency towards worse OS in the high-risk IPSET group, but statistically significant only when compared directly to the low risk group (100-month OS: 60.2% *vs.* 96.6%; P=0.049). We could not analyse OS for the IPSET-T group, since all of them are still alive. There were no differences in OS in any of the remaining variables tested, including JAK2V617F mutation.
Summary and Conclusions: In this series, the 2 models seemed to be comparable. However, we were not able to validate the risk groups defined by IPSET and IPSET-T due to the limited number of pts and thrombotic events. It is emphasized that the presence of anemia (not incorporated in any of the models; and not correlated to a specific risk group, demonstrating its independent value) may play a role in stratifying pts with TE.

P826

BCR/ABL EXPRESSION IN CHRONIC MYELOPROLIFERATIVE DISORDERS
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Background: We have found earlier (EHA 2010, #1504) a high frequency of BCR/ABL gene expression in selected group of pts with chronic myeloproliferative disorders (CMDs) having long lasting disease duration (more than 5 years after diagnosis) and with clear signs of disease progression and resistance to conventional therapy of INFa+HU. Our findings suggest that BCR/ABL gene emergence due to diseases continuation may play a crucial role in CMD progression. Recently a number of papers were published reporting a few cases of simultaneous persistence of Jak2 V617F and BCR/ABL-positive clones in the same patients having controversial diagnosis of CML or one of CMDs. We believe that phenomenon of imultaneous existence of these two molecular markers and two corresponding tumor clones in one patient with myeloproliferative disorder may sometimes course a difficulty for diagnosis and for choosing correct therapy approach. It seems that this phenomenon still require further elucidation. One more issue that has yet to be solved is discrepancy of incidence of Jak2 V617F in CML and BCR/ABL in CMD. The latter is obviously more frequent than the former. We suppose that BCR/ABL gene emergence in CMD is due to activation of V(D)J recombination by Jak2 V617F mutation.
Aims: To compare frequency of BCR/ABL expression in primary CMD pts and CMD pts with long disease duration and progression. The other goal was to evaluate gene expression of V(D)J components RAG1 and RAG2 in Jak2 V617Fpositive granulocytes and in granulocytes of normal donors.
Methods: To perform this study we have exploited qualitative and quantitative PCR detection of BCR/ABL fusion gene expression by means of RT PCR and RQ PCR. We have analyzed gene variants responsible for p190, p210 and p230 types of BCR/ABL protein synthesis. We have also performed detection of Jak2V617F, Jak2-ex12 and MPLW515L/K using AS PCR and direct PCR fragment. Allelic Jak2V617F burden was tested by RQ PCR. After receiving informed consents blood samples (N=175) of CMD pts with unfavorable clinical course and of primary CMD pts (N=67) were collected.
Results: We have examined 175 pts with CMD in progression (35(20%) - PV, 38(22%) - ET, 102 (58%) -IMF). BCR/ABL expression was found in 47 of 175 (27%) CMD pts. Most of the cases were p210, only 3 were p190, p230 was not found. Jak2V617F was in 139 pts (79%), Jak2-ex12 was in 2 (1%), MPLW515L was in 1 case (0,5%); in 33 (19,5%) pts common Jak2 and MPL mutations were not found. In the group of CMD primary pts (N=67) 26 (47%) were PV, 21(38%)–ET, 8(15%)–IMF. Jak2 V617F mutation was found in 55/67 (82%). BCR/ABL p210 expression was found only in 2/55 (3,6%) Jak2 V617F-positive primary CMD pts. RAG1,2 expression was observed in most of the Jak2 V617F positive CMD cases which were tested (46/49 - 94%). In granulocytes of normal donors RAG1,2 expression was found only in 7% (3/42).
Summary and Conclusions: Incidence of BCR/ABL expression gradually increases during CMD progression. Our datasuggest that frequent expression of RAG1,2 in Jak2 V617F-positive granulocytes is responsible for genomic instability coursing t(9;22) and BCR/ABL expression in CMD pts with progression.

P827

A CASE STUDY OF RESENSITIZATION TO RUXOLITINIB, A JAK1/JAK2 INHIBITOR, IN A PATIENT WITH MYELOFIBROSIS (MF)
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Background: Ruxolitinib (RUX) is a potent JAK1/JAK2 inhibitor that has demonstrated rapid and durable improvements in splenomegaly, disease-related symptoms, and quality of life in the phase 3 COMFORT studies. Long-term follow-up confirmed that spleen volume reductions were sustained and RUX treatment remained tolerable with long-term use. In addition, prolonged survival was observed with RUX compared with both placebo and BAT. JAK2 inhibitor–resistant mutations have been generated *in vitro*. However, a recent report (Koppikar P, *et al. Nature.* 2013) suggests persistent JAK2 signaling despite chronic inhibition may be the result of activation by other JAK kinases

and that this persistence is reversible *in vitro*, with cells becoming resensitized to JAK2 inhibition after a washout period. This report demonstrates resensitization to JAK2 inhibition in a patient (pt) with MF.
Aims: This is a case study of a pt receiving RUX in COMFORT-II who had a response after reinitiation of RUX therapy.
Methods: COMFORT-II is a randomized (2:1), open-label, phase 3 study comparing RUX with BAT. Pts with intermediate-2– or high-risk MF received RUX 15 or 20 mg bid based on baseline platelet (PLT) count (100-200 or >200×10^9/L). Spleen volume was measured by MRI every 12 wk and spleen length by palpation at each study visit.
Results: This report is of a 59-year old, female pt diagnosed with PMF 17 years prior to enrolling in COMFORT-II. At study entry, the pt had intermediate-2–risk MF (2 IPSS risk factors: constitutional symptoms and blasts ≥1%), a palpable spleen 19 cm below the costal margin, and reported pruritus and night sweats. RUX therapy was initiated at 20 mg bid (baseline PLT 258×10^9/L). The initial response to treatment was dramatic. Spleen size reduced to 8 cm at wk 4 (58% reduction by palpation), and pruritus and night sweats resolved; however, PLTs dropped to 90×10^9/L, and the dose was reduced to 10 mg bid. Over the next 2 months following dose reduction, spleen size gradually increased to approximately baseline level, and night sweats (but not other symptoms) returned. Consequently, the dose was increased to 15 mg bid, and she experienced a 35% spleen volume reduction from baseline at wk 24 (key secondary endpoint), and night sweats resolved. Baseline hemoglobin was 10.6 g/dL and ranged from 5.1-12.1 g/dL on study; anemia was managed with transfusions. After ≈3 years of RUX treatment she experienced a return of constitutional symptoms, spleen size increased to baseline levels and she had severe anemia. RUX was tapered over 2 wk, and the pt ultimately discontinued after 152 wk for unsatisfactory therapeutic effect. During RUX tapering, she experienced a severe deterioration of her general condition with worsening of constitutional symptoms, anemia, and an increase in spleen size to 26 cm, and she became wheel chair bound. After 2 days with no treatment, she was rechallenged with RUX 10 mg bid, and her situation again improved dramatically. She became completely mobile again, constitutional symptoms improved, and her spleen size reduced from 26 cm to 14-16 cm.
Summary and Conclusions: This case study is the first report of resensitization to JAK2 inhibitor therapy in a pt with MF. The mechanism by which pts lose response to JAK inhibitors then become resensitized is currently unknown; however, preclinical evidence from Koppikar *et al.* suggests that transactivation of JAK2 via heterodimer formation with other JAK kinases (ie, JAK1 and TYK2) leads to persistent signaling of the JAK/STAT pathway that is reversible upon removal of JAK inhibitor therapy.

P828

PRURITUS IN PRIMARY MYELOFIBROSIS: TREATMENT ATTEMPTS, RESPONSE, AND OUTCOMES

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Background: Pruritus, often aquagenic, is a common and disabling feature of myeloproliferative neoplasms. It's prevalence in both polycythemia vera and primary myelofibrosis (PMF) has been well documented. Historically, a number of medications have been used to treat pruritus in PMF, including hydroxyzine, anti-histamines, hydroxyurea, and selective serotonin re-uptake inhibitors (SSRI's). Recent clinical trials with JAK or mammalian target of rapamycin (mTOR) inhibitors in PMF have identified pruritus as one of the most treatment-responsive disease traits. However, there has not been a comprehensive study comparing treatments for pruritus, response rate, and time to response in a large cohort of patients with PMF.
Aims: 1) Describe treatment strategies for pruritus in a large cohort of patients with PMF; 2) Compare effectiveness of treatments for pruritus in PMF.
Methods: The current study population was comprised of 88 patients taken from a larger primary cohort of patients seen at the Mayo Clinic, Rochester between the years 2000 and 2010 with biopsy proven diagnosis of PMF (n=566) who reported pruritus at any point during their care at Mayo Clinic. Patients with pruritus secondary to underlying skin disorder confirmed by Mayo Clinic Department of Dermatology were excluded from evaluation. Clinical records were reviewed and data relating to pruritus was collected in the following domains: severity, treatment, response rate, and time to response. Treatment was stratified by class of medication, and mean response rate and time to response was calculated.
Results: Eighty eight patients (16% of primary cohort) reported pruritus that was not attributable to an underlying skin disorder. Forty nine of these patients (56%) reported severe pruritus and required treatment. The most commonly used medications to treat pruritus were anti-histamines (n=30), anti-depressants (n=22), topical corticosteroids (n=19), topical non steroids (n=13), hydroxyzine (n=14), JAK 2 inhibitors (n=13), and hydroxyurea (n=8). Other medications included thalidomides (n=6), pain relievers (n=6), prednisone (n=5), UVB phototherapy (n=3), alpha interferon (n=2), gabapentin (n=1), and imatinib (n=1). Among the 49 patients who had treatment for pruritus, the response to treatment was documented in 35 patients. Eight three percent of patients (n=29) reported resolution of pruritus and 17% (n=6) had no improvement. Highest response rates were observed in patients treated with a JAK2 inhibitor (92%), thalidomide (83%), or pain reliever (83%). More traditional therapies including hydroxyzine, anti-histamines, and anti-depressants were moderately effective at relieving pruritus (43%, 37%, and 32% response rates respectively). The average time to pruritus alleviation was 2 months (range: 1 day-25 months).
Summary and Conclusions: Pruritus is a treatment responsive feature of primary myelofibrosis. Over 80% of patients with PMF in our cohort had resolution of pruritus after treatment. JAK2 inhibitors were the most effective medication to alleviate pruritus.

P829

ADDITION OF THE DEGREE OF BONE MARROW FIBROSIS TO IPSS IN NEW SCORING SYSTEM IMPROVE PREDICTION OF SURVIVAL IN PRIMARY MYELOFIBROSIS

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Background: During the past few years a several prognostic scoring systems beside the currently used International Prognostic Scoring System (IPSS) and Dinamic International Prognostic Scoring System (DIPSS) have been proposed for risk stratification of PMF patients which are mainly based on clinical parameters.
Aims: The aim of our study was to identify additional prognostic factors for timely appropriate treatment decision for individual patient at the diagnosis.
Methods: We retrospectively analysed 131 WHO-defined PMF pts who were completely treated and followed in our clinic from January 2000 to January 2011. The median follow-up was 44 months (range 2-156). We analyzed clinical, laboratory, pathological parameters (degree of myelofibrosis (MF) ranging from 1 to 4) as well as influence of concomitant comorbity on survival. In each patient 2 comorbidity index were analyzed: 1) Adult Comorbidy Evaluation-27 score (ACE-27), a 27-item comorbidity index for patients with cancer; 2) The Haematopoietic Cell Transplantation Comorbidity Index (HCT-CI). Patients with degree of MF>2 take additional 1 point and become higher IPSS risk grouP-MF modified IPSS (Figure 1).

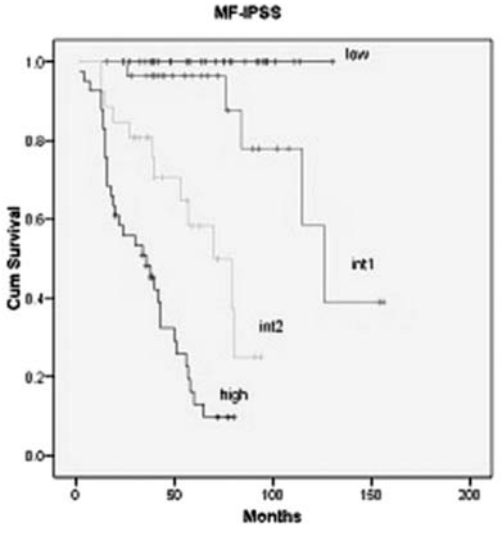

Figure 1.

Results: Median age was 66 years (range 28-84) (M/F=76/55); 66 pts (50.4%) were older than 65 years. According to the IPSS score, 38 pts were stratified as low risk (29%), 40 as intermediate risk-1 (30.5%), 31 as intermediate risk-2 (23.7%) and 22 as high risk group (16.8%). Median survival was 126 months in low-risk group, 80 in intermediate-1, 57 in intermediate-2 and 18 in high risk. Survival was significantly different among the 4 risk categories (P<0.001).According to the degree of MF in 127 (96.2%) analyzed patients distribution was as a follows: MF I 18 patients (14.2%), MF II 53 (41.7%), MF III 43 (33.9%), and MF IV 13 (10.2%). Patients with MF>2 had median survival 51 months while patients with MF≤2 147 months (P<0.001). In MF modified IPSS 33 pts were in low risk group (median survival was not reached), 31 in intermediate risk-1 (median survival 126months), 26 in intermediate risk-2 (median survival 70 months) and 41 in high risk group (median survival 36 months) (P<0.001). Patients with higher both analysed comorbidity scores had significantly shorter survival (ACE- 27 P<0.001 *vs* HCI-CI P=0.002) with better prediction of ACE-27 score. Univariate Cox regression model confirmed the unfavorable influence on survival for age>65 years (P<0.001), male gender (P=0.004), constitutional symptoms (P<0.001),

hepatomegaly (P=0.004), hemoglobin level <100g/L (P<0.001), WBC count>25×10^9/L (P=0.002), Tr≤450×10^9/L (p≤0.001), serum LDH level >700 U/L (P=0.012), peripheral blasts ≥1% (P=0.003), risk groups (P<0.001), MF>2 (P<0.001), MF modified IPSS risk groups (P<0.001), ACE-27 score≥2 (P<0.001), HCI-CI score >2 (P=0.07). Presence of cytogenetic abnormalities, splenomegaly and treatment modality were found not to have statistically influence on survival. Multivariable Cox proportion hazard regression results showed that statistically most important impact on survival had MF modified IPSS risk groups (P<0.001, HR 5.127, 95%CI 3.058-8.594) and ACE-27 score≥2 (P<0.001, HR 5.976, 95%CI 2.451-14.572).

Summary and Conclusions: MF modified IPSS is based on clinical (IPSS) and pathological (degree of MF) parameters and allow a more precise and stronger prediction of survival. Moreover, patients in MF modified IPSS high risk group with ACE-27 score≥2 have a shorter survival than those with only one of these unfavorable scores.

P830

CONTRIBUTION OF SNP ARRAYS TO THE DIAGNOSIS AND PROGNOSIS OF CMML. A STUDY OF PATIENTS WITH LOW RISK CYTOGENETIC FEATURES AND CASES WITH ABSENCE OF METAPHASES

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Background: Chronic myelomonocytic leukaemia (CMML) is a clonal haematopoietic malignancy characterized by features from both myelodysplastic syndromes and myeloproliferative neoplasms, with a median overall survival (OS) of 20 months and approximately 15-30% of progression to acute myeloid leukemia (AML). Clonal cytogenetic abnormalities are found in only 20-30% of patients with this disorder, but none of these alterations is specific of the disease. Most frequent chromosomal abnormalities are trisomy8, abnormalities of chromosome7, loss of the Y chromosomes and complex karyotypes. Some of these alterations have prognostic value. However, most cases still show a normal karyotype.

Aims: To characterize type, frequency and prognostic impact of cytogenetic alterations detected by SNP arrays (SNP-A) in a cohort of 49 patients with low risk CMML according to CPSS (Such *et al.*, 2013). Cases with normal karyotype, -Y or no metaphases were selected. Herein we present the results of 31 preliminary cases. The other 18 patients are currently being analyzed.

Methods: A retrospective study was performed on 31 patients with CMML (26 CMML-1, 5 CMML-2) for whom clinical and biological data were available. Median age at diagnosis was 70 years (48-83), with a male predominance of 1.6:1 and a 29% (9/31) frequency of progression to AML. SNP-A was performed using DNA extracted from bone marrow samples at diagnosis (Cytoscan HD Array, Affymetrix). Survival analysis was performed using Kaplan-Meier estimate and log-rank tests were used for comparisons.

Results: SNP-A revealed 24 (77%) cases with chromosomal alterations which were not detected by conventional G-banding cytogenetics. Chromosome 4 was the most affected chromosome, followed by X,2,3,7, 11 and 16. Among the abnormal cases, copy number alterations (CNA) were detected in 17 (71%) cases. All of them corresponded to gains and losses smaller than 10Mb. Microdeletions in chromosomes7, 12 and 16 were detected more than once, while gains were more frequent in chromosomes 2 and 3. Loss of heterozygosity (CNN-LOH) was detected in 13 (54%) patients. Intersticial LOH (>25Mb) was detected in 8 cases. Among these, chromosome 4 was affected in three cases and chromosome 1 in other 3 different cases. Interestingly, these LOH affected genes where mutations have been described in CMML: TET2 gene, which is one of the most mutated genes in CMML (40-50%), is located in chromosome4, while CBL gene, mutated in 10-20% of CMML, is located in chromosome 11. Survival analysis revealed that the only adverse prognostic factor was CMML-2 subtype. Compared to CMML-1, cases with CMML-2 subtype had worse OS (median OS at 2 years: 18% *vs.* 21%, P=0.003) and progression-free survival (median PFS at 2 years: 21% *vs.* 57%, P=0.001). Due to the fact that some of the cases are still being studied, we will present the prognostic implication of the detection of cytogenetic aberrations detected by SNP-A when the whole cohort is analyzed.

Summary and Conclusions: SNP-A has led to the detection of chromosomal alterations that were not detected by conventional G-banding cytogenetics. Application of this technique in a larger series of patients will allow a better characterization of this disease. Analysis of cytogenetic alterations and mutations detected in the whole cohort may show new prognostic factors, apart CMML-2 subtype.

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P831

EPIDEMIOLOGY OF MASTOCYTOSIS IN ADULTS BASED ON A MULTIDISCIPLINARY DIAGNOSTIC APPROACH

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Background: Mastocytosis is a rare disease. Some epidemiological informations are available about its cutaneous form in children but very few studies addressed the issue of its incidence and prevalence in adults.

Aims: We evaluated the epidemiology of mastocytosis in the adult (≥18 years) population of the Verona province (Veneto region, Italy), based on the activity of the Multisciplinary Mastocytosis Outpatient Clinic, established in Verona in January 2006.

Methods: Patients with suspected mastocytosis are referred to our Clinic from a network of Allergological, Dermatological and Rheumatological Centers in Northern Italy. We focused on all the consecutive adult patients with a confirmed diagnosis of mastocytosis resident in the Verona province. The diagnosis of Cutaneous (CM) or Systemic Mastocytosis (SM) was established according to the World Health Organization Classification. Patients with Urticaria Pigmentosa who did not perform bone marrow evaluation were defined as having Mastocytosis in the Skin (MIS). Written informed consent was obtained from all the enrolled patients. Data about the general population resident in the Verona province from 2008 to 2011 were found at the www.demo.istat.it site. Historical data about the prevalence and incidence of mastocytosis in Veneto were obtained from the Veneto Tumor Registry, Istituto Oncologico Veneto-IRCCS.

Results: Seventy-four adults from a total of 293 patients in our Mastocytosis Registry were identified as living in the Verona province. They were equally distributed by gender (M/F=36/38); median age at first observation was 47.5 (range 20-80) years. The reasons for referral were Urticaria Pigmentosa (n=41), anaphylaxis after hymenoptera sting (n=27) or drugs (n=2) with basal serum tryptase >11.4 ng/mL, unexplained osteoporosis (n=2), other mediator-related symptoms (n=1) and non-MC myeloid neoplasia (n=1). The diagnosis was indolent SM (ISM) in 62 (83.8%) patients; the other cases were CM (n=6), MIS (n=4), aggressive SM (n=1) and SM associated with non-MC disease (n=1). Skin involvement was significantly more frequent in females than in males (78.9% *vs* 47.2%, respectively, P=.007). There was a trend for a higher median basal tryptase in males *vs* females (19.25 *vs* 15.95 ng/mL, respectively, P=.054). D816V KIT mutation was found in 56/64 tested cases (87.5%). We did not find any familial case in this adult population. After a median follow-up of 36 (range 2-112) months all patients are alive. The prevalence of mastocytosis in the Verona province adult population was 9.7 per 100,000 inhabitants, without gender differences. The incidence of new cases did not substantially change from 2008 to 2011 and was 0.93-1.34 per 100,000 inhabitants/year. For comparison, the reported prevalence and incidence in Veneto region before the establishment of our activity (2002-2005) were 0.65 per 100,000 inhabitants and 0.12 new cases per 100,000 inhabitants/year, respectively.

Summary and Conclusions: Although rare, mastocytosis in adults is at least 10 times more frequent than previously reported. Due to the close collaboration with all the Allergology, Dermatology and Rheumatology units of our province, we can assume that virtually all patients with suspected mastocytosis are referred to us. However, the actual prevalence may be higher because ISM diagnosis is understimated especially when skin lesions and/or mediator symptoms are absent. A multidisciplinary diagnostic approach and a wide network of specialists are needed to improve the awareness about this disease and to define its actual epidemiology.

Non-Hodgkin lymphoma - Biology and clinical

P832

HIGHLY SPECIFIC QPCR ASSAYS USING A CONSENSUS LNA PROBE FOR THE DETECTION OF T(11;14) AND T(14;18) IN NON-HODGKIN LYMPHOMA WITH A SENSITIVITY OF 10-5 FOR QUANTIFICATION OF MINIMAL RESIDUAL DISEASE

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Background: In mantle cell lymphoma (MCL) the reciprocal translocation t(11;14)(q13;q32), juxtaposing the *BCL1* gene to the immunoglobulin heavy chain (*IgH*) locus, is present in about 80% of the patients. This results in an overexpression of cyclin D1 giving rise to increased cell proliferation. In follicular lymphoma (FL), the t(14;18)(q32;q21) balanced translocation causes an elevated expression of *BCL2* rendering the cells less prone to apoptosis. In addition, a subpopulation of diffuse large B-cell lymphomas also carry t(14;18). Importantly, the above aberrations can be employed for the detection of minimal residual disease (MRD), but optimal MRD measurements in both peripheral blood (PB) and bone marrow (BM) requires a validated and sensitive assay which can, moreover, be easily applied in a clinical setting. Employing a very sensitive assay enables the use of PB samples for analysis in addition to BM or lymph node biopsies facilitating easy access to cancer cells.
Aims: By employing chemically modified RNA nucleotides, such as locked nucleic acids (LNA), we wanted to design and validate highly sensitive and specific qPCR assays to detect t(11;14) and t(14;18). We set out to use consensus primers avoiding the generation of patient-specific priming in order to monitor MRD in subgroups of NHL patients over time.
Methods: We obtained PB and BM samples at the time of diagnosis and/or relapse as well as during treatment. A consensus reverse primer and probe targeting all six J-segments of the *IgH* locus were applied in the qPCR assays. The probe, consisting exclusively of LNA bases, enabled the design of a short amplicon, thus improving the efficiency of the assay. The forward primer targeting the *BCL1* gene was located in the major translocation cluster. Likewise, the *BCL2* forward primer was designed to cover the various breakpoints of the major breakpoint region. The *TCF20* gene was used as a reference gene, and the cell lines JVM2 and DOHH2 were employed as positive controls. All clinical samples were normalized to a diagnostic or a relapse sample defining the MRD levels at diagnosis or relapse as 1. Samples with Cq values >40 were defined as negative.
Results: We employed the JVM2 and DOHH2 cell lines to establish the t(11;14) and t(14;18) qPCR assays and subsequently validated the assays in 22 MCL and 9 FL patients. The assays successfully quantified MRD in 11/22 MCL patients and 9/9 FL patients. Quantification of t(11;14) and t(14;18) was possible down to less than 10^{-5} and 10^{-4}, respectively, as illustrated in Figure 1 which shows representative longitudinal MRD curves of two patients. The level of MRD reflects the clinical courses of the patients based on pathology and flow cytometry. As indicated in Figure1, high levels of MRD correspond to clinically detectable disease, and low levels of MRD correspond to complete remission (CR).

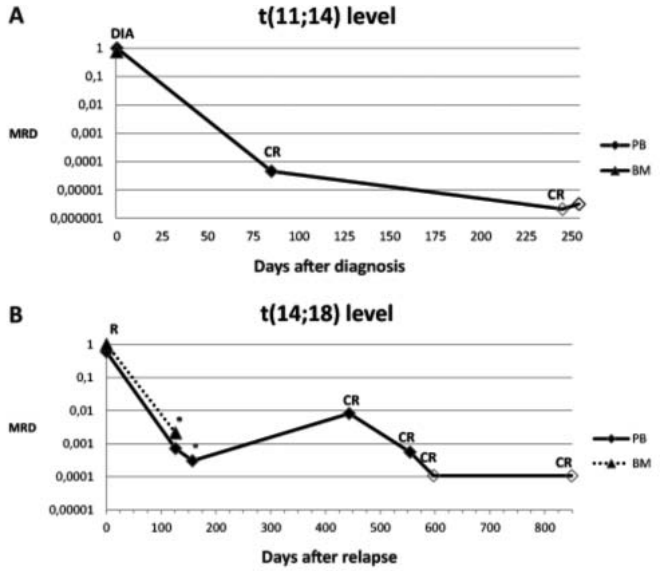

Figure 1. Representative MRD curves of A) an MCL patient and B) an FL patient. Open symbols signify values with a Cq >40. DIA: diagnosis, CR: complete remission, R: relapse, *: clinical status unknown.

Summary and Conclusions: The fraction of quantifiable patients using these assays reflects the presence of multiple translocation breakpoints within the *BCL1* and *BCL2* loci. As the assay is based on consensus primers the time-consuming process of designing and optimizing patient-specific qPCR assays is avoided, and the sensitivity is still down to 10^{-5}. Hence, the present qPCR assays for t(11;14) and t(14;18) show a high degree of sensitivity and specificity and hold great potential as an easily applicable methodology for MRD quantification.

P833

COMPARISON OF NEXT-GENERATION SEQUENCING AND ASO-PCR METHODS FOR MRD DETECTION IN MANTLE CELL LYMPHOMA

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Background: The prognostic value of MRD detection using allele-specific oligonucleotide (ASO)-PCR has been demonstrated in mantle cell lymphoma (MCL) patients treated with autologous stem cell transplant (ASCT) or with chemotherapy alone (Pott *et al.*, Blood 2010). ASO-PCR requires preparation of clonotype-specific primers for each individual which is laborious and time-consuming. We developed the LymphoSIGHT™ platform, a high-throughput sequencing method, which universally amplifies immune receptor gene segments and can identify all lymphoma-specific sequences at diagnosis, allowing monitoring of disease progression during therapy (Faham *et al.*, Blood 2012).
Aims: In this study, we compared the sequencing and ASO-PCR methods for measuring MRD in follow-up samples from 22 MCL patients.
Methods: Using the sequencing assay, we analyzed diagnostic blood and bone marrow samples from 22 MCL patients for clonal rearrangements of immunoglobulin (IGH-VDJ, IGH-DJ, IGK) genes. Clonal rearrangements had been previously detected in all 22 patients using ASO-PCR methods. We assessed MRD at the IGH and/or IGK locus in 159 follow-up samples, and analysis of the concordance between MRD results obtained by the sequencing method and ASO-PCR is ongoing.
Results: Sequencing detected the presence of a high-frequency clonal rearrangement of at least one receptor ("calibrating receptor") in 100% of the 22 MCL patients; 20 patients had at least 2 calibrating receptors at diagnosis, and 2 patients had 3 calibrating receptors. Following the identification of a calibrating receptor, we analyzed follow-up samples for the presence of MRD. Sequencing detected MRD levels of >10% in 1 sample, 1-10% in 3 samples, 0.1-1% in 8 samples, 0.01-0.1% in 7 samples, 0.001-0.01% in 21 samples, and <0.001% in 23 samples. MRD was undetectable by sequencing in 96 samples.
Summary and Conclusions: This high-throughput sequencing method enables MRD detection without the need for development of patient-specific reagents. Concordance data between sequencing and ASO-PCR will be presented.

P834

NR4A1 MEDIATED APOPTOSIS SUPPRESSES LYMPHOMAGENESIS IN A XENOGRAFT MOUSE MODEL

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Background: *NR4A1 (Nur77)* and *NR4A3 (Nor-1)* are two members of the orphan nuclear receptors (NRs) whose function as critical tumour suppressor genes was demonstrated by the rapid development of acute myeloid leukemia (AML) in the *NR4A1* and *NR4A3* double knock out mouse and by their reduced expression in leukemic blasts from human AML patients.
Aims: The aim of our study was to comprehensively investigate *NR4A1* and *NR4A3* expression and function in lymphoid malignancies.
Methods: *NR4A1* and *NR4A3* expression were determined on mRNA- and protein levels in most lymphoid malignancies of B cell type by semi-quantitive RQ PCR, Western Blot and immunohistochemical analyses. For functional characterization *NR4A1* was over expressed in a Sc-1 lymphoma cell line by using an inducible lentiviral construct followed by apoptotic assays (cleaved caspase3, Sub-G1 peak determination, and the Annexin V staining) and by xenograft mouse experiments.
Results: We found a more than 50% reduced expression of both, *NR4A1* and *NR4A3* in B-CLL (71%) in follicular lymphoma (FL) (70%), and in diffuse large B-cell lymphoma (DLBCL) (74%). In aggressive lymphomas (DLBCL, FL III) low *NR4A1* expression was significantly associated with a non-germinal centre B-cell subtype and with poor overall survival. To investigate the function of NR4A1 in lymphomas, we over-expressed *NR4A1* in the lymphoma cell line Sc1 by using an inducible lentiviral expression system. Induction of *NR4A1* expression led to a significantly higher proportion of induced Sc1 cells undergoing apoptosis as demonstrated by caspase 3-, DNA cleavage and Annexin V staining.

To test the tumor suppressor function of *NR4A1 in vivo*, the stably transduced Sc1 lymphoma cell lines were further investigated in the NOD scid gamma (NSG) mouse model. Induction of *NR4A1* in Sc1 abrogated tumor growth in the NSG mice, in contrast to vector control- and uninduced Sc1 cells, which formed massive tumors.
Summary and Conclusions: Our data suggest that *NR4A1* has a pro-apoptotic function in aggressive lymphoma and defines *NR4A1* as novel tumor supressor involved in lymphoma development.

P835

EZH2 EXPRESSION DETERMINED BY IMMUNOHISTOCHEMISTRY DOES NOT CORRELATE WITH THE PRESENCE OF EZH2 MUTATIONS IN A SERIES OF DIFFUSE LARGE B-CELL LYMPHOMAS (DLBCL)
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Background: Advances in DNA sequencing technology have recently enabled the characterization of genomes and transcriptomes at sufficient resolution for identification of somatic point mutations that are involved incancer. This knowledge has led, not only to enhance understanding of the mechanisms of pathogenesis, but also to improve the treatment of these patients by designing drugs that specifically act on these targets. Somatic mutations of *EZH2* have been involved in cancer and lymphomagenesis by affectingthemechanisms implicated in the regulation of gene expression. Gain-of-function mutations involving Y641 amino acid have been described in B-cell lymphomas derived from follicle germinal center cell phenotype (GCB) including follicular lymphoma (FL) (7%) and diffuse large B-cell lymphomas (DLBCL) of GCB type (22%). More recently, mutations in the A677 amino acid have been reported in a B-cell lymphoma cell line.
Aims: Our aim was to study the mutational status of *EZH2* gene in a series of DLBCL, and to correlate the mutational status with EZH2 expression by immunohistochemistry in the neoplastic cells. In addition, we correlated the mutational status of *EZH2* gene with clinical characteristics at presentation and follow-up.
Methods: We included 111 cases of DLBCL diagnosed between 2000 and 2011 according to WHO 2008 diagnostic criteria, corresponding to 61 DLBCL of GCB type and 50 DLBCL of the activated B-cell type (ABC). *EZH2* mutational study was assessed in DNA extracted from paraffin-embedded tumour tissue. Regions where mutations have been previously described (amino acids Y641 and A677 of *EZH2* gene) were analysed by Sanger sequencing. Expression of EZH2 protein was performed by immunohistochemistry using clone SP129 (Ventana Medical Systems, Tucson, AZ) in 27 cases, 14 *EZH2* mutated and 13 *EZH2* unmutated, using the detection system of Optiview BenchMark XT (Ventana Medical Systems).
Results: Of the 111 cases included, 14 samples had mutations in the *EZH2* gene (12.6%) (GCB type in 11 cases and ABC type in 3 cases). In all cases, mutations were detected in the aminoacid Y641 (Y641D:n=7, Y641S:n=4, Y641H:n=3, Y641D:n=1). No mutations were observed in the A677 position. Inmunohistochemical analysis of EZH2 expression showed positivity in neoplastic cells in all cases examined (n=27) with no qualitative or quantitative differences regardless of *EZH2* mutational status. Mutational status of *EZH2* gene was not associated with the following clinicopathologic characteristics: age >60 years, gender, Ann Arbor stage, International Prognostic Index (IPI), ABC-GCB type. Overall survival at 3 years was inferior in high or intermediate-high IPI patients when compared with low or intermediate-low IPI cases (46% *vs* 79%, *P*=0.005), but no differences in survival were observed between patients with mutated and unmutated *EZH2* gene (80% *vs* 59%, respectively, *P*=0.376).
Summary and Conclusions: In our series, *EZH2* mutations were present in the 12.6% of cases, with higher prevalence in the GCB subgroup (18%) but were also observed in the ABC profile (6%). All mutations were detected in the aminoacid Y641 of the *EZH2* gene. No differences were seen between the mutational status of *EZH2* and the pattern of EZH2 protein expression determined by immunohistochemistry in the tumor tissue. Finally, mutational status of *EZH2* gene was not associated with clinicopathologic characteristics and did not have influence on outcome.

P836

DLBCL BIOLUMINESCENT XENOGRAFT MODEL FOR NON-INVASIVE FOLLOW-UP OF ANTITUMOR EFFECT
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Background: Diffuse large B cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma subtype. Addition of rituximab to the classical chemotherapy has improved response rates. However, potential cure is achieved in only 30–50% of patients. The heterogeneous nature of DLBCL and the lack of appropriate animal models are obstacles to further understand the pathology and to identify new drugs for this disease. Although xenograft models of DLBCL are widely used to test new drugs against this neoplasia, most of them are subcutaneous xenografts which do not show a disseminated disease. We describe bioluminescent and disseminated xenograft models of both Germinal Center B cell (GCB) and Activated B Cell (ABC) subtypes of DLBCL. As described previously by our group, the subcutaneous conditioning increased GCB-DLBCL cells capacity to engraft and disseminate. Hence, we performed a subcutaneous passage of the cells before their intravenous injection in mice. The dissemination of the disease was non-invasively followed by *in vivo* imaging.
Aims: To develop bioluminescent xenograft mouse models of GCB and ABC and to validate the DLBCL models using cyclophosphamide
Methods: Toledo (CGB) and RIVA (ABC) DLBCL cell lines expressing GFP-Luciferase (GL) were subcutaneously injected in 6-10 female NOD/SCID mice. Tumors (600 mm3) were extracted and disaggregated. Then, 12-20 mice were randomly divided in two groups and intravenously injected with cultured *in vitro* cells (Toledo GL or RIVA GL) or with the subcutaneously-conditioned cells (Toledo-GL-Sc or Riva-GL-Sc). For cyclophosphamide treatment, mice were injected with subcutaneously-conditioned Toledo-GL-Sc cells and randomized in two groups. Drug (60mg/mL) or vehicle was intravenously injected every 2 days for a total of 7 administrations. *In vivo* images of bioluminescence were taken using IVIS Imaging System once a week.
Results: 1) Subcutaneous passage of Toledo-GL and RIVA-GL cell lines increases aggressiveness: Mice injected intravenously with RIVA-GL-Sc cells showed higher engraftment and dissemination rate than the RIVA-GL intravenously injected group; a significant increase of dissemination to lymph nodes, bone marrow and central nervous system was detected (Figure 1). Aggressiveness of the Toledo-GL-Sc cells also increased with the subcutaneous conditioning. Survival of mice was significantly decreased when lymphoma cells were submitted to the subcutaneous passage (Kaplan-Meier survival curves not shown). 2) Generation of xenograft bioluminescent mouse models of GCB and ABC DLBCL: The intravenously injected cells, Toledo-Sc and RIVA-Sc, generated a disseminated disease in NOD/SCID mice. As observed in Figure 2, most mice showed lymph node and bone marrow infiltration. Macroscopic infiltration was observed mostly in cervical, caudal, inguinal and renal lymph nodes. When tissues were analyzed microscopically, multiple lymph nodes, bone marrow and central nervous system infiltration was observed in most mice (Figure 2D-F) corresponding with the bioluminescent images (Figure 2C). As observed by immunohistochemistry, the infiltrated lymph nodes were CD10+ in Toledo-GL injected mice and CD10-, BCL6+, MUM1+ in RIVA-GL injected mice, confirming its germinal center and activated phenotype, respectively. 3) Validation of DLBCL bioluminescent xenograft model using cyclophosphamide: In order to validate the DLBCL bioluminescent mouse model, cyclophosphamide (Genoxal®) or vehicle were administered to mice. The bioluminescence images (Figure 3) showed decreased dissemination of cells in the treated group. In addition, mice survival significantly increased with cyclophosphamide treatment (Kaplan-Meier survival curves not shown).

	Riva GL	Riva-GL-Sc	Toledo GL	Toledo-GL-sc
Mice dead by lymphoma, n (%)	5/6 (83.3)	6/6 (100)	9/9 (100)	10/10 (100)
Survival time, days	85.2 ± 12.2	52.7 ± 8.1	37.8 ± 6.2	27.7 ± 1.5
Mice with LN infiltration (macro or micro), n (%)	3/5 (60)	6/6 (100)	8/9 (88.8)	8/10 (80)
Mice with BM involvement, n (%)	5/6 (83.3)	6/6 (100)	9/9 (100)	10/10 (100)
Column or Legs, n (%)	0/6 (0)	5/6 (83)	9/9 (100)	10/10 (100)
Cranium, n (%)	5/6 (83.3)	6/6 (100)	9/9 (100)	10/10 (100)
Mice with CNS infiltration, n (%)	5/6 (83.3)	6/6 (100)	9/9 (100)	10/10 (100)

Figure 1.

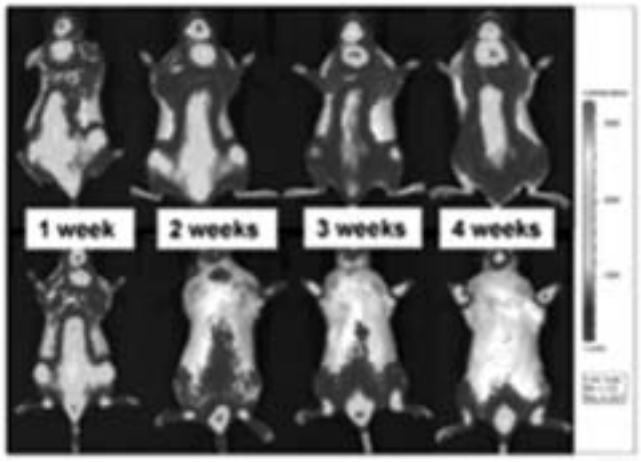

Figure 2.

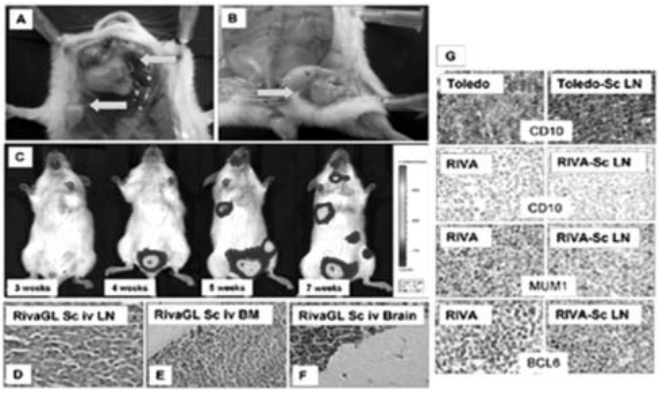

Figure 3.

Summary and Conclusions: We have successfully generated bioluminescent xenograft mouse models for the study of GCB and ABC-DLBCL. Our xenograft mouse models were successfully validated with a drug which is routinely given to patients. The obtained models may be helpful to study the mechanisms of dissemination in DLBCL pathology and to non-invasively monitor the effect of new therapies in the context of a disseminated disease.

P837

BLOOD AND LYMPHATIC MICROENVIRONMENT, MACROPHAGE COMPONENT AS PROGNOSTIC MARKERS IN FOLLICULAR LYMPHOMA

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Background: Follicular lymphoma (FL) has considerable clinical heterogeneity. There is a need for easily quantifiable prognostic biomarkers. There is no consensus on the prognostic significance of microvessel density and number of macrophages.
Aims: To characterize the blood and lymphatic microvessel density, the number of macrophages of tumor tissue basing on immunohistochemical study (IHC) of sections from paraffin blocks of lymph nodes biopsies in two groups of patients with different clinical course.
Methods: The study included 59 patients with follicular lymphoma: 39 women (67%) and 20 men (33%). The mean and median age was 53 years (range: 27-83 years). 49 patients were observed in the National Research Center for Hematology (Moscow) from April 2001 to May 2011 and 10 patients - in Cancer Research Center (Moscow) at the same period of time. The first group–good outcome (1) included 28 patients followed during 2 years or more without therapy; with remission of the underlying disease; with relapse in five or more years from the start of their treatment (late relapse). The second group–poor outcome (2) included 31 patients who died due to tumor progression in the first 1-2 years from the time when this diagnosis was put; with resistance to the passage of the tumor; with relapsed FL in the first year from the start of treatment (early relapse). Patients in both groups received the same initial treatment. The vascularization of tumor tissue was assessed by IHC on sections from paraffin blocks of tumor biopsies of lymph nodes. For the visualization of blood and lymphatic vessels antibodies CD34 and D2-40 were used. CD34 is an endothelial marker of bloodvessels. D2-40 is a new lymphatic marker (podoplanin), which recently was found to express at a high level in lymphatic endothelial cells. CD68 is a marker, which is used to visualize activated macrophages. Morphometric analysis was performed using light microscopy and a digital camera Leica (vol. × 40). The pictures are processed by a computer program "VideoTesT-Morphology 5.2" in order to estimate the specific area of vessels in relation to the tumor tissue (%) during the researcher's visual inspection. IHC - specimen evaluation was carried out using a Table of random numbers. Counting the number of macrophages was realized by using light microscopy (magnification × 40) in nodular, nodular and diffuse areasof tumor growth. Macrophages were counted on 1 mm^2 of tumor tissue. The number of positive cells was determined intrafollicular and interfollicular space.
Results: 5-year OS rate in group 1 was significantly higher than in group 2 (83±7% *vs* 28±13%; P=0,03) (Figure 1A). Evaluation of the specific area of blood and lymph vessels was conducted in two comparable groups. The specific area of blood vessels in group 2 was significantly higher than in group 1: 0,04 (95% CI, 0,03-0,05) *vs* 0,02 (95% CI, 0,01-0,03) (Figure 1B). The specific area of the lymph vessels in group 2 was significantly higher than in group 1: 0,06 (95% CI, 0,04-0,07) *vs* 0,03 (95% CI, 0,01-0,04) (Figure 1C). The number of CD68 - positive macrophages with nodular and diffuse growth of tumor in group 2 was significantly higher than in group 1: 800 (95% CI: 380–1222) *vs* 79 (95% CI: 10–566) (Figure 1D).

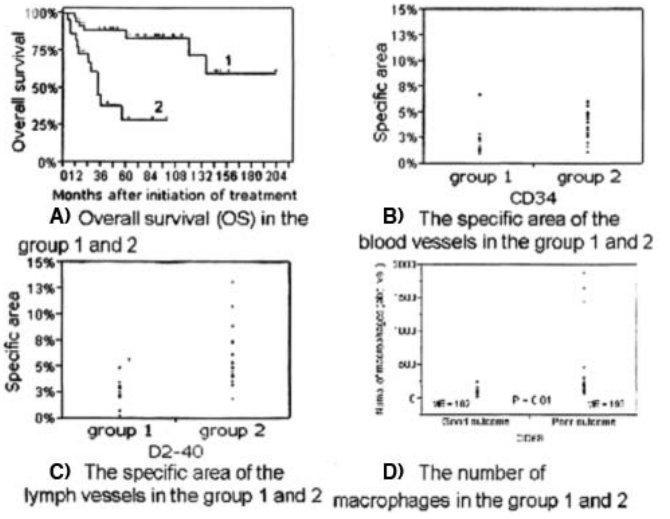

Figure 1.

Summary and Conclusions: The microvascular density can be estimated using the markers CD34 and D2-40. Our results demonstrate an association between prognosis and angiogenic sprouting and reveal higher angiogenic activity in the poor prognostic group. The higher number of macrophages is also associated with a worse prognosis. Probably, these results will let determinate the nature of the follicular lymphoma.

P838

ANALYSIS OF SOX11, SOX4 AND SOX12 IN MANTLE CELL LYMPHOMA

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Background: The related transcription factors SOX11, SOX4 and SOX12 share significant homology at the gene and protein level and are classified as the SOXC family. In mantle cell lymphoma (MCL), SOX11 is expressed in approximately 90% of cases. Lack of SOX11 expression has been associated to a different biological behavior in MCL. Little is known on how SOX4 and SOX12 are expressed in SOX11 positive and SOX11 negative MCL. SOX11 has been suggested to be epigenetically regulated in lymphoma, but there are partly conflicting results considering the role of promoter methylation.
Aims: To investigate the role of promoter methylation in SOX11 expression. To investigate how SOX4 and SOX12 are expressed in SOX11 positive and SOX11 negative MCL.
Methods: Expression of SOXC transcription factors was analyzed by Q-PCR

in primary MCL (n=29), in MCL cell-lines (n=4) and in non-malignant lymph node, spleen and peripheral blood B lymphocytes (PB). These samples were also investigated for promoter methylation using McrBC-digestion and pyrosequencing. The expression of SOXC transcription factors in MCL cell lines was also investigated after treatment with 5-azacytidine.
Results: SOX11 was expressed in all but three primary MCL (median 500-fold expression compared to PB) and in 3/4 MCL cell lines while non-malignant lymphocytes lacked SOX11 expression. SOX4 was variably expressed; 13/29 MCL expressed lower levels and 10/29 expressed higher SOX4 levels than PB. SOX12 was expressed at higher levels in 27/29 MCL compared to PB. Three SOX11 negative primary MCL all expressed SOX12. In SOX11 positive MCL there was a highly significant correlation between expression of SOX4 and SOX11 (Spearman´s correlation coefficient 0,56; P<0,005) and between SOX12 and SOX11 (Spearman´s correlation coefficient 0,63; P<0,005). The promoter region of SOX11 was hypomethylated in SOX11 positive MCL as well as in non-malignant B-lymphocytes and heterogeneously methylated in the three SOX11 negative MCL cases. Treatment with 5-azacytidine reduced SOX11 expression in 2/3 SOX11 positive MCL cell lines and induced expression of SOX4 but not SOX11 in the SOX11 negative MCL cell line JVM2.
Summary and Conclusions: SOX11 expression in MCL is not regulated by promoter methylation but methylation at other sites might be of importance for sustaining high SOX11 expression levels. SOX12 are expressed at higher levels in SOX11 positive and SOX11 negative MCL compared to non-malignant lymphocytes. Finally, the expression of SOXC transcription factors is strongly correlated in SOX11 positive MCL. This might be highly biologically relevant since the members of the SOXC family have been reported to activate similar target genes.

P839

HIV ASSOCIATED DOUBLE–HIT B-CELL LYMPHOMAS PREFERENTIALLY INVOLVE THE MYC AND BCL6 GENE LOCI

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Background: Double-hit (DH) lymphomas have recently attracted attention due to their aggressiveness, their possible under diagnosis previously and the need to better characterize these disease in order to develop novel treatment strategies. A number of case studies and case series on DH lymphoma show that these diseases are very aggressive, are often classified as lymphomas with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL) and typically have translocations of the *MYC* and *BCL2* genes which partner with the immunoglobulin loci. Double hits involving *MYC* and *BCL6* translocations are much more rarely described in the international literature. HIV infection significantly increases the risk of developing high grade B-cell lymphoma. These often present with atypical features posing challenges in diagnosis. In particular, lymphomas presenting with L3-like morphology have other features that best fit the diagnosis of the intermediate class of lymphoma introduced in the WHO 2008 classification (B-Cell lymphoproliferative disorder, NOS, with features intermediate between BL and DLBCL).
Aims: To characterize the clinical, morphological, immunological and cytogenetic features of HIV associated double-hit B-cell lymphoma.
Methods: Reports of patients who presented with HIV-associated high grade B-Cell Lymphoma (particularly Burkitt-like and atypical Burkitt lymphoma /leukaemia), between 2008 and 2012 were reviewed for the presence of DH lymphoma on cytogenetic/FISH analysis. Cytogenetics studies were performed using standard methods and GTG banding, chromosomes were classified according to ISCN 2013. Fluorescence *in situ* hybridization (FISH) was performed using LSI c-MYC/IGH dual colour dual fusion translocation probe and/or LSI c-MYC dual-colour breakapart rearrangement probes, LSI BCL6 dual colour breakapart rearrangement probes and LSI BCL2/IGH dual colour dual fusion translocation probe. Flow cytometry and immunohistochemistry were performed as per standard protocols.
Results: Seven patients were diagnosed between 2008-2012. This included five adults (3 males and 2 females) with an age ranging from 25 to 41 years, (mean age of 35 years) and two male children (6 and 7 years old respectively). All patients were HIV positive and had systemic disease at presentation. Five patients (3 adults, 2 children) had morphology/histology with a mixture of cells of intermediate to large size best fitting the diagnosis of B-cell lymphoma with intermediate features between DLBCL and BL. Two adults patients had a more typical L3 morphology. A complex karyotype with more than 3 structural and/or numerical chromosomal aberrations was seen in every patient. MYC was rearranged in a classical t(8;14)(q24;q32) in four cases and in a variant Burkitt translocation t(8;22)(q24;q11) in three cases. BCL6 was involved in a t(3;14)(q27;q32) in 4 cases and in a t(3;22)(q27;q11) in 2 cases. In one case BCL6 rearrangement was not seen on karyotype but was detected by FISH in 50% of cells. The most common additional aberration was trisomy 1 or partial trisomy/tetrasomy for chromosome 1q. MYC and BCL6 rearrangements were all confirmed by FISH. BCL2 rearrangement was not detected. One case had a triple hit with a t(8;14), t(3;22) and a t(2;14)(?q33;q32) involving an unknown partner gene on chromosome 2. Follow-up cytogenetics obtained in case 1 and2, (after 2 and 1 month respectively), revealed a rapidly evolving karyotype reflecting the aggressiveness of the disease. Immunophenotypically all cases had a CD19 B-cell lineage but weak CD10 and CD20 signals. Interestingly, immunohistochemistry performed in 3 cases for CD38 and CD138 showed weak positivity for CD38 in all 3 cases and a dim CD138 in 2 cases, with focal positive regions in the third case. All patients had a highly aggressive course of disease and died within few months of initial diagnosis.
Summary and Conclusions: In our serie, HIV associated DH lymphoma occured in young people, preferentially involved MYC/BCL6 translocations and had an aggressive course of disease with extremely poor survival. There is a need to characterize these tumours to better understand their pathogenesis and develop novel therapeutic strategies.

P840

LOSS OF NIPA LEADS TO DEFECTS IN THE REPAIR OF DNA DOUBLE STRAND BREAKS IN MICE

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Background: Regulated oscillation of protein expression is an essential mechanism of cell cycle control. The SCF class of E3 ubiquitin ligases is involved in this process by targeting cell cycle regulatory proteins for degradation by the proteasome. We previously reported the cloning of NIPA (Nuclear Interaction Partner of ALK) in complex with constitutively active oncogenic fusions of ALK, which contributes to the development of lymphomas and sarcomas. Subsequently we characterized NIPA as a F-Box protein that defines an oscillating ubiquitin E3 ligase targeting nuclear cyclin B1 in interphase thus contributing to the timing of mitotic entry.
Aims: To study *in vivo*-function of the G_2/M checkpoint NIPA in greater detail, we inactivated the gene *Nipa* using a conditional knockout strategy.
Results: *Nipa*-deficient animals are viable, but show a lower birth rate and a reduced body weight. Furthermore, *Nipa*-deficient males were sterile due to a block of spermatogenesis during meiotic prophase. Virtually no spermatocytes progress beyond a late-zygotene to early-pachytene stage and reach an aberrant stage, with synaptonemal complex disassembly and incomplete synapsis. *Nipa*$^{-/-}$ females are sub-fertile with an early and severe meiotic defect during embryogenesis with extensive apoptosis in early prophase (E13.5-E14.5).
Here we report, that *Nipa*$^{-/-}$ meiocytes exhibit persistent cytological markers for DNA double strand break repair proteins (like DMC1, RAD51) in meiotic prophase with more than twice as many DMC1 foci as control animals. Kinetic analysis of the first wave of spermatogenesis showed increased DMC1/RAD51 foci in *Nipa*$^{-/-}$ cells as soon as early-pachynema cells appear (13-14 days post partum). Moreover, we show that *Nipa* deficiency does not lead to a defect in meiotic sex chromosome inactivation despite epithelial stage IV apoptosis. *Nipa*-deficient spermatocytes exhibit numerous abnormalities in staining of chromosome axis associated proteins (like SYCP3 and STAG3) indicating that chromosome axis defects were associated with compromised chromosome axis integrity leading to overt chromosome fragmentation.
Summary and Conclusions: Taken together, the phenotype of *Nipa*-knockout mice is a definitive proof of the meiotic significance of NIPA and our results show a new, unsuspected role of NIPA in chromosome stability and the repair of DNA double strand breaks.

P841

IDENTIFICATION OF DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) SUBTYPES DEFINED BY THE EXPRESSION OF STROMAL PROTEINS AND THEIR ASSOCIATION WITH MICRORNAS INVOLVED IN ANGIOGENESIS

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Background: The identification of subtypes of DLBCL defined by expression of genes related to tumor stroma (Lenz *et al.*, 2008) may determine new paths in the treatment of these diseases using anti-angiogenic drugs. The relationship of these genes with the pro-angiomiRs is still unknown.
Aims: 1) to classify DLBCL cases according to the signature *stromal-1* and *stro-*

mal-2 using tissue microarray (TMA) and immunohistochemistry; 2) to evaluate the expression of pro-angiomiRs in paraffin embedded tissues affected by DLBCL and correlate them with the signatures of the tumor stroma.
Methods: 111 DLBCL (NOS and variants, all HIV negative) admitted to the Hospital São Paulo between 2000 and 2010 were included in this study. Expression of CD68 (*stromal-1* marker) was classified into four (0-3) levels. To analyze the expression of CD34 (MVD, *stromal-2*), we conducted a manual count of microvessels in the entire field of TMA. We performed miRNA extraction from paraffin samples using RecoverAll ™ Total Nucleic Acid Isolation Kit for FFPE Tissues (Applied Biosystems). Real-time quantitative PCR was performed using TaqMan ® Small RNA kit Assays and normalized with U18. Proangiomirs miR-92a, miR-126, miR-130a, miR-210, miR-378 and miR-296 were selected for assessment of angiogenesis and were considered differentially expressed when tumor samples levels were 1.2 times higher or lower than normal samples.
Results: *Stromal-1*cases had high expression of CD68 (scores 2-3) and low expression of CD34 (quartiles I-II) (34.4% of cases), and as *stromal-2* cases had low expression of CD68 (scores 0-1) and high expression of CD34 (quartiles III-IV) (12.5% of cases). The 38 cases that had high expression of both CD68 and CD34 (39.6%) were additionally considered as *stromal-2*, due to high MVD scores. Cases of low expression of CD68 and CD34 (13.5% of cases) were not scored. We observed a statistically significant relationship between the median expression of CD34 and Ann Arbor stage (I-II *versus* III-IV), with a predominance of high MVD expression in patients with advanced stage disease (III-IV). The median of vessels in stages I-II was 82 (range 9 to 240) and in stages III-IV was 111 (range 12-297) (P=0.0183, Mann-Whitney). For the marker CD68 (*stromal-1*) and for the other variables, there were no statistically significant associations between groups. 101 of 111 initial cases were considered suitable for analysis of microRNAs. We observed overexpression of miRNAs miR-296, miR-378, miR-92a, miR-210, miR-130a and miR-126 in 55.5%, 50.5%, 42.6%, 30.7%, 19% and 9.9% of cases, respectively. We also observed increased expression of miR-296 in DLBCL classified as stage III-IV (P=0.0355, Mann-Whitney). For other variables, there were no statistically significant differences between groups.
Summary and Conclusions: *Stromal-2* signature was found in 52.1% of cases. The higher MVD in cases of DLBCL with advanced stage (Ann Arbor III-IV) shows a possible association between angiogenesis and more aggressive/disseminated disease, being a possible target for anti-angiogenic therapy in cases that do not achieve a complete response with R-CHOP. The miR-296, directly responsible for decreased levels of hepatocyte growth factor and indirectly by the upregulation of VEGFR2 and PDGFRβ, showed overexpression in 55.5% of DLBCL and association with advanced stage disease. Our study provides new information about the importance of the role of microRNAs in the development of DLBCL, which can be exploited as a therapeutic target and prognostic factor for patients with this malignant disease of high prevalence. (Supported by FAPESP 2010/17668-6).

P842

DISTINCT GENE EXPRESSION PROFILES IN A CANINE MODEL OF HUMAN DLBCL

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Background: Diffuse large B-cells lymphoma (DLBCL) is the most common type of lymphoma in adults. In humans, several studies have categorized DLBCL lymphomas into different morphological and molecular subtypes. Gene expression profiling provided a tool to reveal the presence of at least 3 subtypes of DLBCL; active B-cell (ABC-DLBCL), germinal center B-cell (GCB-DLBCL), and primary mediastinal B-cell Lymphoma (PMBL) with specific signatures correlating to the clinical course of the disease.
Aims: Evaluate the potential benefits of a canine model as an experimental platform to improve treatment and evaluate new therapies for both humans and dogs with DLBCL. In particular, the dog presents an ideal model with spontaneous occurrence of the disease. Indeed, Non-Hodgkin lymphoma is the most common hematopoietic disease in dogs that shares morphological and immunophenotypic features with human DLBCL and a highly aggressive course of the disease. Here we characterized at morphological, phenotypic, and molecular levels dogs with DLBCL to assess the factual likeness of these malignancies between human and dog patients.
Methods: More than one-hundred DLBCLs were collected during a three years period (2009-2012) at the diagnostic service of the Department of Veterinary Sciences and Public Health in Milan (IT). The diagnosis was based on morphological criteria and expression of CD20, CD21 and CD79. For twenty-one dogs with the morphological diagnosis of DLBCL that yielded high-quality extracted RNA, gene expression profiling was performed, using Affymetrix Canine 2.0 platform (Affymetrix, Santa Clara, CA, USA). Five samples of normal lymph nodes were also analyzed. Data analyses were generated by R Bioconductor software and public databases of gene signatures (http://Lymphochip.nih.gov/signaturedb/ and http://broadinstitute.org/gsea/msigdb/index/jsp) used to cross-compare human and dog signatures.Written informed consent was obtained from the dog owners.
Results: In dogs with DLBCL, the whole gene expression profiling analysis identified two clusters, a minor and a major one, with distinct signatures, providing the first example of molecular subgroups in Canine DLBCL not observed with the current standards of classification. No differences in age, gender and breed were observed for the 2 subgroups. Pathway analysis revealed activation of different pathways; the minor clusters showed decrease of apoptosis, DNA-damage response and down regulation of cell cycle progression genes suggesting a link with the human GCB-DLBCL signature. Moreover, the major cluster showed activation of *NFKB2* and *FUT8* genes together with a high expression of genes related to proliferation as revealed by cross comparison analysis between human and dog signatures, more correlated with ABC-DLBCL. Dogs DLBCL showed a general down-regulation of *BCL-6* gene compare to normal lymph nodes with a further difference between the two clusters.
Summary and Conclusions: In conclusion, in this study we analyzed gene expression profiles of dogs with DLBCL to determine if the dog with spontaneous occurrence of the disease can represent a useful study model for human DLBCL. We showed that as in human lymphoma molecular subtypes of DLBCL could be distinguished also in dogs. Further studies are needed to translate the relevance of the canine model of DLBCL to clinical research in human lymphoma.

P843

REAL-WORLD TREATMENT PATTERNS AND OUTCOMES IN ELDERLY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA: SEER-MEDICARE ANALYSIS

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Background: A disproportionate number of newly diagnosed diffuse large b-cell lymphoma (DLBCL) occurs in elderly patients
Aims: We set out to evaluate real-world treatment patterns and outcomes in elderly patients with DLBCL in the United States.
Methods: We utilized a retrospective cohort analysis of 9333 DLBCL patients in the linked Surveillance, Epidemiology, and End Results (SEER)-Medicare database. Patients were diagnosed between 1/1/2000 - 12/31/2007, >66 years, and continuously enrolled in Medicare Part A and B in the year prior to diagnosis. There were 4565(49%) R (rituximab) +Chemo, 2181(23%) Chemo-only, and 467(5%) R-mono patients who initiated treatment within 3 months after diagnosis. The remaining 2120(23%) did not receive any treatment. Statistical comparisons were made between R+Chemo *vs.* Chemo-only, and R-mono *vs.* No Treatment. Logistic regression modeling assessed patient characteristics predictive of not receiving treatment. Kaplan-Meier curves and Cox proportional hazards regression assessed overall survival by treatment type. The median follow-up time was 2.4 years and date of last follow-up was 12/31/2009.
Results: Patients receiving R+Chemo were slightly younger (≤75yrs: 50% *vs.* 46%; P<0.05), more likely white, and married compared to those receiving Chemo-only. Patients receiving R-mono were older (>80yrs: 60% *vs.* 50%; P<0.01), and more likely female compared to those Not Treated). In general, untreated patients were older at diagnosis (mean age 80 *vs.* 77; P<.0001), more likely non-white (14% *vs.* 12%; P<.01), diagnosed at late stage (51% *vs.* 44%; P<.0001), and had higher comorbidity burden than treated patients (P<.0001). As age increased, the treatment rate significantly decreased especially among patients >80yrs. In the adjusted logistic regression model, increasing age, non-white race, male gender, higher comorbidity score, unmarried and lower income were predictive of not receiving any treatment. The unadjusted median overall survival was 96 months in the R+Chemo group *vs.* 13 months in Chemo-only group (log rank P<.0001); and 18 months in the R-mono group *vs.* 2 months in the Not treated group (log rank P<.0001). Among the treated, there were more R+Chemo and R-mono treatment cycles than Chemo-only cycles (5 *vs.* 4 cycles; P<.0001) delivered in the first-line. In multivariate survival analysis, patients receiving Chemo-only had a 2-fold increased risk of death (HR=2.20; 95%CI=2.04-2.37) compared to R+Chemo patients. Increasing age and comorbidity score were associated with significant increases in mortality. A higher mortality risk was noted with receipt of <6 cycles (HR=1.40; 95%CI=1.22-1.62) compared to 6 cycles. R-mono patients had a 69% lower risk of death (HR=0.31; 95%CI=0.28-0.35) compared to Not treated patients. Almost identical mortality risk reductions with R+Chemo and R-mono were also noted in a subgroup of patients >80yrs.
Summary and Conclusions: Twenty-three percent of elderly DLBCL patients were not receiving treatment for their disease. We observed that R+Chemo and R-mono significantly decreased mortality risk in elderly DLBCL patients relative to chemo only and no treatment, respectively. The benefits were maintained even among patients >80yrs, who were less likely to receive treatment. Suboptimal durations of curative intent therapy (<6 cycles) were found to be associated with poorer outcomes.

P844

CLINICAL SYMPTOM OR SIGN-DIRECTED SURVEILLANCE CAN BE MORE USEFUL TO DETECT RELAPSE COMPARED TO ROUTINE IMAGING IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA AFTER COMPLETE REMISSION

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Background: Although the prognosis of patients with diffuse large B-cell lymphoma (DLBCL) has improved after introduction of rituximab, about one-third of the patients still experience eventual disease relapse. As there exists the second chance of cure by salvage therapy followed by autologous stem cell transplantation (ASCT), the optimal follow-up strategy to detect relapse early enough to proceed salvage therapy with maintenance of good performance status should be established for patients who have achieved a complete remission (CR) after frontline therapy. Despite lacking evidences, routine imaging has been widely adopted in surveillance of patients with lymphoma after CR.
Aims: The current study aimed at analyzing the patterns and their outcomes of out-patient department (OPD) follow-up for surveillance and evaluating the role of routine imaging and the result of unplanned early OPD visit in patients with DLBCL after CR.
Methods: Patients were included if they 1) diagnosed as DLBCL according to 2008 WHO criteria, 2) achieved CR by 2007 Revised Criteria after R-CHOP immunochemotherapy with or without consolidative therapy such as radiotherapy or ASCT, and 3) had at least 1 OPD visit for the surveillance of relapse. Routine imaging was defined as scheduled computed tomography (CT) or Positron Emission Tomography (PET)/CT at least 3 months prior to actual scanning by physician without any suspicious symptom or sign of the patients.
Results: From May 2004 to Feb. 2012, 106 of 151 patients with DLBCL (70.2%) obtained CR and fifteen of them (14.2%) experienced relapse, with a median follow-up duration of 38.1 months. A total of 856 OPD visits (median6, range 1-25) were recorded from the 106 patients: 501 visits (median4, range 0-15) were OPD surveillance with routing imaging (332 visits with CT alone, 94 with PET/CT alone, and 75 with both CT and PET/CT) and 322 visits (median2, range 0-14) were planned OPD surveillance with only history taking and physical examination (Hx & P/Ex). Thirty-three visits (33/856=3.9%) were unplanned early visit by patiensts due to any abnormal symptom or sign. Routine imaging showed a perfect sensitivity and negative predictive value but low positive predictive value (PPV) due to frequent false-positive visits. Six of seven patients with false-positive routine CT scan and 17 of 23 patients with false-positive surveillance PET/CT received unnecessary further evaluation (please see the image). Compared to planned OPD surveillance with routine imaging (3 relapses out of 501 visits) and planned OPD visit with Hx & P/Ex (1 relapse out of 322 visits), early visits by patients due to any symptom or sign were highly related to disease relapse: 11 out of 33 visits (33.3%) resulted from relapse of DLBCL. Although survival from the time of relapse was not compared because of small sample size, no tendency of survival benefit in 3 patients with asymptomatic detection by routine imaging was observed (Figure 1).

Routine imaging with CT

	No Relapse	Relapse
CT normal	397	0
CT abnormal	7	3

Sensitivity: 100% (95% CI 30.5 ~ 100.0)
Specificity: 98.3% (95% CI 96.5 ~ 99.3)
PPV : 30.0% (95% CI 7.0 ~ 65.2)
NPV : 100% (95% CI 99.1 ~ 100.0)

7 patients with false positive
2 short-term CT follow-up
2 MRI correlation
1 biopsy with local anesthesia
1 biopsy with general anesthesia
1 close observation

Routine imaging with PET/CT

	No Relapse	Relapse
PET/CT normal	142	0
PET/CT abnormal	23	3

Sensitivity: 100% (95% CI 30.5 ~ 100.0)
Specificity: 86.1% (95% CI 79.8 ~ 91.0)
PPV : 11.5% (95% CI 2.6 ~ 30.2)
NPV : 100% (95% CI 97.4 ~ 100.0)

23 patients with false positive
7 CT or ultrasonogram correlation
3 ENT exam correlation
2 gynecologic exam correlation
3 biopsy with local anesthesia
1 biopsy with general anesthesia
1 diagnosed as infection (liver abscess)
6 close observation

Figure 1.

Summary and Conclusions: Surveillance routine imaging has limited value because of low PPV and its resultant unnecessary procedures such as additional imaging or biopsy. Considering the risk of radiation exposure, financial cost, and anxiety of the patients (Thompson CA *et al.*, *Ann Oncol* 21:2262-2266), routine imaging seems not to be an appropriate strategy for patients with DLBCL after CR. It is mandatory to perform thorough Hx & P/Ex, and adequate further active investigation when a patient visit OPD earlier than planned schedule and it have to be emphasized that patients should not hesitate to visit hospital earlier if they have any discomfort.

P845

VIROLOGICAL SUCCESS IS ASSOCIATED WITH FAVORABLE PROGNOSIS IN PATIENTS WITH HEPATITIS C ASSOCIATED B-CELL NON-HODGKIN LYMPHOMAS, NATIONAL ANRS HC-13 LYMPHO-C STUDY RESULTS.

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Background: Hepatitis C virus (HCV) associated B-cell non-Hodgkin lymphomas (B-NHL) are a model of lymphoma induced by chronic antigenic stimulation. Lymphomagenesis mechanisms and treatment management remains matter of debate.
Aims: The national Lympho-C study collected clinical and biological data from HCV associated B-NHL aiming to better understand lymphomagenesis, treatment and outcome.
Methods: Adult patients with B-NHL and active HCV infection were included in this observational multicenter study. Patients co-infected with HIV could not be included. Data were collected from patients with either ongoing (prospective) or past (retrospective) history of HCV associated B-NHL. Cytological and histological samples were collected for centralized review and molecular analyses. Informed consent was required for each patient.
Results: Between 2006 and 2012, 138 consecutive patients were enrolled in 26 French hospitals. Subsequently 13 patients were excluded from analysis. 59 out of 125 (47%) patients were included at B-NHL diagnosis. At lymphoma diagnosis, median age was 61 years $_{IQ25-75}$[50-71] and genderratio was 0.9. Genotype HCV distribution was genotype 1 (51%), 2 (19%), 3 (9%), 4(10%), 5 (1%) or unknown (10%). Median delay between HCV infection and B-NHL diagnosis was 25 years $_{IQ25-75}$[21-31] (unknown in 41/125 patients). Cyto-histological pattern was: diffuse large B cell lymphoma (DLBCL): 49/125 (39%), marginal zone lymphoma (MZL): 48/125 (38%), follicular lymphoma: 16/125 (13%) and others types 13/125 (10%). Among DLBCL, 17/49 (35%) were transformed from underlying indolent lymphoma. Patients in DLBCL group displayed frequent digestive tract (17/49; 35%) and hepatic (11/49; 22%) involvements. Patients in MZL group showed frequent spleen (23/48; 48%) and hepatic (13/48; 27%) involvements. The MZL patients had higher levels of rheumatoid factors (P=0.001) and more frequent mixed cryoglobulinemia vasculitis (11/48; 23%) (P=0.042). Median follow-up since lymphoma diagnosis was 31 months $_{IQ25-75}$[19-71]. At the end of follow-up, 79/125 (63%) had received antiviral therapy since lymphoma diagnosis, and sustained virological response (SVR) was obtained in 48/79 (61%). Significant association was shown between SVR and hematological response in LZM group (P<0.001) but not in DLBCL group (P=0.83). However in the overall population, patients with SVR had better event free survival (EFS) (P=0.06) and overall survival (OS) (P=0.006) than patients without SVR (Figure 1). During the follow-up, 16 (13%) deaths were recorded, related to lymphoma progression (n=7), infection (n=5), cirrhosis (n=3) or cardiovascular disease(n=1).

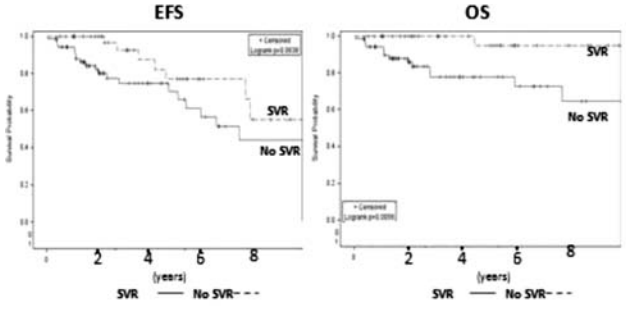

Figure 1. Impact of virologic response on HCV associated B-NHL prognosis.

Summary and Conclusions: This study underlines the heterogeneity of HCV associated B-NHL subtypes, with predominance of DLBCL and LZM. Virological success and hematological response are associated among LZM patients. Moreover, virological success appears to improve prognosis of HCV associated B-NHL patients.

P847

HIGH-DOSE METHOTREXATE, HIGH-DOSE CYTARABINE AND TEMOZOLOMIDE FOR THE TREATMENT OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Background: treatment of primary central nervous system lymphoma (PCNSL) associates with low response and survival rates using conventional radio and chemotherapy.Due to its favorable toxicity profile,Temozolomide has emerged as a new option for the treatment of PCNSL.In this study we report a consecutive series of PCNSL patients treated with an innovative regimen combining high-dose of both Cytarabine and Methotrexate with Temozolomide without Radiotherapy or intra-thecal chemotherapy
Aims: to evaluate a new intensive chemotherapy with a new drug Temozolomide,trying to assess response rate and progression free survival and if the results are promising we are aiming ate evaluating the overall survival rate at 5 years taking into consideration the toxicity profile.The study was performed at al Bairouni university hospital and al Shahbandar medical center in Damascus (SYRIA).
Methods: we have included 40 patients under 60 years old newly diagnosed with PCNSL histologically confirmed by a reference pathologist,biopsies were also cultured and Karyotype was performed for almost all patients.An induction chemotherapy was started (Methotrexate 3 gr/m2 infused over 12 hours on day 1+Cytarabine 3 gr/m2 twice daily,short infusion on day 1+Temozolomide 100 mg/m2 from day 2 through day 6).A total of 6 cycles were given on a monthly basis.
Results: among the 40 patients included in the study,a complete response was observed in 34 patients (85%) *versus* a partial response seen in the remaining 6 patients (15%).Disease was progressed in 8 out of 40 patients (20%) while 31 patients were still living after 5 years making the overall survival reaching (77%).Grade II nephrotoxicity was observed in 2 patients while grade II and IV hematologi toxicity were seen in 5 patients.
Summary and Conclusions: our study has reached the 5-years overall survival comparing with other regimens not exceeding 35 months taking into consideration the lack of both radiotherapy and intra-thecal chemotherapy,also the good toxicity profile makes it an acceptable regimen.However,more prospective studies are warranted to confirm or reject such results.

P848

MALE SEX EVOLVES AS A SIGNIFICANT RISK FACTOR IN DLBCL TREATED WITH RITUXIMAB ONLY IN ELDERLY, BUT NOT IN YOUNG PATIENTS: CORRELATION WITH RITUXIMAB (R) PHARMACOKINETICS

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Background: Sex and weight influence R clearance in elderly DLBCL patients (Mueller *et al.*, Blood 2012).
Aims: Therefore, we now investigated the influence of sex and weight on R pharmacokinetics and outcome in young DLBCL patients.
Methods: We analyzed the impact of sex on R pharmacokinetics and outcome of 1222 elderly pts of the RICOVER-60, 823 young (18 to 60 years) aaIPI=0,1 pts of the MInT, and 275 aaIPI=2,3 pts of the Mega-CHOEP trials. R pharmacokinetics was determined in 33 young and 49 elderly patients. Population pharmacokinetic modeling was performed with nonlinear mixed-effect modeling software (NONMEM VI).
Results: R clearance was independent of tumor mass (IPI), but weakly correlated (0.2, R^2linear=0.045) with increasing age in male, and moderately inversely correlated (-0.5, R^2linear=0.207) with age in female DLBCL patients, resulting in similar R clearances in young female and male patients (9.88 *vs.* 10.38 mL/h; P=0.238), but a significantly slower R clearance in elderly males compared to females (10.50 *vs* 8.25 mL/h; P=0.006). In the RICOVER-60 trial, elderly females had a higher 3-year PFS (68% *vs.* 61%) and OS (74% *vs.* 68%) than male pts. due to a greater outcome improvement by the addition of R in females. In a multivariable analysis adjusting for IPI, the male hazard for progression was not significantly increased after CHOP (HR=1.1; P=0.348), but was significantly higher after R-CHOP (OR=1.6; P=0.004). In contrast, young males treated in the MInT and Mega-CHOEP trials benefitted as much as females from the addition of rituximab, with a similar male hazard after CHOP and R-CHOP (HR=1.2) with no significant difference to female patients (HR_{PFS}=1.2, P=0.552; HR_{OS}=1.0; P=0.898).
Summary and Conclusions: While no differences in R clearance and benefit from rituximab were found in young female compared to male patients, the reduced benefit of adding R to CHOP in elderly male DLBCL pts. who have a shorter rituximab serum half life and hence lower serum levels suggests that this subpopulation is suboptimally dosed when R is given based on body surface area at 375 mg/m^2. Appropriately designed prospective DSHNHL studies investigate whether higher R doses for pts. with a shorter R serum half life can improve the outcome of the respective patients.
Supported by Deutsche Krebshilfe and Roche.

P849

THE BCL-2 INHIBITOR ABT-199 (GDC-0199) IS ACTIVE AND WELL-TOLERATED IN PATIENTS WITH RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA AND OTHER NON-HODGKIN LYMPHOMAS

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Background: New treatment options are needed for patients with relapsed or refractory lymphomas, particularly those such as mantle cell lymphoma (MCL) where curative options are limited. The anti-apoptotic protein BCL-2 is highly expressed in many non-Hodgkin lymphoma (NHL) subtypes, including MCL. Because BCL-2 plays a critical role in NHL pathogenesis and treatment resistance, it is a promising target for therapeutic intervention. A first-generation BCL-2 inhibitor, navitoclax, showed modest activity in indolent lymphoma, but co-inhibition of BCL-x_L resulted in dose-limiting thrombocytopenia, precluding the full exploration of BCL-2 inhibition with this agent in NHL. ABT-199 is an orally bioavailable, second-generation BH3-mimetic that potently inhibits BCL-2 (K_i<0.10 nM). Unlike navitoclax, which has similar activities against BCL-2 and BCL- x_L, ABT-199 has 500-fold less activity against BCL-x_L (K_i=48 nM). It demonstrates potent activity against a variety of human cell line and xenograft models of a range of B-cell NHL, including MCL.
Aims: The primary objectives of this phase-I dose-escalation study are to evaluate the safety and pharmacokinetics (PK) of ABT-199, determine a maximum tolerated dose and recommended phase 2 dose, and assess efficacy and biomarkers. We report results to date for patients in the R/R NHL arm.
Methods: Adult patients (pts) requiring therapy, with ECOG performance status ≤1, and adequate marrow function (ANC ≥1.0 × 10^9/L, Plts ≥50 × 10^9/L) received ABT-199 on Week 1 Day -7 (W1D-7), followed by continuous once-daily dosing from W1D1, until progressive disease or unacceptable toxicity. Due to concerns for potential tumour lysis syndrome (TLS), a 2 to 3 wk lead-in period with stepwise escalation to the target cohort dose was implemented. Evaluations include adverse events (AE; NCI-CTCAE-V4), PK parameters and disease responses (IWG 2007 criteria). Dose cohorts up to 900 mg have been evaluated to date.

Table 1.

Characteristic	All NHL n=31	MCL Subset n=8
Age (yr)†	68 [35-85]	72 [35-75]
Male#	20 (64.5)	6 (75)
Number of prior therapies†	3 [1-7]	2.5 [1-7]
Bulky disease (>= 5 cm)#	13 (42)	6 (75)
Bulky disease (>= 10 cm)#	4 (13)	1 (13)
Time on study (days)†	143 [14-448]	160 [56-291]
Response		
Time to first 50% reduction in nodes (days)†	42 [36-113]	42 [36-113]
Best response‡	n=29	n=8
CR	1 (3)	0 (0)
PR	15 (52)	8 (100)
SD	8 (28)	0 (0)
PD	5 (17)	0 (0)
Response Rate (CR + PR)	55%	100%

†median [range]; #n(%); ‡Evaluable pts completed at least a W6 assessment
CR achieved in 1/7 pts with diffuse large B-cell lymphoma (DLBCL)
PRs achieved in 3/3 Waldenstrom macroglobulinaemia, 2/9 follicular lymphoma and 2/7 DLBCL pts

Results: As of January 2013, 31 pts have been enrolled, 8 (26%) with MCL (Table 1). The most common AEs of any grade (≥15% of pts) were nausea (36%), diarrhea (26%), dyspepsia, vomiting, fatigue, pyrexia and cough (16% each). Grade 3/4 AEs occurring in >1 pt were anaemia, neutropenia (4 pts each), febrile neutropenia (2 pts) and thrombocytopenia (3 pts). Gr 3/4 throm-

bocytopenia was not dose-dependent. Two of 14 pts in cohort 5 experienced DLTs (Gr 3 febrile neutropenia and Gr 4 neutropenia) at the target dose of 600 mg. Gr 3 TLS was seen after the initial dose in 1 pt with bulky MCL (largest lymph node >10 cm). 17 pts have discontinued drug: 13 due to progression, 2 due to AEs and 2 proceeded to stem cell transplantation in ongoing response. After a single dose with high-fat meal, ABT-199 had T_{max} ~ 8 hrs and $T_{1/2}$ ~ 15 hrs. Food increased ABT-199 bioavailability by 3-4 fold.
Preliminary efficacy data are summarised (Table 1).
Summary and Conclusions: ABT-199 shows a high efficacy signal in MCL, with all 8 pts to date achieving PR. The drug is generally well-tolerated, and shows activity in several NHL subtypes, with an overall response rate of 55% in this R/R population. Additional dosing and scheduling modifications are being explored in both monotherapy and combination studies in NHL to optimize the balance between the safety and efficacy of this active new agent. Updated results will be presented. (study status see clinicaltrials.gov).

P850

LENALIDOMIDE PLUS RITUXIMAB-CHOP21 IN ELDERLY UNTREATED DIFFUSE LARGE B-CELL LYMPHOMA IS PROMISING: LONG TERM RESULTS OF THE PHASE II REAL07 STUDY OF THE FONDAZIONE ITALIANA LINFOMI (FIL)

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Background: Standard treatment for elderly diffuse large B-cell lymphoma (DLBCL) patients is R-CHOP21, however up to 40% of patients fail. In the phase I trial REAL07 conducted by FIL, 15 mg lenalidomide from day 1 to day 14 was identified as the maximum tolerated dose (MTD) in combination with R-CHOP21,showing that the association of Lenalidomide plus R-CHOP21 (LR-CHOP21) is safe and feasible in elderly untreated DLBCL patients.
Aims: Based on the phase I results, FIL conducted a phase II trial REAL07 aimed at evaluating efficacy of LR-CHOP21 combination. Primary endpoint was an improvement of overall response rate (ORR) of 15% in LR-CHOP21 compared to 70% of standard R-CHOP21.
Methods: Phase II was designed according to Simon's two stage design. Response was evaluated according to 2007 Cheson criteria. PET scan was mandatory at the end of the treatment; patients in partial remission (PR) who underwent radiotherapy were considered as failure in progression free survival (PFS) analysis. Inclusion criteria were: age 60-80 FIT at the comprehensive geriatric assessment; untreated CD20+ DLBCL; Ann Arbor stage II/III/IV; international prognostic index (IPI) at low-intermediate/intermediate-high/high (LI/IH/H) risk. Treatment plan was: R-CHOP21 plus 15 mg lenalidomide from day 1 to 14 for 6 courses. All cases were reviewed by expert pathologists and tissue samples were centrally collected. With the aim to correlate outcome with cell of origin profile and biological markers, immunohistochemistry for Hans' and Choi criteria and gene expression profile were planned and are in advanced status of analysis at the time of the submission of the present abstract.
Results: From April 2010 to May 2011, 49 patients were enrolled. Clinical characteristics were: median age 69 years (range 61-80); stage III/IV 43 (88%), performance status >1 31 (63%), IPI IH/H 30 (61%). At the end of 6 LR-CHOP21, ORR was 45/49 (92%). Complete remissions (CR) were 42 (86%) and PR 3 (6%); 3 patients (6%) did not respond and one (2%) died for violent death. At a median follow-up of 22 months, 2-year overall survival (OS) was 92% (95%CI: 79-97) and 2-year PFS was 73% (95%CI: 57-84). According to IPI risk, 2-year PFS for IPI LI was 84% (95%CI: 59-95) and for IPI IH/H 65% (95%CI: 41-81). PFS analysis according to cell of origin profile is ongoing. Hematological toxicity was mild: grade III/IV thrombocytopenia occurred in 13% of the 277 courses performed, anemia in 5% and neutropenia in 31%, with only 4% of febrile neutropenia. No grade IV extra-hematological toxicities were observed. Grade III non-hematological toxicities were reported in 7 patients: cardiologic, gastroenteric and renal in one patient respectively, grade III neurological toxicities, sensory and motorial neuropathy in two, thromboembolic event in one and infection in one. No toxic deaths occurred during treatment. One patient died three months off therapy while in CR, due to aeromonas hydrophila sepsis and multi-organ failure.
Summary and Conclusions: With a still limited follow-up time, our data compare favorably with historical R-CHOP21 data. LR-CHOP21 is able to induce a high 2-yr PFS (73%) and it is effective also in poor risk patients (65%). The addition of lenalidomide to R-CHOP21 is safe without unexpected toxicities. These encouraging data warrant a future phase III randomized trial comparing LR-CHOP21 *vs.* R-CHOP21 in elderly untreated DLBCL.

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PHASE 1B STUDY COMBINING IBRUTINIB WITH RITUXIMAB, CYCLOPHOSPHAMIDE, DOXORUBICIN, VINCRISTINE, AND PREDNISONE (R-CHOP) IN PATIENTS WITH CD20-POSITIVE B-CELL NON-HODGKIN LYMPHOMA (NHL)

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Background: Ibrutinib, a first-in-class oral Bruton's tyrosine kinase inhibitor, has demonstrated single-agent activity in a variety of relapsed or refractory B-cell malignancies with limited toxicity, making it an appropriate drug to combine with standard R-CHOP chemotherapy in patients with previously untreated NHL.
Aims: The primary objective of this study was to determine the recommended phase 2 dose (RP2D) of ibrutinib in combination with standard R-CHOP (IR-CHOP). Secondary objectives were to assess safety, overall response rate, pharmacokinetics, and pharmacodynamic biomarkers.
Methods: Patients received a daily oral dose of ibrutinib (280, 420, or 560 mg) in combination with standard doses of R-CHOP (rituximab, cyclophosphamide, doxorubicin, and vincristine on day1, and prednisone on days 1 through 5 of each 21-day cycle for up to 6 cycles).
Results: Seventeen patients (7,4, and 6 in increasing ibrutinib doses) were enrolled: 59% male, median age 65 (range 46-81) years, diffuse large B-cell lymphoma 47%, mantle cell lymphoma 29% and follicular lymphoma 24%. In the 280 mg cohort, 2 patients had dose-limiting toxicity (DLT): 1 with transient syncope and 1 with periorbital cellulitis; at 560 mg, 1 patient had gastritis (grade 2). The RP2D was established at 560 mg ibrutinib. The most common (≥20% of patients) adverse events (AEs) were neutropenia (77%), thrombocytopenia (65%), vomiting (59%), anemia (53%), nausea (47%), fatigue (35%), headache (29%), constipation (24%), diarrhea (24%), and dizziness (24%). To date, 6 patients completed 6 cycles of treatment, and 2 patients discontinued treatment (1 due to noncompliance with study drug and 1 due to non-DLT AE). At the time of this analysis, of the 10 patients with at least one post-baseline tumor assessment, the overall response rate was 100% (7 complete and 3 partial responses).
Summary and Conclusions: The combination of IR-CHOP has an acceptable safety profile. No new toxicities were noted with adding ibrutinib to R-CHOP. An expanded 560 mg ibrutinib cohort (RP2D) is being opened to further explore the safety and efficacy of IR-CHOP in patients with newly diagnosed diffuse large B-cell lymphomas.

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PROSPECTIVE COHORT STUDY FOR SECONDARY CENTRAL NERVOUS SYSTEM INVOLVEMENT IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS TREATED WITH RITUXIMAB-CHOP

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Background: Secondary central nervous system (CNS) involvement is a serious complication encountered during the management of diffuse large B-cell lymphoma (DLBCL) patients. Although many studies were performed to evaluate the secondary CNS involvement in DLBCL, the majority of data were from retrospective analyses. Thus, there is a few data about the prospectively monitored patients who were treated with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone).

Aims: We analyzed the incidence of secondary CNS involvement in pathologically confirmed DLBCL patients enrolled in the Prospective Cohort Study (Risk-adapted Central Nervous System Evaluation in DLBCL, PROCESS study, NCT01202448).

Methods: Patients should be treated with at least one cycle of R-CHOP, and provide written informed consents. We assessed the risk of CNS involvement based on previously reported risk factors: serum LDH elevation, the number of extranodal involvements, serum albumin, bone marrow invasion, HIV positivity, the involvement of testis, breast, paranasal sinus, bone, retroperitoneal lymph nodes, orbit, and epidural space. If patients had any of these risk factors, they underwent CSF study to screen the CNS involvement. If necessary, brain MRI was also done. CNS prophylaxis was done with intrathecal chemotherapy with methotrexate for patients who had positive findings of screening evaluation or were determined to have a risk of CNS involvement based on physicians' decision.

Results: 600 patients were enrolled between 2010 and 2012 from 27 institutions belonged to the Consortium for Improving Survival of Lymphoma. Seven patients including withdrawal of informed consents were excluded. Thus, 593 patients were prospectively monitored with the median follow-up duration of 12.8 months. The median age was 59.5 years old (range 20-89 years), and stage III/IV accounted for 49.2% (109/186). 208 patients (34.7%) involved two or more than two extranodal sites including bone marrow (n=76, 12.7%) and 280 patients showed the elevation of serum LDH (46.7%). Thus, 204 patients (34.4%) belonged to high or high-intermediate risk of the International Prognostic Index (IPI). 390 patients had at least one of risk factors for CNS involvement (65.8%), and 198 patients underwent CNS evaluation at diagnosis. The screening CNS evaluation including CSF exam and brain MRI revealed that 19 patients initially had the evidence of CNS involvement even though they did not have any neurologic symptoms: positive cytology (n=15) and brain parenchyma lesion (n=4). During follow-up, 14 cases of additional CNS involvement including brain parenchyma (n=8), leptomeningeal (n=5), and ocular invasion (n=1) were observed. The median time to CNS event in these 14 patients was 7.5 months (range 1.2–15.9 months). Thus, 33 cases of secondary CNS involvement were documented in our study population at the time of analysis (5.5%) including 19 cases at diagnosis (3.2%) and 14 cases during follow-up (2.4%). Out of 390 patients with any risk factors for CNS involvement, 28 patients developed CNS involvement (28/390, 7.2%) whereas only 5 cases of CNS involvement were found in 203 patients without risk factors (2.5%). The univariate analysis for evaluation of risk factors demonstrated serum LDH, the number of extranodal involvements, bone marrow invasion, and the involvement of retroperitoneal lymph node, breast, paranasal sinus and orbit were significantly associated with CNS involvement. The high/high-intermediate risk of IPI was also predictive of CNS involvement ($P<0.05$).

Summary and Conclusions: The incidence of secondary CNS involvement in DLBCL patients treated with R-CHOP was around 5%, and a half of cases had CNS involvement at diagnosis. Considering a particular risk of CNS involvement of disease-related factors, risk-adapted active screening against CNS involvement may help to improve treatment outcome of patients with DLBCL.

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INTERIM PET-CT SCAN AFTER 2 CYCLES SUCCESSFULLY PREDICTS OUTCOME IN DLBL BUT INTRODUCES SIGNIFICANT SELECTION BIAS.

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Background: Cure rates of 60-80% have been achieved with R-CHOP in DLBL, but robust prognostic indicators are lacking. Following reports that interim PET-CT negativity after 2 chemotherapy cycles (iPET2) is strongly associated with improved PFS, we adopted iPET2 as a standard investigation in our centre. A potential bias of iPET2 is that patients with highly aggressive disease who die before iPET2 or are considered too frail for standard management have not been included in recent analyses.

Aims: The aims of this study were to evaluate the predictive value of iPET2 and investigate the possibility of patient selection bias in iPET2 patients.

Methods: We performed a retrospective study of DLBL patients treated with R-CHOP in our centre examining the relationship of iPET2 to PFS. We then compared outcomes of patients who had iPET2 to a historical control group to investigate the possibility of selection bias in iPET2 patients.

Results: The first 50 patients with DLBL and iPET2 were identified, with a median follow up of 24 months. 17 (34%) were iPET2 positive/indeterminate and 33 (66%) were iPET2 negative. 1/33 (3%) iPET2 negative patient had relapsed and 2/33 had died from non-NHL causes. 7/17 (41%) iPET2 positive patients had relapsed (6/7 biopsy-proven, $P=0.001$). At the time of analysis iPET2 negative patients had a significantly better PFS than iPET2 positive patients (47 *vs* 24 months, $P=0.009$, see Figure 1).

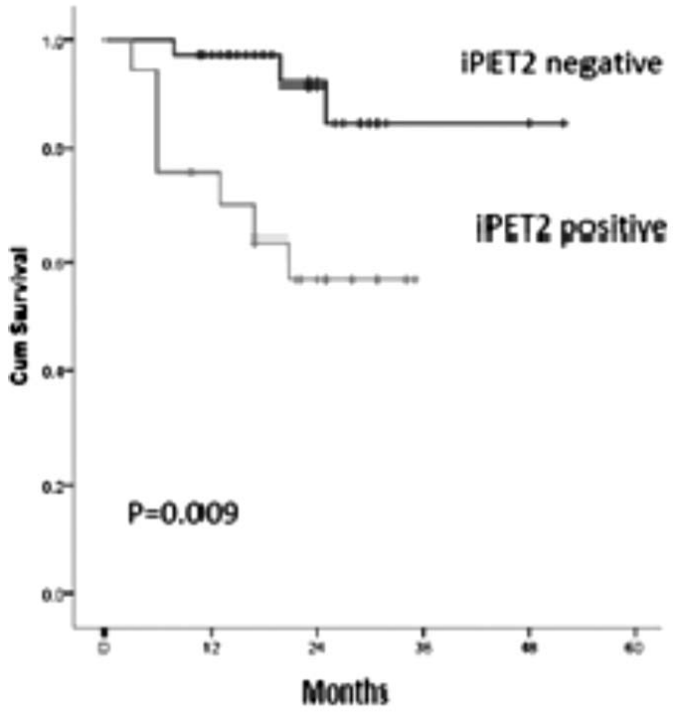

Figure 1. PFS of iPET2 negative *vs* iPET2 positive patients.

There was no significant difference in OS between iPET2 negative and positive patients at this follow up point (P=NS, data not shown). Of the 7 patients who relapsed/progressed, 1 has been salvaged and is in remission, 3 are being palliated, 2 have died, and 1 is being salvaged. Comparing the 50 patients who received iPET2 with 301 unselected DLBL patients managed over an 8 year period from 2004, the iPET2 patients have a significantly higher OS (53 *vs* 38 months, $P=0.006$, see Figure 2), indicating that iPET2 patients represent a selected group with a better prognosis.

Summary and Conclusions: These results confirm that iPET2 is valuable in predicting outcome in DLBL, but it is important when evaluating such studies to bear in mind that significant selection bias may be present.

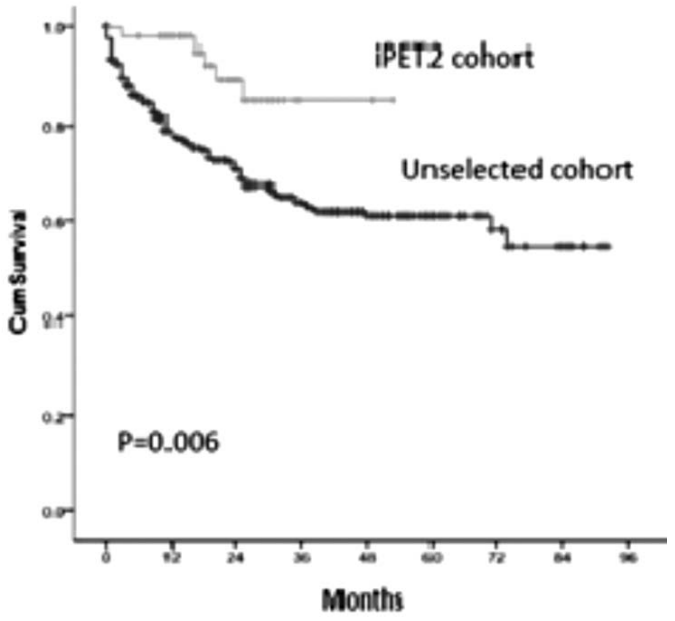

Figure 2. OS of patients receiving iPET2 *vs* unselected cohort.

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STAGING NEWLY DIAGNOSED LYMPHOMAS: COMPARISON BETWEEN WHOLE BODY-MRI/DWIBS AND 18FDG-PET/CT

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Background: FDG-PET/CT is currently regarded as the reference standard in the staging of HL and high-grade NHL. It's role is confirmed also in the staging of Follicular Lymphoma but becomes less important in other histopypes. A recent development of MRI is whole-body diffusion-weighted imaging (DWI), whose potential advantage over conventional MRI sequences in the evaluation of lymphoma is the high lesion-to-background contrast possible because of the relatively low diffusivity of lymphomatous tissue. MRI does not entail ionizing radiation and may be complementary to FDG PET/CT in the staging of lymphoma.
Aims: The aim of this prospective study was to assess the role of whole body-MRI/DWIBS in the staging of newly-diagnosed lymphomas (HL and NHL) in comparison to 18F-FDG-PET/CT.
Methods: 25 consecutive pts were enrolled and underwent WB-MRI (coronal T1-weighted, coronal STIR and axial DWIBS sequences) and 18F-FDG-PET/CT. Axial DWIBS sequences included 3 acquisitions of b factor (0, 500, 1000). Lymph nodes larger than 10 mm in short-axis diameter, in the coronal plane on T1- weighted and STIR images, were considered positive. The agreement between WB-MRI/DWIBS and 18F-FDG-PET/CT for each of the nodal and extranodal sites was evaluated. A statistical evaluation with the Cohen k was performed.
Results: 18F-FDG-PET/CT showed 75 involved nodal and 10 extranodal lesions. WB-MRI/DWIBS showed 80 nodal and 12 extranodal lesions, revealing an overstaging in 2/25 (8%) pts. In 10\25 pts we found differences in the attribution of disease in each of the stations described. Of these 10 pts, 5 had a diagnosis of HL, 5 of NHL (2 DLBCL, 1 FL, 1 SLL/CLL, 1 sMZL). The disagreements were 21: 4 cervical, 2 mediastinum, 5 abdominal, 3 pelvic, 3 femoral stations, 4 extra-nodal sites. In 6\21 disagreements observed, 18FDG-PET/CT was positive for lymph nodes with a diameter less than 1 cm, with negative WB-MRI/DWIBS. In 2\21 discrepancies 18FDG-PET/CT was positive, with negative MRI, for a cervical lymph node localization with a diameter greater than 1 cm and a bone marrow localization. In 13\21 disagreements described MRI was positive, with negative 18FDG-PET/CT, for nodal and extra-nodal localizations (2 cervical, 1 mediastinal, 3 abdominal, 2 pelvic, 2 femoral and 3 extranodal). Then, the two methods were concordant in the staging in 22/25 cases (88%). According to Ann Arbor criteria, the agreement was good. 2/25 (8%, 1 DLBCL and 1 SLL/CLL) patients showed a different stage with the 2 techniques, with an overstaging by WB-MRI/DWIBS (Figure 1).

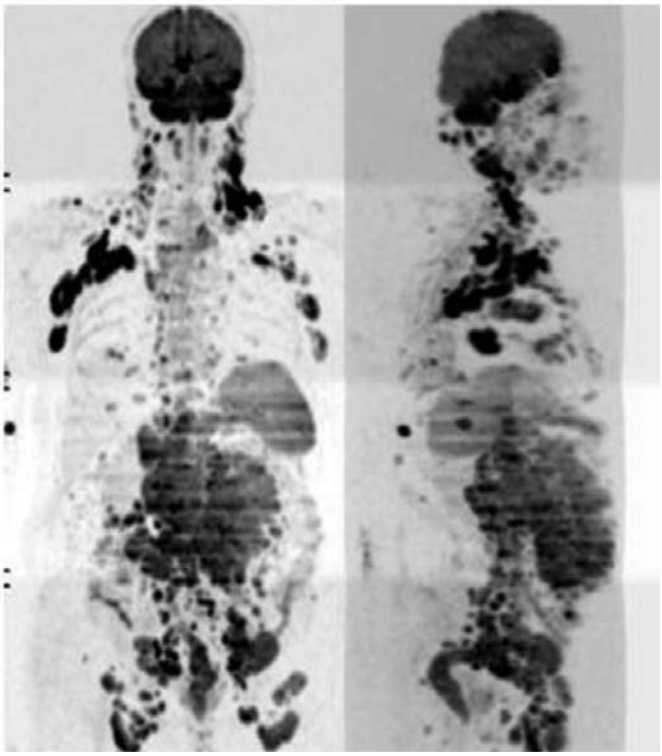

Figure 1.

Summary and Conclusions: Our initial results show a good agreement between whole-body MRI-DWI and 18F-FDGPET/CT in lymphoma staging, both in the evaluation of nodal and extranodal involvement. Although 18F-FDG-PET/CT remains the gold standard for lymphoma staging, WB-MRI/DWIBS may be considered an emerging functional whole-body imaging modality. This new technique may provide complementary information and, due to the low toxicity profile, become a useful tool also in the follow-up.

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CENTRAL NERVOUS SYSTEM RELAPSE IN NON HODGKIN LYMPHOMA: PROGNOSTIC FACTORS AND OUTCOME IN A COHORT OF 304 PATIENTS

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Background: The efficiency and the criteria for prophylaxis of central nervous system(CNS) relapse in non-Hodgkin lymphoma are not well defined. There are conflicting results related to the heterogeneity of analysed patients. It is doubtful whether Rituximab can reduce the frequency of neurological relapse
Aims: To analyse the baseline predictive variables of CNS relapse in a series of unselected patients with clinically aggressive mature B and T cell lymphomas (CAMBTCL), its clinical presentation and outcome
Methods: This is a prospective observational study of patients diagnosed in our Lymphoma Unit with CAMBCL from 1988-January to 2007-December.The follow up was maintained until 2012-December.The patients with Burkitt's lymphoma, AIDS or initial neurological involvement were excluded. We evaluated the usually related variables: bone marrow(BM) disease, two or more extranodal sites(E), testicular or mediastinal involvement, proximity to CNS, advanced clinical stage or IPI, high LDH or B2microglobulin,effect of chemoprophylaxis and, in B-lymphomas, treatment with Rituximab. We scheduled chemoprophylaxis (systemic or intrathecal methotrexate or cytarabine) in patients with unfavorable prognostic factors (histologically affected bone marrow, elevated LDH, testicular, mediastinal or paranasal sinuses involvement).The CNS disease was documented by clinical data, cerebrospinal fluid (cytology and flow cytometry) findings, CT or MNR, and biopsy in case of isolated brain involvement.The CNS disease treatment included high dose methotrexate and triple intrathecal chemotherapy, associated with other drugs in case of extraneurological disease. *Statisticals Methods*: Fisher's exact test, log rank test, Kaplan Meier survival tables and Cox multivariate proportional hazard regression.The study was approved by the hospital ethics committee

Results: 304 patients (165 males, 139 females) were included, with median age of 64 years:227 with diffuse large B cell lymphoma (DLBCL), 35 with mantle cell lymphoma(MCL) and 42 with peripheral T cell lymphoma(PTCL).Involvement of BM (histological or by flow cytometry) was ascertained in 20% of DLBCL, 65.7% of MCL and 50% of PTCL and two or more extranodal involvement(E) was demonstrated in 32%, 43% and 40.5% respectively. Neuroprophylaxis(systemic or intrathecal) was applied to 23.5% of DLBCL patients, 28,6% of MCL and 33,3% of PTCL, and Rituximab in 47%(107 patients) of DLBCL and 51.4% of MCL.The median observation time has been 5 years (2.8 in PTCL) and actuarial mean survival has been 10 years in DLBCL, 5.9 in MCL and 2.5 in PTCL.We found neurological relapse in 12 patients with DLBCL (5%), 2 with MCL (5.7%) and 3 (7%) with PTCL, at a median time of 8.4 months from the diagnosis.There was meningeal involvement in 76% and brain involvement in 35% (two patients had both locations). Six relapsed patients (35%) had received neuroprophylaxis(none with testicular involvement and one of 14 with mediastinal disease).Mean actuarial survival from relapse was 4.4 months. In multivariate analysis of DLBCL only two prognostically significant variables were found: involvement of two or more extranodal areas(p:0.001; HR:12) and treatment with rituximab (p:0.048; HR: 0.25);also in univariate analysis bone marrow involvement (LRT: p: 0.03) and IPI advanced (LRT: p: 0.02) were significant
Summary and Conclusions: The relapse rate in our series (5.6%) is at the low level ofthe published range.Despite a high proportion of patients with neuroprophylaxis, two or more extranodal involvement implies bad prognostic.On the contrary in our patients treatment with Rituximab reduces the likelihood of neurological relapse, as it has been reported in other studies

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LYMPHOCYTE COUNT AT DIAGNOSIS IN DIFFUSE LARGE B-CELL LYMPHOMA IN THE ERA OF IMMUNOCHEMOTHERAPY WITH RITUXIMAB: IS 1000/MM3 STILL THE BEST CUT-OFF VALUE TO USE TO DEFINE LYMPHOPENIA ?

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Background: Lymphopenia is a well-established prognostic value in advanced Hodgkin lymphoma, and constitutes one of the parameters included in the International Prognostic Score. In non Hodgkin lymphoma, lymphocyte counts have mostly been evaluated in aggressive histology and low levels have been shown to be associated with inferior overall survival.
Aims: In this multi-center collaborative study on a large cohort of 1191 patients with diffuse large B-cell lymphoma (DLBCL) we assessed the prognostic significance of the absolute lymphocyte count (ALC) at diagnosis, in an attempt to verify if the widely used cut-off level of 1000 /mm^3 if retained it's prognostic significance in the era of immuno-chemotherapy with rituximab. In addition we also examined the possible correlation between ALC values and International Prognostic Index(IPI).
Methods: In our research all relevant clinical and laboratory data at diagnosis were retrieved from the medical records of consecutive untreated patients with DLBCL followed in different centers in Haifa, Israel and Italy during 1993-2010. After merging the data from independent but homogeneous database sources, we were able to evaluate a final cohort of 1017 patients. The median follow up was 48 months and the 5 year overall survival (OS) rate was 68%; 521 (51%) patients were treated with CHOP or CHOP- like regimens in combination with rituximab.The influence of rituximab on the prognostic power of ALC was evaluated by interacting ALC with rituximab use after adjusting by IPI score.
Results: The median ALC at diagnosis was 1450/mm^3 (300-3730). After correlation with IPI in patient groups with scores 0-1, 2 and 3-5, the median ALC was 1645, 1347, and 1195/mm^3, respectively (P<0.001). The threshold value of ≤1000/mm^3 is widely used to define lymphopenia in cases of aggressive lymphoma. In the large study cohort the difference in terms of OS at 5 years for patients with ALC ≤ or >1000/mm^3 did not reach statistical significance (P=0.082), with HR of 1.23 (95% CI 0.97-1.55). In multivariate analysis the ALC level of ≤1000/mm^3, after interaction with rituximab use and adjusting by IPI score, did not have a prognostic impact, both in patients treated with chemotherapy with or without the addition of rituximab to the combination regimen (HR 1.10, 95% CI 0.79-1.52 and HR 1.09, 95% CI 0.78-1.53, respectively). However when we defined lymphopenia as an ALC ≤840/mm^3, (as reported in our previous studies), we found that the difference in OS in all patients with ALC ≤ or >840/mm^3 was statistically significant (P=0.002). An ALC level of ≤840/mm^3 was found to be significant only in patients treated with rituximab (P<0.001), compared to those treated with chemotherapy alone (P=0.360).
Summary and Conclusions: In this study on a large cohort of patients with DLBCL, ALC values correlate with IPI score (decreasing values of lymphocytes are related to IPI high risk groups) but, unlike previous studies, ALC ≤1000/mm^{3}did not provide convincing support for its use as a reference value to define lymphopenia. It appears that in the rituximab era of immunochemotherapy we need to reassess the reference cut-off value utilized for ALC, which remains a simple but important tool to identify high–risk patients with a worse survival in newly diagnosed DLBCL.

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TREATMENT OUTCOMES IN AIDS-RELATED DIFFUSE LARGE B-CELL LYMPHOMA AT A PUBLIC TEACHING HOSPITAL IN SOUTH AFRICA

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Background: Non-Hodgkin lymphoma is the second most common acquired immune deficiency syndrome (AIDS)-defining malignancy. Sub-Saharan Africa is the worst affected region in the HIV pandemic, and many South Africans are at risk of developing AIDS defining non-Hodgkin lymphomas (ADL) such as Burkitt lymphoma (BL) or Diffuse Large B-cell Lymphomas (DLBCL). The high frequency of ADL in this region is, in part, due to incomplete community-wide antiretroviral coverage. Curative therapy for AIDS-related diffuse large B-cell lymphoma (DLBCL) is feasible in settings with available combination antiretroviral therapy (cART). However, given limited haemato-oncology resources, outcomes for AIDS-associated DLBCL in South Africa are unknown.
Aims: Our primary objective was to document 2-year overall survival (OS) in South African patients with AIDS-related DLBCL treated with CHOP or CNOP at an academic institution. Secondary objectives included evaluation of response rates, progression free survival (PFS) and prognostic factors for death.
Methods: We performed a retrospective analysis of survival in patients with newly diagnosed AIDS-related diffuse large B-cell lymphoma (DLBCL) treated at a tertiary teaching hospital in Cape Town, South Africa with CHOP or CHOP-like chemotherapy (January 2004 until Dec 2010). HIV and lymphoma related prognostic factors were evaluated. This study was approved by the Health Research Ethics Committee and complies with Principles of the Declaration of Helsinki.
Results: 36 patients were evaluated; median age was 37.3 years, 52.8% men and 61.1% black South Africans. Median CD4 count was 184 cells/μL and 80% were high-risk according to the age-adjusted International Prognostic Index. Concurrent Mycobacterium tuberculosis infection was 25%. Two-year overall survival (OS) was 40.5% (median OS 10.5 months, 95%CI 6.5–31.8). ECOG performance status of 2 or more (25.4% *vs* 50.0%, *P*=0.01) and poor response to cART (18.0% *vs* 53.9%, *P*=0.03) predicted inferior 2-year overall survival. No difference in 2-year OS was demonstrated in patients co-infected with either Mycobacterium tuberculosis or Hepatitis B.
Summary and Conclusions: Two-year OS for patients with AIDS-related DLBCL treated with CHOP like regimens and cART is comparable to that seen in the US and Europe. Important factors effecting OS in AIDS-related DLBCL in South Africa include performance status at presentation and response to cART. Patients with co-morbid Mycobacterium tuberculosis or hepatitis B seropositivity appear to tolerate CHOP in our setting. Additional improvements in outcomes are likely possible.

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ELEVATED SERUM ADENOSINE DEAMINASE LEVELS IN HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Background: Hemophagocytic lymphohistiocytosis (HLH), a potentially fatal hyperinflammatory condition, is caused by activated T cells and macrophages. Adenosine deaminase (ADA) plays an important role in immune regulation, especially in the proliferation, maturation and differentiation of lymphocytes. Its role has been studied in inflammation and malignancy.
Aims: Here we present for the first time the alteration of ADA in secondary HLH (sHLH).
Methods: Serum ADA levels were measured in 20 cases with lymphoma-associated hemophagocytic syndrome (LAHS), 15 with infection-associated hemophagocytic syndrome (IAHS), 6 with macrophage activation syndrome (MAS) as experimental group. Additionally, we enrolled 20 cases with lymphoma, 15 with infection, 6 with autoimmunity who were all not associated with HLH as conditional control group and 20 healthy subjects as blank group. We also demonstrated the ADA levels in 20 LAHS cases and 21 benign disease-associated HLH cases (IAHS and MAS).

Results: Serum ADA levels were 150.29±76.08, 91.33±44.38 and 57.40±11.30 U/L in patients with LAHS, IAHS and MAS, respectively. In control groups, the levels of ADA were 19.13±11.78, 20.57±8.10, 22.62±8.69 and 12.36±4.07 U/L for patients with lymphoma, infection, autoimmune disease who were not associated with HLH and healthy people. Serum ADA levels were significantly higher in LAHS, IAHS, and MAS patients compared to the control groups (P<0.05). Serum ADA levels of LAHS patients were significantly higher than IAHS and MAS cases (P<0.05). The optimum ADA cut-off point for LAHS was 89.25 U/L, with a sensitivity and specificity of 85.0% and 76.2% respectively.
Summary and Conclusions: In our study, serum ADA levels were increased in sHLH suggesting a partial role of activated T-cell response in the disease pathophysiology. Serum ADA levels were particular high in LAHS and it may be a new serum marker for the diagnosis of LAHS.

P859

PROGNOSTIC FACTORS IN VERY OLD PATIENTS (≥75 YEARS) WITH FOLLICULAR LYMPHOMA: A MULTICENTRE RETROSPECTIVE STUDY
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Background: The incidence of B cell Non Hodgkin Lymphoma (NHL) is steadily increasing with age and about 40% of cases occur in patients aged over 70 years. Some series have reported that low grade NHL lymphomas represent about 35% of all B NHL in elderly patients. Follicular lymphoma (LF) is the most common indolent lymphoma. Data on outcome, prognostic factors, and treatment for elderly LF is sparse.
Aims: The aim of our study was to analyse the overall survival (OS) and the progression free survival (PFS) of elderly LF and to evaluate the prognostic impact of different clinical and biological factors.
Methods: We conducted a multicentre retrospective analysis of LF patients ≥75 years followed between 2006 and 2012. Detailed characteristics were obtained including age adjusted Charlson Comorbidity index (aaCCI), FLIPI and biological parameters.
Results: We identified 69 patients: median age was 79 years (75-98); 49% were FLIPI 1-2 and 51% FLIPI 3-5. Median ECOG was 1 (0-4). One third presented B symptoms; 46% had extranodal site (most frequently pleural, skin and bone lesions), 27% presented elevated LDH. 48% presented with aaCI ≥5. Fourty-nine patients received chemotherapy: 64% with rituximab, 39% RCHOP like therapy. Overall response rate (CR+PR) was 77% with 52% of CR. Median follow up was 26.4 months (0.5-78), 2-year overall survival (OS) was 73%: 88% and 58% for FLIPI 1-2 and 3-5 respectively (P=0.003). Two-year progression free survival (PFS) was 74%: 94% and 52% for FLIPI 1-2 and 3-5 respectively (P=0.0007). 24 deaths were reported: 12 of them secondary to FL progression, 8 to toxicity. After RCHOP and RCVP 15 pts (34%) had grade 3/4 haematological toxicity, 10 (23%) had febrile neutropenia and 3 (7%) acute cardiac failure. Univariate analysis identified aaCCI ≥5 (hazard ratio (HR) 4.5; 95% IC 1.6-12.8; P=0.002), FLIPI ≥3 (HR 5.3; 95% IC 1.9-14.1; P=0.003) and serum albumin (SA) ≤35g/l (HR 2.9; 95% IC 0.9-9.2; P=0.03) as predictive factors for shorter OS. On multivariate regression FLIPI ≥3, SA ≤35g/l and aaCCI ≥5 predicted inferior OS (P=0.02, P=0.04, P=0.002 respectively) (Table 1).

Table 1.

Median Age (years)	79 (75-98)
Median ECOG	1 (0-4)
FLIPI 1-2	34 (49%)
FLIPI 3-5	35 (51%)
Elevated LDH	17 (27%)
AaCCI ≥ 5	33 (48%)
SA ≤ 35g/l	16 (29%)
Watchful Waiting	12 (17%)
R-CHOP like regimen	27 (39%)
R-CVP	16 (23%)
R-Bendamustine	1 (1%)
Total R-containing regimen	44 (64%)
Chlorambucil	5 (7%)
Radiation	4 (6%)
Surgery only	2 (3%)
Treatment refusal	1 (1%)
Death before any treatment	1 (1%)

Summary and Conclusions: In this large cohort of elderly FL patients, we identified FLIPI ≥3, aaCCI ≥5 and SA ≤35g/l as three key predictive factors for inferior OS. Prospective studies incorporating geriatric tools are warranted for this population.

P860

EFFICACY AND SAFETY OF LENALIDOMIDE WITH/WITHOUT RITUXIMAB OR STEROIDS IN HEAVILY PRETREATED NON-HODGKIN'S B-CELL LYMPHOMAS: RESULTS OF A RETROSPECTIVE STUDY
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Background: Relapsed or refractory B-cell non-Hodgkin's lymphomas (NHL) had a poor prognosis, however for patients not eligible to high dose chemotherapy, salvage treatment is not yet clearly defined. Lenalidomide monotherapy exhibits significant activity in patients with relapsed aggressive NHL.
Aims: On this basis, we conducted a monocentric retrospective study to investigate efficacy and safety of lenalidomide monotherapy or in combination with rituximab (R) in patients with heavily pretreated B-Cell NHL.
Methods: The primary end point of the study was Overall Response Rate (ORR) defined as complete response (CR), partial response (PR), stable disease (SD) and duration of response (DOR); secondary ends points were feasibility and safety of treatment. Inclusion criteria were: relapsed/refractory B-Cell NHL patients treated between August 2007 to June 2012 with 25 mg lenalidomide daily for 21 days every 4 weeks as single agent or in association to weekly dexamethasone (20 mg bolus) or 20 mg lenalidomide daily for 21 days every 4 weeks in combination with R (375 mg/sqm). Patients were treated until disease progression or unacceptable toxicity.
Results: A total of 54 patients were included in the analysis: 34 diffuse large B-cell lymphomas (DLBCL), 11 mantle cell, 6 follicular, 2 primitive mediastinum B-cells and one Burkitt lymphoma. Clinical characteristics at relapse before starting lenalidomide were: stage III/IV in 36 patients (67%); intermediate high/high risk International Prognostic Index in 28 (52%); bone marrow involvement in 11 (20%) and bulky disease in 15 (28%). Prior treatments were: 8 patients (15%) received lenalidomide at first relapse while 25 (46%) underwent more than 3 previous lines of treatment. Fourteen patients (26%) did autologous stems cell transplant, one (2%) allogenic transplant and 4 (7%) did both, before receiving lenalidomide. All patients analyzed received lenalidomide: 32 (59%) underwent single agent lenalidomide, 11 (21%) received a combination of lenalidomide plus R and 11 (20%) were treated with lenalidomide plus steroids. Median time from diagnosis to the beginning of lenalidomide was 25,3 months (range3,7- 145,9), while median time from last previous therapy to lenalidomide treatment was3,2 months (range 0,4- 38,0). Responses were: CR in 8 patients (15%), PR in 5 (9%), SD in 5 (9%); 32 (60%) patients did not respond and 4 were not evaluable for response. Concerning DLBCL: ORR was 41% (n=14: CR5, PR4, SD 5). Median DOR for all patients was11,7 months (range 0,2-23,7). At a median follow-up of 20 months 5 patients were alive in CR, 9 continued lenalidomide, 11 relapsed and 28 died. A total of 259 cycles of lenalidomide were performed, of which 25 were prematurely interrupted and 49 were reduced in dose or duration; 50% of patients had at least one interruption in the planned treatment, nevertheless 91% of the expected dose was given. The most common grade 3-4 adverse events for the 54 patients were neutropenia (19%), anemia (17%) and thrombocytopenia (17%); 5 patients had grade 3-4 infections, one a grade 3 thromboembolic event and 2 died because of pneumonia and heart failure, respectively.
Summary and Conclusions: Salvage treatment with oral lenalidomide as single agent or in combination with R or steroids is effective with a good safety profile in patients with heavily pretreated B-Cell NHL.

P861

RESULTS OF A PROSPECTIVE PHASE II TRIAL OF ORAL LOW DOSE BEXAROTENE AND ULTRAVIOLET A PHOTOCHEMOTHERAPY (PUVA) FOR EARLY AND ADVANCED STAGE MYCOSIS FUNGOIDES
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Background: Bexarotene, the first synthetic retinoid that selectively binds retinoid X receptors has shown efficacy and safety for the treatment of refractory or relapsed cutaneous T cell lymphomas (CTCL) both in monotherapy and in association with other treatments. Bexarotene and PUVA seemed to be promising in the early studies performed with low dosages of Bexarotene on small groups of patients. Recently EORTC randomized trial reported similar

response rates in the PUVA arm *versus* the combination arm; however the treatment duration was limited to a maximum of 16 weeks and the trial was not designed to address the role of a maintenance therapy.
Aims: The aim of this study was to assess the efficacy and safety of a 2 stage-protocol (induction and maintenance) with low dose Bexarotene (Targretin) and PUVA in refractory and/or resistant patients with Mycosis Fungoides (MF) and Sézary Syndrome (SS).
Methods: A prospective trial was conducted between February 2007 and September 2012 by two Italian academic hospital centers. We enrolled 22 patients with stages I through IV MF; all patients had failed PUVA or several systemic regimens. The treatment schedule consisted of an induction phase with low dose Bexarotene (150 mg/die) plus PUVA with clinical response assessment after two months: patients who achieved CR (complete response) remained on the same dose of Bexarotene, with gradual reduction in the frequency of PUVA sessions (maintenance phase), while patients in very good partial response (VGPR), PR (partial response), MR (minimal response) and stable disease (SD) received an increasing dose of Bexarotene, up to 300 mg/die. Subsequently, patients not achieving at least PR went off-study. Primary endpoints were overall response (ORR) at the end of induction and maintenance; secondary endpoints were safety and event-free survival (EFS). Kaplan-Meyer method and long-rank test was used for statistical analysis.
Results: We enrolled 22 patients: 15 patients were affected by early stage MF (13 with stage IB, 2 with stage IIA), 7 were affected by advanced disease (3 with stage IIB, 2 with stage IIIA, 1 with stage IIIB and 1 with stage IVA). The median age was 69 years (range, 35-82). The global overall response was 86%, higher in early MF (93%) than in advanced disease (72%). At the end of maintenance, CR rate in early stage patients remained 46% and increased from 0 to 14% in advanced stage disease. Significantly, early stage patients treated previously with PUVA monotherapy achieved CR more frequently (83% *versus* 22% respectively; $\chi 2$ test, P=0.020) and more rapidly (median time to maximal response, 4 weeks *versus* 13 weeks respectively, P=0.003) compared with those treated with more than one systemic therapy. Median EFS for the whole group was 33 months. Bexarotene was well tolerated due to side effects prophylaxis and to the progressive dose increase in induction: only one patient dropped out because of hypertriglyceridemia; adverse events were mainly of low and moderate grade; only two cases with grade 3 creatine-phosphokinase (CK) elevation was recorded.
Summary and Conclusions: In conclusion, our study has demonstrated a >80% response rate in patients with all stages of CTCL refractory or intolerant to the previous therapies; the efficacy is more pronounced in patients previously treated with PUVA monotherapy. Starting treatment at lower dose of Bexarotene followed by dose escalation may improve the efficacy and safety of the combination therapy. Moreover, the additional maintenance phase seems to improve the rate and length of response.

P862

SERUM FREE LIGHT CHAINS LEVELS IN PATIENTS WITH HIV-RELATED LYMPHOMA ARE LOWER AT COMPLETE RESPONSE THAN AT DIAGNOSIS

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Background: Increase in serum free light chains (sFLC) has been shown to be a marker of risk of developing HIV-related lymphomas. sFLC are easily measurable and are potential biomarkers for screening and prognosis in HIV-related lymphomas but there is a lack of data on sFLC values in patients in complete response (CR).
Aims: To determine the clinical relevance and the potential use of sFLC measurements as tumor markers in patients with HIV-related lymphomas.
Methods: Retrospective study of HIV-infected patients diagnosed with non-Hodgkin (NHL) and Hodgkin lymphoma (HL) treated in a single institution from 1998 to 2012. Levels of κ and λ sFLC were measured in plasma using a standardized assay (FREELITE, The Binding Site, Birmingham, UK). sFLC were considered elevated when κ, λ, or the sum of κ+λ were above the upper limit of normal and sFLC were considered monoclonal when κ-λ ratio was abnormal. Clinical and biological data were collected from the records.
Results: Sixty-eight patients were studied (53 NHL and 15 HL). At diagnosis (N=46), levels of κ, λ, and sum of κ+λ sFLC were elevated in 87%, 70%, and 80% of patients, respectively, and κ-λ ratio was abnormal in 37%. At CR (N=28), the percentage of patients with elevated κ, λ, and sum of κ+λ was 82%, 46%, and 64%, respectively. The κ-λ ratio was abnormal in 25%.
Mean levels of κ, λ, and κ+λ sFLC at diagnosis were higher than at CR (10.25mg/dL *vs* 7.01mg/dL, P=0.007; 5.56mg/dL *vs* 3.68mg/dL, P=0.034; 15.81mg/dL *vs* 10.69mg/dL, P=0.007; respectively). There were less patients with elevated λ sFLC levels at CR than at diagnosis (P=0.048). The mean values of κ-λ ratio were similar at diagnosis and CR as well as the number of patients with abnormal ratios. The levels of sFLC were compared between groups defined by the clinic-biological features. At diagnosis, less patients with increased λ sFLC were found in the group whose lymphoma was the first criteria of AIDS (N=2/7 *vs* N=30/38, P=0.015) and mean levels of λ and κ+λ sFLC were lower in this group (2.04mg/dL *vs* 6.30mg/dL, P=0.006 and 6.93mg/dL *vs* 17.75mg/dL, P=0.028, respectively). At CR, mean level of λ sFLC was lower in patients whose lymphoma was the first criteria of AIDS (N=4, 1.51mg/dL *vs* N=23, 3.97mg/dL, P=0.048). The mean levels of κ sFLC and κ+λ were higher in patients with detectable HIV loads at diagnosis (N=20) than in those with undetectable loads (N=4) (8.48mg/dL *vs* 1.75mg/dL, P=0.20 and 12.65 *vs* 3.32, P=0.036). Interestingly, all 4 patients with undetectable HIV loads had a normal sum κ+λ sFLC at CR, unlike, only 5 out of 20 patients with detectable HIV loads normalized κ+λ sFLC (P=0.005). Patients treated with cART before lymphoma (N=14) presented a lower mean level of κ sFLC than patients previously untreated with cART (N=14) (2.96mg/dL *vs* 11.05mg/dL, P=0.048). Mean level of κ-λ ratios was lower in patients previously treated with cART (1.20 *vs* 1.70, P=0.031) and all 14 patients had a normal κ-λ ratio unlike previously untreated patients, only 7 out of 14 had a normal ratio (P=0.002). Neither elevated sFLC levels nor the presence of monoclonal sFLC at diagnosis had impact on overall survival.
Summary and Conclusions: In patients with HIV-related lymphomas, sFLC levels at lymphoma diagnosis were higher than at CR pointing sFLC may be used as lymphoma markers. The control of HIV-infection was related to lower sFLC levels. Monoclonal sFLC were undetectable at CR in patients on cART before lymphoma.
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P863

PROGNOSTIC RELEVANCE OF FDG-PET IN PATIENTS WITH PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA

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Background: Primary mediastinal large B-cell lymphoma (PMBCL) present with mediastinal «bulky» tumor and often invades adjacent structures. It is commonly associated with alveolar fibrosis, which was explained frequent residual mass at the end of chemotherapy. The role of consolidative radiotherapy (RT) to the mediastinum remains unclear particularly with the more frequent use of FDG-PET.
Aims: We evaluated the impact of the introduction of PET in the outcome of pts with PMBCL.
Methods: We identified 83 pts with PMBCL treated in N.N. Blokhin Russian Cancer Research Center during last 10 years (2002-2011). The median age was 29 years, 56% of the pts were female, 71% had I/IIE stage, B symptoms had 72%, LDH elevated in 81% pts. All 83 pts treated with R-CHOP or MACOP-B±R, consolidative RT to the mediastinum was routinely administered in 65 (78%) pts. After completion of chemotherapy, residual disease measuring more than or equal to 2.5 cm in diameter was assessed by PET in 50 (60%) pts.
Results: In total, 50 pts had a PET scan: 33 (66%) were PET-negative (neg) (18 received RT) and 17 (34%) were PET-positive (pos) (14 received RT). The 5-y progression-free survival was 91% for PET-neg and 66% for PET-pos patients (P=0,001). The presence of B symptoms and extranodal disease more frequently associated with PET-pos. There were 3 relapses in PET-neg cases (2 without RT) and 7 relapses in the PET-pos cases (4 PD within RT field).
Summary and Conclusions: Positive PET is predictive of a worse outcome in PMBCL; larger prospective studies are needed to introduction of a PET-guided RT approach in PMBCL treatment. Interim PET may be useful for the early identification of pts, who need more intensify therapy.

P864

FC-GAMMA-RECEPTOR IIIA POLYMORPHISM AND GENE EXPRESSION PROFILE DO NOT INFLUENCE TREATMENT OUTCOMES AND SURVIVAL IN DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH R-CHOP PROTOCOL

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Background: The addition of rituximab to conventional chemotherapy has significantly improved the treatment outcome in diffuse large B-cell lymphoma. However, differences in treatment response and survival data can be observed independently from the International Prognostic Index scores.
Aims: The current study evaluated the impact of Fc-gamma-receptor IIIa polymorphism and gene expression profile on the response of DLBCL patients to R-CHOP therapy as well as on their survival results.
Methods: Fifty-one DLBCL patients were involved, thirty-two females, nineteen

males, their median age was 53.1 years. The FCGR3A polymorphism of NK cells at the 158. amino acid position was determined with real time PCR method. Three genotypes, V/V, V/F and F/F were distinguished. Gene expresion profile was screened for CD10, cbl-2, bcl-6 and MUM-1 on lymph node tissue samples using immunhistochemical methods.
Results: The detection of FCGR3A polymorphism showed the following results: VV: 12 cases (23.5%), VF: 29 cases (56.8%) and FF: 10 cases (19.6%), respectively. There was no significant difference between the treatment responses of the three groups. The event-free survival data were less favorable in the F-allele carriers than in V/V homozygous patients, but without any significancy, and the overall survival curves were almost the same. As for the gene expression profile, 20 patients' biopsy specimens showed germinal center and 31 showed non-germinal center characteristics. Treatment results did not differ from each other in the two groups. Both the event-free and the overall survival data were more favorable in the GC group, however the differences were not significant.
Summary and Conclusions: Our results contest the predictive value of Fc-gamma-receptor IIIa polymorphism and gene expression profile in diffuse large B-cell lymphoma.

P865

INTENSIFIED PROGRAM INCLUDING BENDAMUSTINE, HIGH DOSE THERAPY AND AUTOGRAFT FOR PATIENTS WITH RELAPSED OR RESISTANT FOLLICULAR NON HODGKIN LYMPHOMA: RESULTS FROM AN EXPLORATORY GITIL STUDY

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Background: Intensive chemotherapy with autologous stem cell transplantation (ASCT) is a potentially curative treatment for relapsed/refractory Follicular non Hodgkin's Lymphoma (FL-NHL) patients. The combination of Bendamustine and Rituximab proved to be remarkably active in previously untreated and relapsed FL-NHL patients.
Aims: To evaluate the safety of an intensified program including Bendamustine, high dose therapy and autograft for patients with Follicular Non Hodgkin Lymphoma at first relapse or resistant to first line treatment; to evaluate the benefit-risk profile of R-DHAOX and R-bendamustine in inducing a clinical response (CR or PR) in these patients. To evaluate also the feasibility of a salvage therapy in an outpatient setting before autograft.
Methods: From February 2011 to October 2012, 20 patients with relapsed (n=19) or refractory (n=1) FL-NHL were enrolled. The median age was 60 years (range 45-70). Previous therapy consisted of R-CHOP for all patients. The study treatment included: 2 cycles of R-DHAOX (oxaliplatinum 100 mg/m^2 on day1, Rituximab 375 mg/m^2 on day2, aracytin 2 g/m^2×2 and dexametasone 40 mg days 1-4) with peripheral blood stem cell harvest; 4 intensification courses of R-Bendamustine (90 mg/m^2 days1-2 every 28 days); patients who achieving PR or CR received a final consolidation with BEAM (or Mini BEAM for patients older than 65) and ASCT.
Results: Eighteen patients completed the pre-transplant phase, one is still in treatment and one patient dropped out of the study after the first R-DHAOX because a Grade IV non haematological toxicity (renal failure) was reported. After R-Bendamustine, 2 patients had grade III or IV Hematological Toxicity. After R-DHAOX, PBSC were collected in 19 patients with a median number of CD34+ cells >10×10^6/kg (range 4-48.6). The overall response rate (ORR) evaluated at the end of R-Benda intensification, was 100% with 14 CR (77%) and 4 PR (23%). At time of this analysis only 16 patients have performed ASCT and with a median follow up of 7 months (range 1-18) after ASCT, all patients are alive, 15 of them in continuous CR.
Summary and Conclusions: This intensive salvage treatment proved safe, feasible in an outpatient setting and effective in most relapsed FL-NHL. Thus, bendamustine can be safely employed in rescue programs with ASCT for FL. The benefit provided by R-Benda should be confirmed in a prospective randomized trial.

P866

THE INTENSITY AND DURATION OF PAIN ASSOCIATED WITH TREPHINE BIOPSY

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Background: Trephine biopsy is one of the most common diagnostic procedures in hematology; it is frequently performed as part of the diagnostic work-up for lymphoma as well as many malignant and non-malignant hematological conditions. Trephine biopsy is an invasive procedure usually performed under local anesthesia. However, despite the use of local anesthesia, most patients experience some degree of pain during and after the procedure. The intensity and duration of pain caused by this frequently performed procedure has not been reported previously.
Aims: The study aimed to determine the intensity and duration of pain after trephine biopsy as well as to determine factors related to pain.
Methods: Patients underwent trephine biopsy were recruited to the study from 6 participating hospitals in Norway. Patients were included only once in the study. Pain related to the biopsy was measured by Numerical Rating Scale (NRS). The scale ranges from 0 (no pain) to 10 (worst imaginable pain) twice daily at 08:00 and 20:00 (+/- 2 h) for 7 days, except on the day of the examination (day 0) where pain was assessed during the procedure (T0), 1 hour and 8 hours later. At each time point 2 registrations were performed, one at rest and one during activity. Consumption of analgesics was registered at each time point. The study was approved by the regional ethics committee; written informed consent was acquired from all patients. All values are expressed by median and Q1, Q3.
Results: Of 226 recruited patients, only 184 completed and returned the patients' form for assessment of pain and were thus included in the study. Median (Q1, Q3) age was 66 years (58,73); 97 males (52%). Maximum score was measured at time of biopsy (T0); median NRS score was 3 (1, 5). Pain intensity was substantially lower on day1, see Figure 1. Median duration (Q1, Q3) of pain at rest was 36 hours (0.5–72) and in activity 36 (0.5-84). Pain was localized at the site of biopsy in 50%, perceived in the lower limb in 10%, at both localizations in 10%, localization not specified in 30%. Analgesics comprising paracetamol +/- codeine was consumed by 4.4% at day 0 +1 h; 8.5% at day 0 +8h; 6.3% at day1, 8 am and 5.4% at day 1, 8 pm and by 2-4% in the remaining period. All patients received lidocain 2% as local anesthetic agent. Median injected volume was 10 mL (10, 13). Median time from application of lidocain to start of biopsy was 3 minutes 26 seconds and median duration of biopsy was 2 minutes. A significant correlation was found between NRS score at T0 and age (r=-0.21; P=0.006). Simultaneous aspiration of bone marrow was also found to be associated with greater pain at T0 (P=0.009). No significant associations were found between the intensity of pain and gender, length of biopsy, time of anesthesia to biopsy or BMI. The duration of pain in activity did correlate with NRS score at T0 (r=0.35; P<0.0001), BMI (r=-0.16; P=0.03), age (r=-0.22, P=0.004) and the localization of pain, which was longer in those with pain radiating pain (P<0.04). Median duration of pain was significantly longer in females (60 h) as compared to males (8 hours) P=0.001.

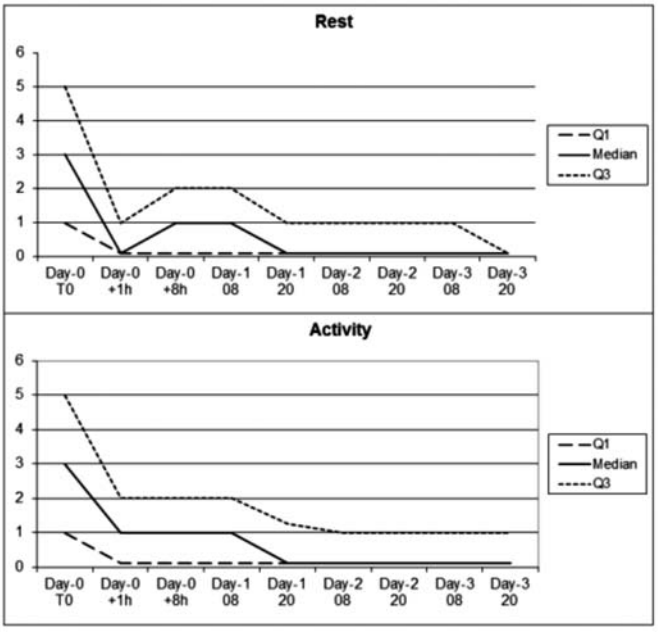

Figure 1.

Summary and Conclusions: Trephine biopsy is a painful procedure. Local anesthesia did not abolish the pain completely as 50% of the patients still experienced pain intensity of ≥3 points, and 25% ≥5 points during the procedure. The intensity of pain was negatively correlated with age, indicating that younger patients may require more analgesics. Pain lasted less than 2 days in half of the patients. Longer duration of pain was associated with greater NRS at T0, female gender, lower BMI and pain involving the lower extremity.

P867

NON-GERMINAL CENTER ORIGIN WITH BCL-2 EXPRESSION PREDICT UNFAVORABLE OUTCOME IN DIFFUSE LARGE B-CELL LYMPHOMA WITH HIGH IPI

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Background: In diffuse large B cell lymphoma (DLBCL), international prognostic index (IPI) was used to identify the patients with high-risk disease. The patients with identical IPI scores may still exhibit striking variability in outcome, suggesting the presence of significant heterogeneity within each IPI. Complexity and heterogeneity of DLBCL were demonstrated by the cell of origin (COO). DLBCL could be divided into germinal center B-cell (GCB) lymphoma and post-germinal center (non-GCB) lymphoma by gene expression profiling (GEP). However, GEP was not applicable to routine clinical practice and approaches using immunophenotypic algorithms with small panels of biomarkers were developed. Bcl-2 was an antiapoptotic protein and was implicated in resistance to chemotherapy. With COO, Bcl-2 was a candidate prognostic marker in DLBCL.
Aims: To evaluate the prognostic value of the COO (GCB or non-GCB) by immunophenotypic algorithm and/or Bcl-2 protein in patient with DLBCL especially with high IPI (high-intermediate and high risk of IPI).
Methods: Among 126 DLBCL patients receiving R-CHOP, 50 patients with high-intermediate and high risk of IPI were enrolled for retrospective analysis. For defining COO, Han's algorithm using CD10, Bcl-6 and MUM-1 was used. 50% cutoff for Bcl-2 expression by immunostaining was used. We plotted survival curves according to the Kaplan-Meier method and made comparisons using the log-rank test.
Results: In 50 patients with high IPI, the median age was 67 years old (range 39-83). 39 (78%) patients completed six cycles of R-CHOP as planned. 12 patients (24%) were classified as GCB subgroup and 38 patients (76%) were non-GCB subgroup. The 3-year PFS of GCB and non-GCB subgroup were 80% and 39%, respectively (P=0.099). 43 patients (86%) were Bcl-2-positive DLBCL and 3-year PFS of them was 42.4%. 7 patients were Bcl-2-negative and all of them did not observe disease progression until last follow-up (P=0.126). When COO combined with Bcl-2 expression, 68% of enrolled patients (34 of 50 patients) were non-GCB with Bcl-2-positive DLBCL. Interestingly, 3-year PFS of them was significantly inferior to that of others (33.3% and 85.7%, respectively; P=0.027). Consequently, Bcl-2 expression combined with non-GCB subgroup was found to have an adverse effect on the prognosis of patients in DLBCL with high IPI.
Summary and Conclusions: COO with Bcl-2 protein expression is reliable prognostic factor in DLBCL with high IPI although it needs confirmation by a prospective study.

P868

COMP-R AS FRONT LINE IN ELDERLY PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA AND G3 FOLLICULAR LYMPHOMA. THE SPANISH EXPERIENCE

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Background: The current standard treatment used on Diffuse Large B Cell Lymphoma (DLBCL) and Grade 3 Follicular Lymphoma (G3FL) patients (pts) is R-CHOP (Rituximab, Cyclofosfamide, Doxorubicin, Vincristine and Prednisone). Despite the fact that Doxorubicin is essential, with elderly patients (70+) we avoid using it due to the inherent cardiac toxicity. Liposomal Doxorubicin has shown less toxic profile compared to the standard formulation in breast cancer studies. Several phase II studies have explored it in pts with Lymphoma.
Aims: The aim of the present study is to establish the role of R-COMP21 (off label use) in elderly DLBCL/G3FL pts analyzing effectiveness and toxic profile.
Methods: Fifteen Spanish centres participated in this retrospective study. Inclusion criteria were as follows: pts ≥70 at the time of treatment, confirmed diagnosis of DLBCL or G3FL and treated with R-COMP21 (Rituximab 375 mg/m2/day1, Cyclofosfamide 750 mg/m2/day1, Vincristine 1.4 mg/m2/day1, Non Pegylated Liposomal Doxorubicin 50 mg/m2/day1 and Prednisone 60 mg/m2/day1-5) between September 1st 2006 and August 31st 2011. Endpoints were overall response (OR) and complete response (CR) rate as *Revised Response Criteria for Malignant Lymphoma v2007,* toxicity as *CTCAE v3.0 of NCI scale* and overall survival (OS) and progression free survival (PFS). Left ventricular ejection fraction (LVEF) was assessed by echocardiogram visual estimation method prior to and after R-COMP21 treatment. This study was approved by the Spanish Medicines Agency (AEMPS, code MDA-RCO-2012-01).
Results: One hundred pts (85 DLBCL/15 G3FL) over 70 years of age treated with R-COMP21 were included. Mean age was 77.8 (range 70-91), male/female 52/48 pts.ECOG≥2: 51pts, Ann Arbor stage III or IV: 66 pts, extranodal involvement 60 pts (≥2 areas 29 pts), IPI ≥3: 63 pts, hypertension 67 pts, diabetes 22 pts, cardiomyopathy 32 pts, pretreatment echocardiogram was available in 80 pts, LVEF <50% in only 5 pts. Four pts had a pretreatment creatinine ≥2mg/dL and 2 pts Bilirrubine ≥2 mg/dL. Efficacy: Ninety six pts completed the treatment and 87 were evaluated for effectiveness. With a mean of 5.5 (range 3-8) R-COMP21 cycles administered the OR rate was 83.3% (56 pts achieved CR, 11 pts uCR, 13 PR) and 7 pts (8%) PD. In 10 cases the response was consolidated, with radiotherapy 6 cases and with radio-inmunotherapy (90Y Ibritumomab Tiuxetan) 4 cases. No predictive factor of response was found in univariate analysis among age (cut-off at 80 yrs), sex, histologic subtype, Ann Arbor stage (I-II *vs* III-IV) or IPI (0-2 *vs* 3-5). Toxicity: Ninety two pts received primary G-CSF prophylaxis,37 pts presented febrile neuthropenia and 6 cases resulted in death due to infection. Five pts presented cardiac adverse events: 2 pts received a pacemaker implant and 3 cases of heart failure was documented. IPI (0-2 *vs* 3-5) and LVEF (<50% *vs* >50%) were predictors for OS (p0.0033 and p 0.0011 respectively) IPI did also predict PFS (p0.0036).With a mean follow up of 20.64 months, 57 ptsare alive and in CR.
Summary and Conclusions: In summary this retrospective study suggests that R-COMP (off label use of Non Pegylated Liposomal Doxorubicin) can be effective, and well tolerated in elderly (70+) DLBCL/G3FL pts.
Disclosures: Off Label Use: Non Pegylated Liposomal Doxorubicin is approved for metastatic breast cancer

P869

THE ROLEOF PET/CT FOR EVOLUATION OF THE BONE MARROW INVOLVEMENT IN LYMPHOMA PATIENTS

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Background: Bone marrow involvement in lymphoma patients is an important factor for staging and prognosis. Bone marrow biopsy is a standard procedure to evaluate infiltration but it is an invasive and painful. For staging and response to treatment in lymphoma patients, 18F-FDG PET CT is now widely used
Aims: At this present study, we retrospectively analyzed our PET- CT results for bone marrow infiltration comparing with biopsy in newly diagnosed lymphoma patients.
Methods: PET-CT and bone marrow biopsy of 367 lymphoma patients (222 non- Hodgkin and 145 Hogdkin lymphoma) at the time of diagnosis were analyzed retrospectively. The (SUV) which was more than SUV of liver was considered as bone marrow involvement in PET-CT evaluation.
Results: Both PET-CT and biopsy revealed infiltration in 37 patients. We could not demonstrate infiltration in 276 patients neither with biopsy nor PET- CT. There was disconcordance between the procedures in 54 patients; in 18 patients, although the biopsy revealed positive results, no increased activity was observed in PET- CT. When we compared the PET- CT with biopsy; it had a 67,2% sensitivity, 88.4% specificity, 51% positive predictive value and 93,8% negative predictive value. The power of the procedure was calculated as 85%. The subgroup analysis of the group was shown at Tables 1 and 2.

Table 1. PET–CT at dıffuse large B cell lymphoma patients.

Sensitivity	89,4%
Spesificty	95%
Negative predictive value	98,5%
Positive predictive value	70.8%
Power of the test	94,4%

Table 2. PET–CT at Hodgkin lymphoma patients.

Sensitivity	70%
Spesificity	82.2%
Negative predictive value	97.3%
Positive predictive value	22,5%
Power of the test	81,3%

Summary and Conclusions: In this study, we evaluated the role of PET-CT for demonstrating the bone marrow involvement in lymphoma patients. Although it is not useful in all lymphoma patients, when we performed subgroup analysis, we realized that it could be a possible test for diffuse large B-cell lymphoma and Hodgkin lymphoma (Tables 1 and 2). But prospective large cohort studies with these subgroups are needed before it becomes a standard procedure.

P870

ANALYSIS OF THE BONE MARROW INFILTRATION IN A GROUP OF PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA BY COMPARING BONE MARROW BIOPSY AND 18F-FDG PET/CT

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Background: Bone marrow (BM) biopsy is the standard procedure for depicting BM involvement in diffuse large B-cell lymphoma (DLBCL). However, it is an invasive method that explores a limited part of the bone.
Over the past decade, ^{18}F-2-fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET)/CT has become the most valuable imaging modality for the initial staging of lymphomas, showing to be sensitive and specific. Recently, it has also been reported to be highly specific in the evaluation of BM infiltration in lymphoma patients.
Aims: Evaluate the value of ^{18}F-FDG PET/CT in the detection of BM involvement when compared to BM biopsy, in patients with the diagnosis of DLBCL.
Methods: Data from patients with DLBCL diagnosed between January 2004 and December 2011 were reviewed. All patients included in our study were submitted to BM biopsy and ^{18}F-FDG PET/CT at diagnosis or relapse, before any specific treatment.
Results: 97 patients were included, 55% men, median age of 55 years (range 25-90 years). In 84 patients (86.6%) there was concordance between results from BM biopsy and from ^{18}F-FDG PET/CT: 66 patients without bone marrow involvement and 18 patients with positive infiltration. In 8 patients, ^{18}F-FDG PET/CT was positive for bone marrow involvement, although BM biopsy showed negativity. Of these discordant cases, all patients had multi-focal 18F-FDG bone marrow uptake except one patient that showed uptake in a unique site. In 3 patients, BM infiltration was not confirmed by results from ^{18}F-FDG PET/CT.
Summary and Conclusions: The final results were concordant in 86.6% of the patients included in this study. The observed discordance seems to be related with the multi-focal characteristics of BM infiltration and with the methodological particularities of BM biopsy, executed on the posterior superior iliac crest. This causes the possibility of missing the affected portion of the bone. These results suggest ^{18}F-FDG PET/CT as a useful exam in the staging of DLBCL that should complement the BM biopsy.

P871

COMPARISONS OF B-CELL AND T-CELL LYMPHOMA-ASSOCIATED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Background: Lymphoma-associated hemophagocytic lymphohistiocytosis (HLH) is a fatal hematologic disorder that initially presents as fever, hepatosplenomegaly, cytopenia, hypertriglyceridemia, hyperferritinemia, and hemophagocytosis in bone marrow or other reticuloendothelial organs. Comparisons of clinical characteristics and treatment outcome between patients with B cell and T cell lymphoma-associated HLH remain unclear and need further investigation.
Aims: The aims of this study was to compare the clinical characteristics and treatment outcome between patients with B cell and T cell lymphoma-associated HLH.
Methods: We retrospectively analyzed 30 lymphoma patients with HLH and stratified them into B cell (n=13) and T cell lymphoma groups (n=17).
Results: Our results showed the age, performance status, Epstein-Barr virus infection, international prognostic index, presence of disseminated intravascular coagulopathy, serum triglyceride, fibrinogen, and lactate dehydrogenase levels were not significantly different in patients between B cell and T cell lymphoma groups. Patients in T cell lymphoma group, however, had higher serum ferritin levels than those in B cell lymphoma group (11525.6 *versus* 3790.6 ng/mL, P=0.043). The median survival time for patients in B-cell and T-cell lymphoma groups was 330 and 96 days, respectively (P=0.198). A trend of a better survival in patients with B cell lymphoma-associated HLH was observed and could be from use of rituximab (P=0.045) and better treatment response (P=0.112). Our limited experiences also showed allogeneic hematopoietic stem cell transplantation might provide survival benefits to patietns with lymphoma-associated HLH.
Summary and Conclusions: In summary, our study showed patients with B cell lymphoma-associated HLH had a trend toward a better survival outcome than those with T cell lymphoma-associated HLH. This survival benefit could be from better chemotherapy sensitivity in patients with B cell lymphoma, as well as introducing rituximab to B cell lymphoma treatment. Allogeneic hematopoietic stem cell transplantation could possibly provide survival benefit to patients with lymphoma-associated HLH by graft verses lymphoma effect, especially for those whose underlying malignancy was T cell lymphoma.

P872

A SERUM LACTATE DEHYDROGENSE WITH SYSTEMIC INFLAMMATION SCORE IS A USEFUL INDICATOR TO PREDICT RESPONSE AND SURVIVAL IN PATIENTS WITH NEWLY DIAGNOSED DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Systemic inflammatory responses as evidenced by C-reactive protein (CRP) and albumin have been reported as useful indicators of prognosis in patients with advanced cancers. Serum lactate dehydrogenase (LDH) also well-known prognostic biomarker in patients with lymphoma
Aims: In this study, we added serum lactate dehydrogenase (LDH) level as another GPS parameter. The usefulness of theLDH with GPS (L-GPS) was evaluated as a prognostic indicator in patients with newly diagnosed DLBCL
Methods: We evaluated the relationship between the LDH with systemic inflammation score and survival in 213 patients with diffuse large B cell lymphoma (DLBCL) receiving the first-line R-CHOP chemotherapy. Patients were classified into three groups based on serum CRP, albumin and lactate dehydrogenase (LDH) level at diagnosis, as specified by the LDH with Glasgow prognostic score (L-GPS). Patients with elevated CRP (>1.0 mg/dL), hypoalbuminemia (<3.5 g/dL) and elevated LDH (>1´ upper normal value) were assigned a score of 2. Patients in whom one or two of these biochemical abnormalities was present were assigned a score of 1. Patients in whom none of these abnormalities was present were assigned a score of 0
Results: In multivariate analysis, the independent poor prognostic factors for progression free survival (PFS) were L-GPS 2 (HR 5.415, 95% CI 2.028-14.454, P=0.001), ECOG performance status (PS) ≥2 (HR 3.504, 95% CI 1.655-7.419, P=0.001), and bulky lesion ≥10 cm (HR 2.030, 95% CI 1.037-3.972, P=0.039). In addition, the independent poor prognostic factors for overall survival (OS) were L-GPS 2 (HR 5.898, 95% CI 2.028-14.454, P=0.001) and ECOG PS ≥2 (HR 3.525, 95% CI 1.642-7.567, P=0.001). The overall response rate for the R-CHOP chemotherapy was decreased by according to the L-GPS; 96.7% at L-GPS 0, 87% at L-GPS1, and 75% at L-GPS 2 (P=0.009) (Figure 1).

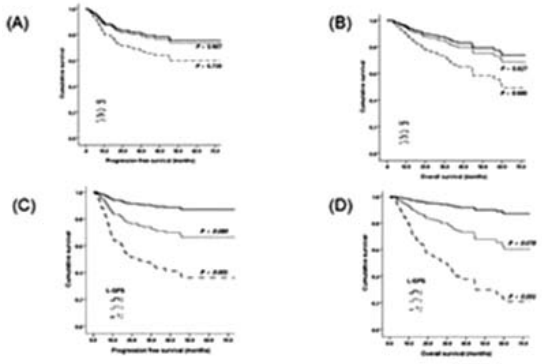

Figure 1. Progression free survival (PFS) and overall survival (OS), according to Glasgow prognostic score (GPS) and LDH with Glasgow prognostic score (L-GPS) in patients with newly diagnosed diffuse large B-cell lymphoma (DLBCL) receiving R-CHOP chemotherapy.

Summary and Conclusions: This study suggest that the L-GPS based on systemic inflammatory indicators may be useful clinical prognostic indicator for OS and PFS, and reliably predicts the response for R-CHOP chemotherapy in patients with newly diagnosed DLBCL

P873

HIGH DOSE THERAPY WITH AUTOLOGOUS STEM CELL SUPPORT FOR PRIMARY CNS LYMPHOMA: A RETROSPECTIVE ANALYSIS FROM THE ADULT LYMPHOMA WORKING GROUP OF THE JAPAN SOCIETY FOR HEMATOPOIETIC CELL TRANSPLANTATION

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Background: The prognosis of primary CNS lymphoma (PCNSL) still remains dismal, with high rates of local relapse and consequent death.Given the excellent results obtained with high dose therapy(HDT) with autologous stem cell transplantation (ASCT) regimens in PCNSL, ASCT is not widely used for PCNSL in Japan due to unavailability of key drugs.
Aims: We report the results of ASCT for PCNSL from the JapanSocietyforHematopoieticCellTransplantation (JSHCT) Registry.
Methods: DatafromtheJSHCT registrywereretrospectivelyanalyzed. Thirty-one patients with immunocompetent PCNSL received HDT/ASCT between 2007 and 2010 were evaluated. Overall survival (OS) and progression free survival (PFS) were calculated using Kaplan-Meier method.
Results: Median age at diagnosis was 54 years old (range 20 to 74). Median time from diagnosis to transplant was 22.2 months (range 2.6 to 51.7). Disease status at transplant: 1st CR 15 patients (pts), 1st PR 5 pts, 2nd CR 3 pts, 2nd PR 3 pts, 3rd CR 1 pts and refractory 3 pts. The median CD34+ cell number collected was 3.0×10^6/kg body weight (range, 1.5 to 19.5). No patient died of toxicity related to the procedure within 100 days after ASCT. With 1.3 years (range 0.1 - 4.3) of median follow-up period after ASCT, OS and PFS at 2 years were, respectively, 72% (95%CI: 45-88%) and 57% (95%CI: 29-77%) for 1st CR/PR pts, 100% and 63% (95%CI: 14-89%) for 2nd CR/PR pts, and 67% (95% CI: 5-95%) and 67% (95%CI: 5-95%) for other pts. High dose therapy regimens were varied: Busulfan(Bu)+Thiotepa (TT): 7 pts, Bu+TT+cyclophosphamide(CY): 7 pts, Bu+Cy: 2 pts, MCNU containing regimens 8 pts, LEED (melphalan, etoposide, CY, dexamethazone) 4 pts. In univariate analysis, LDH, age, performance status, use of TT and MSKCC prognostic score did not exert significant effects on survival (Figure 1).

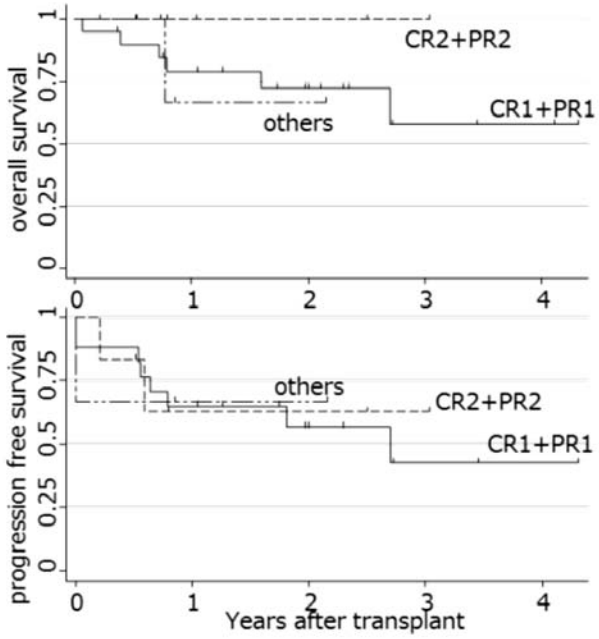

Figure 1.

Summary and Conclusions: Although there is the limitation related to unavailability of CNS penetrating drugs in Japan (*e.g.*, TT since 2010, BCNU), our results are encouraging and comparable to previous reports. Further prospective studies for PCNSL patients are warranted to confirm the efficacy of HDT/ASCT.

P874

A MULTICENTRE REVIEW OF THE OUTCOMES OF PATIENTS ABOVE THE AGE OF 75 WITH PRIMARY DIFFUSE LARGE B CELL LYMPHOMA

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Background: Diffuse large B-cell lymphoma (DLBCL) is the commonest lymphoid malignancy in the western world[1]. Its incidence increases with age and 40% of reported cases occur in patients aged over 70. However, elderly patients especially those over the age of 80 are excluded from most trials. There is only one prospective study that explored the outcomes of this age group[2] and unsurprising it reported that outcomes of such patients were inferior to younger patients (At 2 years CR/CRu of 62% and an OS of 59%). Nevertheless some elderly patients do have a complete response to treatment and have a long-term survival.
Aims: Patients enrolled into clinical trials are often selected population. We therefore aim to evaluate the outcome of an unselected group of patients with the below mentioned characteristics and their outcomes with treatment.
Methods: A retrospective, multicentre review of all patients aged above 75 who have a new diagnosis of DLBCL between January 2008 and January 2012 at 3 large centres in the North West of England. Patients for curative treatment were identified at a multidisciplinary meeting. Patients who had relapsed or transformed disease and those for palliative treatment were excluded. 50 patients were identified as suitable for the review. However, 2 patients died due to unrelated causes prior to commencing treatment (GI bleed in patient with cirrhosis, post laparotomy pneumonia & sepsis). Therefore the review will explore the outcomes of 48 patients.
Results: The patient cohort included 23 male and 25 females with a median age of 83 years (range 76-91). The cohort was well distributed across performance status (PS), stage and the age adjusted IPI (AA-IPI). 8 different treatment regimens were used (see Figure 1). Standard RCHOP includes 375mg/m² Rituximab, 750mg/m² Cyclophosphamide, 50mg/m² Doxorubicin, and 1.4 mg/m² Vincristine on day 1 of each cycle, and 40 mg/m² Prednisolone on days 1–5, cycle repeated every 21 days). Where "RCHOP 50%" was used, the dose of rituximab and prednisolone remained unchanged, while the dose of cyclophosphamide & doxorubicin was reduced by 50% and vincristine to 1mg. Where etoposide was used (RCEOP), it was given at 100mg/day for 14 days while gemcitabine in RGCVP was given at 750 mg/m² dose on day 1 & 8. 77% of the patients (n=37) completed their planned chemotherapy and only 6% of patients required an attenuation or alteration in their planned treatment regime. Response to treatment was assessed based on clinicians' choice (majority of patients underwent a CT scan, a few received PET scan). CR/CRu: 35 (73%), PR: 3 (6%), Refractory: 2 (4%), Died during chemotherapy: 8 (17%). At the follow up period of 12 months 60% (n=27) of the patients were noted to be in remission at 12 months while 19% (n=9) had relapsed. All of the patients with refractory (n=2) or relapsed (n=9) disease were identified as having poor prognostic factors. (All were stage III/IV and 9 had aa-IPI of 2 or 3). 14.4% (n=7) of the patients did not survive the treatment course and died due to toxicity. Of these interesting only 28.5% (n=2) had a PS of 3 or more. Peyrade *et al.* (2011) concluded in their study that the presence of low albumin prior to commencement of treatment was a strong indicator of poor outcome. We looked at the impact of albumin on the outcome of our patient cohort. At diagnosis 14 (29%) patients had a an albumin <35 g/L and of these, 85% (n=12) had an adverse outcome. (5 relapsed, 2 were refractory, 5 died due to chemotherapy toxicity).

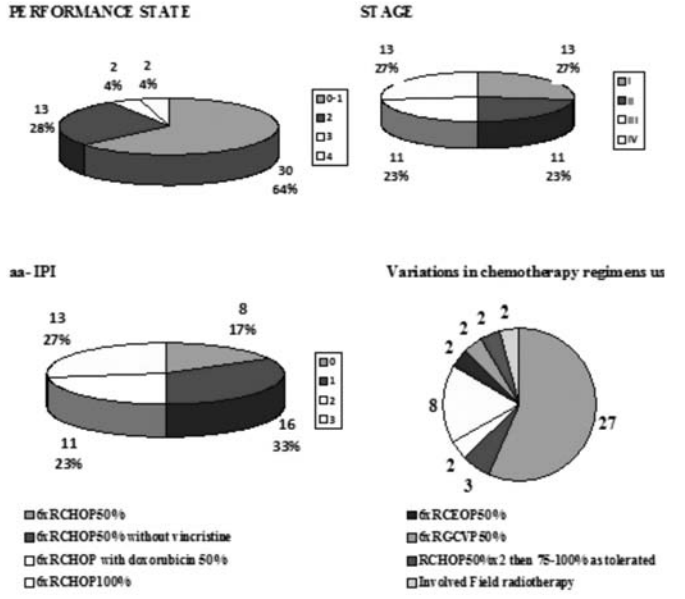

Figure 1.

Summary and Conclusions: With the increasingly ageing population, a large increase in the number of new lymphoma cases is anticipated. Our review suggests that in selected patients above the age of 75, variations of RCHOP chemotherapy can be administered with curative intent offering a good balance between efficacy and safety, with a substantial proportion of patients being disease free at 1 year. Moreover it also confirms the key role of aa-IPI and disease stage as prognostic indictors for outcome while lending support to the value of albumin as an indicator of elderly patients to tolerability to chemotherapy the treatment.

Stem cell transplation - Experimental

P875

HUMAN CORD BLOOD CD45+ CELLS PROTECT MICE BRAIN AFTER CLOSED HEAD INJURY

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Background: Traumatic brain injury is a major health care problem and a significant socioeconomic challenge worldwide, causing death and disability for at least 1.4 million patients in the United States alone each year. Regrettably, no therapeutic neuroprotective agents are clinically available to date. Cell therapy by umbilical cord blood (CB) transplantation has been lately suggested for the treatment of brain trauma, but although CB contains different stem cell populations, only the potential of mesenchymal stem cells was evaluated for this aim.
Aims: To explore the protective potential of CB CD45$^+$cells for the treatment of brain trauma.
Methods: In the present study, we used a closed head injury (CHI) mice model, in which a focal blunt injury over an intact skull is being induced by a standardized weight-drop device. The resulting mechanical impact triggers a profound neuroinflammatory response within the brain with high consistency and reproducibility, leading to neurological and cognitive impairment and breakdown of the blood–brain barrier. CB-cells were separated according to their CD45 expression and the derived populations were than transplanted into CHI mice. Animals were evaluated for their neurologic severity score (NSS) (motor ability, balancing, alertness) and under rotarod test (motor deficits and ataxia).
Results: We demonstrated that CB derived mononuclear cells (MNC) and CD45$^+$, but not CD45$^-$ -cell subset reduced the neurobehavioral and motor deficits which typically occur in a mouse model of CHI. Transplantation of CB-cells was given at day 1 or 7 post-trauma and their therapeutic effect was observed up to 35 days. A significant reduction in brain anatomical damage and head wound area were measured in treated mice from 2 to 7 days after cell transplantation, as evaluated both *ex vivo* and by non-invasive near-infrared measurement of the hemorrhage surface. CB cells which were administered either intracerebroventricularly or intravenously (iv) displayed similar efficacy. Transplanted cells, labeled with near-infrared dye and infused iv were detected at the site of injury, indicating their homing properties. Head *in vivo*and brain *ex vivo* imaging, taken at short times after cell transplantation indicated a fast increase in brains fluorescence 1.5 h after iv cells transplantation, which was reduced shortly thereafter (5 h). Acute (2 days) and chronic (35 days) after transplantation, differential levels of the BDNF, NGF and VEGF were measured in the ipsilateral and contralateral hemispheres. Finally, anti CD45 antibodies blocked the beneficial effects of the cord blood derived MNC, strengthening the notion that CD45$^+$ cells are responsible for the protective effect.
Summary and Conclusions: Altogether, these findings demonstrate the potential of the wide therapeutic window and protective properties of CB derived CD45$^+$ cell fraction in animal model of brain trauma. Based on the minimal manipulation for CD45$^+$ cells isolation from CB, their ability to reduce neurological deficits even when transplanted 7 days after the insult and their ability of fast homing to the brain, we propose that CD45$^+$ cells should be considered for translational therapy in treating patients with brain trauma.

P876

PYROSEQUENCING IS A NEW PROMISING APPROACH TO PERFORM HLA TYPING IN A QUICKLY, SIMPLE AND ACCURATE WAY

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Background: The HLA system is characterized by an high number of alleles and haplotypes because the HLA genes are the most polymorphic in the human genome. A variety of methodologies have been developed for HLA typing at the protein and nucleic acid level, but ambiguity can affect the possibility of the right call for each HLA-locus. Particularly phase ambiguities arise from the incomplete genomic coverage or the contemporary Sanger sequencing of two heterozygous alleles that determines different haplotypes. The new generation sequencing (NGS) technologies have the potential to perform HLA-typing in a rapid and accurate way without phase ambiguities.
Aims: A simple and reliable method to implement the knowledge of HLA compatibility between donor and recipient would be necessary to obtain a rapid and accurate HLA-typing and to reduce phase ambiguities. To this purpose in this study we evaluated the feasibility, reliability and robustness of the HLA-typing by NGS in 40 samples.
Methods: We performed high-resolution HLA-typing using pyrosequencing and subsequent bioinformatic analysis. Fourtheen amplicons for sample were synthesized using two custom assay. The output file was then uploaded into JSI SeqPilot software to align all sequences with the reference database (ref 3.9 2012).
Results: Using the method of Multiplex Identifier (MID) tag, we can pooling amplicons from different samples; so we have pooled 5 different samples into 8 sequencing runs (total 40 samples). The PCR reactions generate 560 amplicons who correspond to HLA-A/B/C exons2, 3 and4, DQB1 exons 2 and 3 and DPB1, DQA1, DRB1/3/4/5 exon 2. We have obtained over of 150 reads for most amplicons. The assignment of unambiguous genotype was possible on 45.5% of alleles. The ambiguities were related to the assay design (above all for class-II). Notably, some ambiguities on the locus B and C have a little biological importance because both alleles coding for the same peptide binding domain, instead the genomic differences between the two alleles were located on the transmembrane domain coding region. Ten cases analyzed in this study were also genotyped using conventional strategies, for the most part ssp with a concordance of 100%.
Summary and Conclusions: Clonal amplification and pyrosequencing strategy is a feasible and reliable method to perform the HLA-typing. This method discriminates very well the alleles that determines differences on the peptide binding domain. Using a NGS technique we have obtained a high-resolution HLA-typing for the most important loci of the samples, quickly and without phase ambiguities. This work was supported by Lions Club " Bassa Bresciana", BCC Pompiano e Franciacorta Founds and by Roche.

P877

A RANDOMIZED DOUBLE BLIND CONTROL TRIAL COMPARING FILGRASTIM AND PEGFILGRASTIM IN CYCLOPHOSPHAMIDE PERIPHERAL BLOOD HAEMATOPOIETIC STEM CELL MOBILIZATION–PRELIMINARY ANALYSIS

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Background: There were very few randomized trials published in full article comparing filgrastim and pegfilgrastim in peripheral blood haematopooietic stem cell mobilization (PBHSCM). None of them to our knowledge examined cyclophosphamide as single chemotherapy (CT) agent, which is the commonest CT mobilization in our centre.
Aims: Hence, we conducted a trial to compare filgrastim (F) and pegfilgrastim (PF) in cyclophosphamide PBHSCM. (NMRR ID: NMRR-10-755-6906)
Methods: This was a randomized double blind control trial. Only patient without previous history of apheresis was eligible. Cyclophosphamide 2g/m2 was given on Day (D) 1 On D3 onwards, patient received a daily subcutaneous injection at 6pm, which was either Arm 1: F 5µg/kg till completed apheresis, Arm 2: PF 6mg on D3, normal saline (NS) D4-10, or Arm 3: PF 6mg on D7, NS D3-6 & D8-10. Peripheral blood CD34+ cell count (PB34)(/µL) was checked in the early morning on D8,11, and onwards till stop apheresis. Only if PB34 was ≥10, apheresis was done and repeated until CD34+ cell collection of $\geq 2 \times 10^6$/kg.
Results: There was 153 patients enrolled between 1st September 2009 till 31st December 2012 (Arm 1 49 (32%), Arm 2 50 (33%), Arm 3 54 (35%). Basic characterics: M:F=75:78, Malay:Chinese:Indian:Others=88:49:10:6; diagnosis were acute leukemia, myeloma, and lymphoma=23 (15%), 33 (22%), and 97 (63%), respectively; mean (SD) weight (kg) 60.4 (16.6); median age (range) 41 (12-66). There was no significant difference of sex, ethnicity, diagnosis, status of disease (new, relapse, relapse ≥2), and response of disease during PBHSCM (CR, PR, primary refractory, relapse refractory), weight and age between the 3 arms. The successful mobilization rate for Arm 3 was 1.5 times higher than Arm 2 (RR=1.51, 95% CI=1.09, 2.09). Arm 1 seems to give 1.3 times higher than Arm 2 but the result was not significant (RR=1.29, 95% CI=0.90, 1.85). In comparison to Arm1, Arm 3 seems to have a higher mobilization rate but the result was not significant (RR=1.17, 95% CI=0.90, 1.53).
Summary and Conclusions: Pegfilgrastim 6mg given on D7 resulted in more successful PBHSCM than D3 probably because of the surge of neutrophil resulting in degradation of PF when given in D3. There was a favourable trend towards PF 6mg D7 compared to F 5µg/kg from D3 onwards.

P878

DIFFERENT DOSE OF ATG INDUCTION THERAPY IN HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION: LONG TERM EFFECT ON TH17 AND CONVENTIONAL T CELLS, NOT ON NK RESPONSES

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Background: Anti–T lymphocyte globulin (ATG) have been demonstrated to play important roles in graft-*versus*-host disease (GVHD) prophylaxis after myeloablative unrelated or HLA-mismatched transplantation resulting in a reduction of GVHD without compromising anti-leukemia activity. However, the risk of infectious complications and post-transplantation Lymphoproliferative disorder (PTLD) were shown to be significantly elevated. Therefore, to further explore the effect of ATG on immune responses would be help to explore the optimal dose of ATG induction in HLA-mismatched transplantation.
Aims: Based on the open-label, prospective, randomized trial to compare two different total doses of ATG (6 mg/kg, ATG-6 group) *vs.* (10 mg/kg, ATG-10 group) in patients receiving myeloablative conditioning prior to allo-HSCT from haploidentical donors in our center, weevaluated the effect of the two different doses of ATG in conditioning regimen on immune reconstitution.
Methods: Using flow cytometry, we prospectively collected 29 standard-risk patients (15 received 6mg/kg ATG and 14 received 10mg/kg ATG) every 1 months until half-year post-transplant continuously to investigate the reconstitution kinetics of NK cells and T cells subsets as well as Treg cells, T cells functions such as CD4 helper activity, *in vitro* cytokine responses ie IL-17 and IFN-γ.
Results: The reconstituted kinetics of CD3+, CD4+ T-cell and CD56+ NK-cell abolute numbers in peripheral blood were similar between patients in ATG-6 group and ATG-10 group during half-year after transplantation. There were no differences in the expression of KIR, NKG2C, NKP30, and CD57 within the CD3-CD56+ NK lymphocyte compartment between the patients in ATG-6 group and ATG-10 group no matter at day15, day 30, day 60, day 100 or day 180 after transplantation. Meanwhile, the cytotoxic function or the IFN-γ secretion of NK cells reconstitution between the patients in ATG-6 group and in the ATG-10 group were similar during half-year post-transplant. However, patients in ATG-6 group showed faster Th17 cells and conventional T cells reconstitution at day15, day 30, day 60, day 100 or day 180 after transplantation. The proportions of Treg cells tended to be high in patients of ATG-10 group compared to those of ATG-6 group.
Summary and Conclusions: ATG 10mg/kg applied in conditioning regimen showed impared Th17 cells and conventional T cells reconstitution, therefore would reduce the GVHD occurrence compared to ATG 6mg/kg after transplantation.

P879

CIRCULATING ENDOTHELIAL CELLS (CEC) ARE A RELIABLE AND DYNAMIC BIOMARKER OF ACUTE GVHD (AGVHD) IN PATIENTS UNDERGOING ALLOGENEIC STEM CELLS TRANSPLANTATION.

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Background: Acute GVHD (aGVHD) is one of the major cause of morbility and mortality in allogeneic stem cell transplantation (allo-SCT). Clinical and physiopathological evidences have shown that vascular endothelium could be a target of aGVHD in very early phase; therefore markers of endothelial damage are warranted as valuable support in aGVHD diagnosis and to monitor its response to immunosuppressive treatments.
Aims: The primary endpoint of our study was to investigate the values of CEC count in peripheral blood to diagnose and predict aGVHD in patients submitted to allo-SCT.
Methods: We conducted an explorative and prospective study to evaluate CEC count at different time points: before and at the end of the conditioning regimen, at engraftment, at aGVHD onset and at 1 and/or 2 weeks after corticosteroids therapy administration. The CellSearch System® was used to capture and enumerate CEC. Magnetic particles conjugated to anti-CD146 are used to capture CEC from 4.0 mL of peripheral blood. Enriched cells are stained with DAPI and anti-CD105-PE antibody. APC conjugated anti-CD45 is used to exclude leukocytes. Enriched and stained cells are dispensed into a MagNest® cartridge for magnetic mounting. The cartridge is scanned and individual images of cells are presented for review and scored as CEC, based on CD146+, CD105+, DAPI+ and CD45- phenotype and cell morphology.
Results: We studied 10 healthy subjects (controls) and 41 patients with hematologic neoplastic diseases (7 HL, 1 NHL, 2 AL, 12 AML, 5 ALL, 8 MM, 3 CLL, 1 CML, 2 SAA) undergoing allo-SCT from either HLA-matched familial (n=11) or unrelated donor (n=30). The median count in controls was2,5 CEC/mL (range 1-14). The median CEC/mL pre-allo-SCT was 20 (n=33, range 4-718, P<0.0001 compared to controls), going up to 33 CEC/mL (n=39, range 3-648, P=NS compared to pre-allo-SCT) at the end of the conditioning regimen. At aGVHD onset the median CEC/mL was 58 (n=17, range 16-299, P=0.0009 compared to pre-allo-SCT), while at aGVHD response the median CEC/mL decreased to 31 (n=12, range 6-107, P=0,0079 compared to aGVHD onset) after 1 week of steroids therapy, and to 10 (n=5, range 5-29, P=0,0017 compared to aGVHD onset) after 2 weeks of steroids therapy (Figure 1).

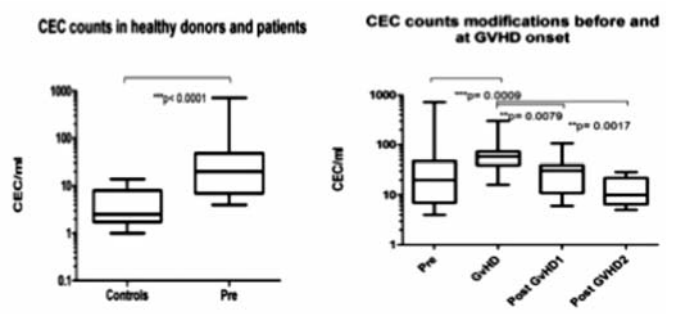

Figure 1.

Summary and Conclusions: Circulating endothelial cells can represent a promising marker to monitor endothelial damage in patients undergoing allo-SCT. We have showed a statistical significant increase in CEC numbers at aGVHD onset with a normalization at treatment response. CEC count helped us to settle between aGVHD and other transplant complications such as infections or autoimmune diseases leading to endothelial injuries: *e.g.* in a patient with diarrhea and low number of CEC, colon biopsy diagnosed infective colitis. The confirmation of the clinical utility of CEC counts, together with the use of a semiautomatic, standardized and reproducible technology, will allow a valuable help in the diagnostic definition of aGVHD in early phase and an information on the response to treatment.
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P880

EFFECT OF GRANULOCYTE COLONY-STIMULATING FACTOR MOBILIZATION ON THE EXPRESSION OF REGULATORY GAMMA DELTA T CELLS

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Background: The immune modulatory effect of granulocyte colony-stimulating factor (G-CSF) on T cells resulted in an unexpected low incidence of graft-*versus*-host disease (GVHD) in allogeneic peripheral blood stem cell transplantation. Our previous studies demonstrated that G-CSF mobilization influenced the distribution and clonality of TRGV and TRDV repertoire (T cell receptors of gamma delta [GD]Tcells), and significant positive correlation was observed between the invariable clonality of TRDV1 gene repertoire after G-CSF mobilization and low incidence of GVHD in recipients (P=0.015, OR=0.047) (Li Xuan *et al.* Journal of Translational Medicine 2011). Regulatory GD T cells (GD Tregs), which express Foxp3 and primarily belong to $CD27^+CD25^{high}$phenotype, are a novel subset of cells with immunosuppressive function (Xiaoyan Li *et al.* Journal of Immunology 2012). However, whether G-CSF could influence the expression of GD Tregs remains unknown.
Aims: To investigate the effect of G-CSF mobilization on the expression of GD Tregs.
Methods: The immunophenotyping of GD Tregs was analyzed in peripheral blood mononuclear cells (PBMCs) from 20 donors before and after G-CSF mobilization, using flow cytometry.
Results: Compared with that before mobilization, the proportions of Vdelta 1 and $CD25^+$ subsets were significantly increased (P=0.012, P=0.032), whereas the Vdelta 2 proportion was significantly decreased after G-CSF mobilization (P=0.002). The proportions of total GD T cells, $CD27^+$ and $Foxp3^+$ subsets were similar between the two groups (P=0.133, P=0.110, P=0.780, respectively). In addition, there was a significant increase in the proportions of $Foxp3^+$Vdelta 1 and $CD25^+Foxp3^+$subsets (P=0.038, P=0.013), and a significant decrease in the proportions of $CD27^+$Vdelta 2 and $CD25^+$Vdelta 2 subsets after G-CSF mobilization (P=0.013, P=0.022). The proportions of $CD27^+$GD T, $CD25^+$GD T, $Foxp3^+$GD T, $CD25^+CD27^+$, $CD27^+Foxp3^+$, $CD27^+$Vdelta1, $CD25^+$Vdelta 1 and $Foxp3^+$Vdelta 2 subsets were similar before and after G-CSF mobilization (P=0.422, P=0.342, P=0.724, P=0.070, P=0.503, P=0.053, P=0.386 and P=0.097, respectively). We then compared the Foxp3, CD27 and CD25 phenotypes in total GD T cells, Vdelta 1 and Vdelta 2 subsets. We observed a significant increase in the proportion of $CD27^+Foxp3^+$Vdelta 1 subsets after G-CSF mobilization (P=0.036). The proportion of $CD27^+Foxp3^+$GD T and $CD27^+Foxp3^+$Vdelta 2 subsets before mobilization were similar to that after mobilization (P=0.539, P=0.507). The proportion of $CD25^+Foxp3^+$GD T, $CD25^+Foxp3^+$Vdelta1, $CD25^+Foxp3^+$Vdelta2, $CD25^+CD27^+$ GD T, $CD25^+CD27^+$Vdelta 1 and $CD25^+CD27^+$Vdelta 2 subsets were also similar between the two groups (P=0.249, P=0.539, P=0.507, P=0.934, P=0.209 and P=0.061, respectively).

Summary and Conclusions: G-CSF mobilization significantly increased the proportions of Vdelta 1 subsets, including Foxp3+Vdelta 1 and CD27+Foxp3+Vdelta 1 subsets, whereas decreased the Vdelta 2 proportion.

P881

ENUMERATION OF RECENT THYMIC EMIGRANTS AFETER AUTOLOGOUS HSCT ACCORDING TO THE TYPE OF CONDITIONING: IRRADIATION VERSUS CHEMOTHERAPY

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Background: Stem cells present n the graft are predominant source of T cell recovery after HSCT.
The process, however requires proper function of the thymus, which may be negatively affected by many factors including toxicity of the conditioning regimen. Experimental data suggest that irradiation may be more harmful to the thymus compared to myeloablative chemotherapy.
Aims: The goal of the current analyusis was to verify this hypothesis in a clinical setting of autologous HSCT.
Methods: The thymic function was evaluated by enumeration of circulating recent thymic emigrants (RTEs) determined by flow cytometry as CD4+CD31+CD45RA+CD62L+ cells. It was done before start of conditioning and on day +100 after autoHSCT. A total of 57 patients with hematologic malignancies 9mm, n=32, NHL, n=22; other, n=3) were included in the study. Median age was 57 (22-66) years. Patients were divided in 4 groups according to the type of conditioning; total body irradiation (TBI, 12Gy) monotherapy (n=20), TBI+CHT (n=11), total marrow irradiation (TMI) monotherapy (n=11) and chemotherapy alone (BEAM or high-dose melphalan, n=15). The groups did not differ in terms of age.CD34+ cell was significantly higher for TBI+CHT compared with remaining groups.
Results: RTEs could be detected in peripheral blood of all subjects prior to HSCT (median 21, range 2-261cells/uL) with no significant differences between groups (Kruskall-Wallis test, P=0,95). On day +100 after autoHSCT the highest number of circulating RTEs was observed for TBI alone (9,2-36/uL) followed by TMI alone (7,2-15/uL), CHT alone (5,2-24/uL) and TBI+CHT (3,0-14/uL) (Kruskall-Wallis test, P=0,03). In the post-hoc analysis (Umann-Whitney test) the values for TBI alone were significantly higher compared to TBI+CHT (P=0,01) and tended to be higher compared to CHT alone (P=0,11). Analysis of lymphocyte subsets on day +100 revealed no differences between groups for the number of circulating T CD4+ cells (P=0,41), T CD8+cells (P=0,52) and B cells (P=0,24). The number of Treg cells tended to be lower for TBI+CHT compared to other groups (P=0,07) and the number of NK cells was lower for TBI alone than for other groups(P=0,003).
Summary and Conclusions: We conclude that conditioning regimens have variable impact on thymic function after autoHSCT. As opposed to previously published data from animal models we demonstrated that TBI itself is not more harmful to the thymus than high-dose chemotherapy. In contrast, the addition of chemotherapy to irradiation increases significantly the toxicity.

P882

SERUM MICRORNAS SIGNATURES IN ACUTE GRAFT VERSUS HOST DISEASE (AGVHD) AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLO HSCT)

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Background: aGVHD is one of the most frequent and lethal complications after allo HSCT, underscoring the need to develop novel therapies. To achieve this goal, aGVHD mechanisms needs to be further elucidated. Our group recently reported aberrant miRNA expression in donor T cells from animals and patients with aGVHD. Further studies focusing on miR-155 showed that this miRNA is modulating aGVHD. Emerging data also indicate that miRNAs are also present in the human serum and regulate immune responses. Here, we hypothesize that serum miRNAs expression is deregulated in aGVHD and could play a role in aGVHD pathogenesis or serve as biomarkers for the disease.
Aims: To identify miRNAs associated with aGVHD by performing serum miRNA expression analysis using deeP-sequencing in allo HSCT recipients at the time of clinical suspicion of aGVHD.
Methods: Peripheral blood (PB) samples were collected weekly until day 100+ and at the time of clinical diagnosis of aGVHD from allo HSCT patients enrolled into OSU11002 (a biorepositorium trial). After serum separation, total RNA was extracted using Trizol. Libraries were constructed using the small RNA profiling kit and sequenced on the Solid analyzer. A mouse model of aGVHD (B6 mice donor splenocytes and bone marrow cells transplanted to lethally irradiated F1 recipients) was used to assess serum miRNA expression in animals with aGVHD after transplant.
Results: In this study we included 10 patients with aGVHD (bowel aGVHD n=2; skin aGVHD (n=5) and both skin and bowel aGVHD (n=3). Median age was 51.9, conditioning regimens were myeloablative (n=1) and non-myeloablative (n=9) and the type of donors used were unrelated (n=9) and related (n=1). PB samples were obtained from these patients at the time of clinical suspicion of aGVHD. PB samples from allo HSCT patients with no aGVHD and matched for age, disease, conditioning regimen, donor and timing of sample collection were obtained and used as controls. Sequence alignment was performed using miRBase. The average reads count per sample was 875,000. Normalization as reads per million was followed by quantiles. First we compared miRNA expression between all patients with aGVHD (n=10) and controls (n=7) using BRB tools and class comparison. We found 7 miRNAs uP-regulated (miR-146a, miR-323-b, miR-34c, miR-363, miR-4245, miR-29a, miR-181a*) and 3 miRNAs down-regulated (miR-3168, miR-662, miR-550a) (Fold change (FC) >2, P<0.01). Next, we compared miRNA expression of patients with bowel aGVHD (n=5) *vs.* controls (n=7). We found 3 miRNAs uP-regulated (miR-146a, miR-4295 and miR-181a*) and 4 miRNAs down-regulated (miR-3168, miR-582, miR-193a and miR-662) (FC>2, P<0.01). Last we compared miRNA expression between patients with skin only aGVHD (n=5) and controls (n=7). We found 5 miRNAs uP-regulated (miR-323b, miR-34c, miR-3940, miR-3674, miR-4258) and 2 down-regulated (miR-3168 and miR-3678) (FC>2,P<0.01). MiR-3940 and miR-4258 are expressed exclusively in the skin (miRBAse). Remarkably, miR-146a, a miRNA associated with innate immunity, was found uP-regulated as well on serum samples from mice with aGVHD (n=6) compared with controls (n=6) (FC>2,P<0.01). Both, miR-181a and miR-29a, which are known to regulate immunity, were found deregulated in serum samples from patients with aGVHD. Ongoing experiments are being performed to dissect the function of those miRNAs.
Summary and Conclusions: miRNAs related to skin and immune system regulation were found to be de-regulated in the serum of allo HSCT recipients with aGVHD suggesting that they may play a role in the modulation of this process.

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PARALEL STUDY OF CHIMERISM AFTER ALLOGENIC HAEMTOPOIETIC STEM CELL TRANSPLANTATION MONITORED BY TWO DIFFERENT MOLECULAR METHODS

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Background: Over the past decades, allogeneic stem cell transplantation (allo-SCT) has gained increasing importance as a treatment option for patients with both malignant and non-malignant life threatening disorders. Surveillance of chimerism after allo-SCT seems an indispensable tool for the clinical management of transplant recipients. There are many genetic methods used for this purpose. The FISH analysis of chromosome X and Y is applicable only in sex mismatched transplantations. The most widespread method for testing of chimerism is the PCR-based analyses of highly polymorphic short tandem repeats (STR). Real-time PCR of SNP or NPs isn´t used so frequently.
Aims: To compare patient´s chimerism after allo-SCT measured using relative quantification (RQ PCR) of SYBR green-based real-time PCR of SNP or NPs or using PCR-based analysis of STR markers by fragment analysis on an automated genetic analyzer.
Methods: Whole peripheral blood samples were collected for DNA extraction from both the donor and recipient before transplantation in order to determine an informative marker. The blood samples of patients after allo-SCT (N=65 pairs) were collected at regular intervals at the Department of Hematology and Transfusion, Comenius University Medical School, Bratislava, Slovakia and provided for chimerism testing. RQ-PCR was performed by the real-time PCR system using SYBR green and 12 pairs of specific primers for two allelic variants of DNA polymorphism and GAPDH as endogenous gene control. The STR analysis was performed using of commercially available STR multiplex amplification kits with fluorescently labeled PCR primers. The quantification of donor and recipient signal was done by comparing the fluorescence intensity given by the peak area of analyzed fragments.
Results: We screened 65 related and unrelated donor/recipient pairs by both methods and we found at least one informative marker. The quantification of informative markers was provided at the same time by both methods in parallel and estimated chimerisms were compared. We found that our results were identical only in 2% and the discrepancy was noticed also in 2% between the two methods used. In the case of 1-50% mixed chimerism (MC) similar results were obtained. However, complete chimerism (CC) estimated by the fragment analysis was evaluated as mixed chimerism (MC) by the real-time PCR in 94% patients, mainly in the first half of a year of the post-transplantation monitoring. The example of the parallel monitoring of one patient is shown in Figure1. Fig-

ure 1 Monitoring of chimerism after allogeneic stem cell transplantation (allo-SCT) by analysis of STR markers by fragment analysis and by relative quantification (RQ PCR) of SYBR green-based real-time PCR of SNP or NPs.

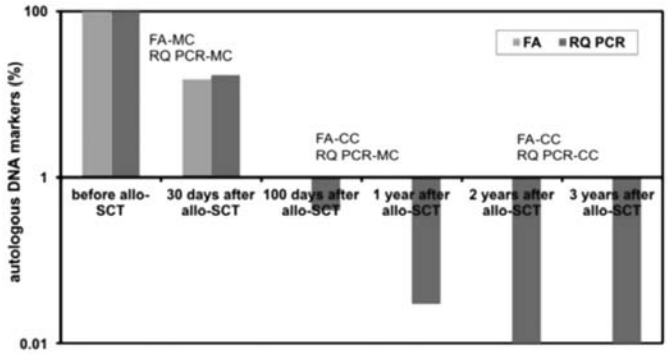

Figure 1.

Summary and Conclusions: Both methods compared above are suitable for chimerism assessment after the allogeneic SCT. Complete chimerism detected by the fragment analysis (FA) and mixed chimerism (MC) detected by the real-time PCR was due by the different sensitivity of two methods used. RQ PCR had the higher sensitivity (<1%) for the detection of the autololougous DNA markers than FA, so it is better for earlier revealing of eventual relaps. On the other hand the quantification of donor´s DNA markers is more precise estimated by the FA.

P884

VENO-OCCLUSIVE DISEASE MAY DEVELOP IN SECONDARY IRON OVERLOADED MICE AFTER TOTAL BODY IRRADIATION.

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Background: The outcome of hematopoietic stem cell transplantation (HSCT) is poor in patients with secondary iron overload (SIO). High incidence of veno-occlusive disease (VOD), acute GVHD, and infection were observed in SIO patients treated with HSCT.
Aims: We evaluated the relation between SIO and VOD in an animal model of HSCT.
Methods: We used 6 week-old female BDF1 (H-$2^{b/d}$) as recipient and male C57/BL6 (H-2^{b}) as donor. Recipient mice were injected intraperitoneally with 10mg of iron dextran according to experimental design (cumulative dose: 50mg, 100mg, and 200mg). All mice were treated with HSCT including total body irradiation designed by dose. Also, some mice without SIO were treated with allogeneic or syngeneic HSCT. We obtained peripheral blood for alanine aminotransferase (ALT) and liver for pathologic findings, lipid hyperoxide (LH), and liver iron content (LIC) as reactive oxygen species on post-HSCT day 1 and day 7. The score for VOD was assessed by pathologic findings (Hepatology, 1999;29:1779-91).
Results: All mice with SIO died within 10 days of HSCT. ALT level was increased depending on cumulative iron dose, with a significant difference between day 1 and day 7 for 200mg iron group (P<0.01). LH level was significantly increased in the 200mg iron group than in other groups (P<0.01). For the 100mg iron group, the LH level depended on radiation dose (P<0.01). There was a statistically significant relation among ALT, LH and LIC parameters (ALT *vs.* LH; r^2=0.911, ALT *vs.* LIC; r^2=0.548, LIC *vs.* LH; r^2=0.564). Also, the pathologic scores for VOD correlated with LIC (P<0.01, r^2=0.597).
Summary and Conclusions: The liver with SIO showed a high level of ROS that depended on cumulative iron dose, which also showed correlations with elevated liver enzyme and LIC. The pathologic score for VOD was associated with LIC. Our results suggest that SIO may induce VOD after HSCT done with irradiation. Iron chelation may improve outcome in SIO model of HSCT.

P885

ASSESSMENT OF CMV SPECIFIC IMMUNITY BY FLOW CYTOMETRY USING A COMBINED APPROACH OF SPECIFIC CMV CELL ENUMERATION AND FUNCTIONAL T CELL ANALYSIS

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Background: CMV reactivation is one of the most frequent infectious complications following allogeneic stem cell transplantation (SCT). CMV specific memory CD8+ cells (CMV CD8+ Tly) play crucial role in the regulation of CMV infection. In practice, CMV specific response can be monitored by detection and quantification of CMV CD8+ Tly using tetramer technology and/or *ex-vivo* assessment of CMV induced T cell function.
Aims: To study the recovery of CMV specific immunity by analysing the functional activity of CMV stimulated CD8+ Tly in terms of simultaneous production of IL-2 and IFNg in correlation with CMV CD8+ Tly enumeration by CMV tetramer test.
Methods: In 37 patients after SCT, we determined the presence of CMV CD8+ Tly using the CMV tetramer test and simultaneously evaluated the functional status of CMV stimulated CD8+ Tly by evaluation of intracytoplasmic presence of both IL-2 and IFN-g using multiparameter flow cytometry (MPFCM).
Results: During the last 4 months, 37 patients were analyzed on day +60, +150, +240, +360 following SCT. In 10 (27%) patients, we demonstrated the presence of CD8+IL-2+INFg+ double positive cells, indicating sufficient CMV specific response of Tly in agreement with literary data. In 7 of 10 cases (70%), we proved the presence of CMV CD8+Tly: 203 c/µl (min. 51 c/µl, max. 785 c/µl). All of these 10 patients didn't experience any CMV complications. In 3 of 37 patients (8%) suffered from severe CMV infection and neither CMV CD8+ Tly, nor CMV CD8+IL-2+,IFNg+ Tly were identified.
Summary and Conclusions: Preliminary results suggest possible correlation between *ex-vivo* functional activities of CMV stimulated Tly and the presence of tetramer positive CMV CD8+Tly in concentration >10 c/µl. Further analysis is necessary to confirm this observation and implement both techniques in clinical practice for laboratory evaluation of CMV specific immunity recovery and optimal timing of anti-CMV therapy.

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SERUM FREE LIGHT CHAIN (SFLC) AN EARLY PROGNOSTIC MARKER OF OUT-COME IN LYMPHOMA AND LEUKEMIA PATIENTS POST ALLOGENEIC TRANSPLANTATION

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Background: Early markers of allogeneic transplant engraftment and graft *versus* host disease (GVHD) may aid patient management. Previously a small study of 47 multiple myeloma patients undergoing allogeneic transplant identified changes in serum free light chain (FLC) production (of the non-clonal isotype) as markers of allogeneic transplant outcome.
Aims: Here we present data looking at the delta changes of FLC compared to baseline and comment on their role as markers of immune-reconstitution in a group of leukemia and lymphoma patients.
Methods: Serum from 19 lymphoma (13 NHL, 6 HL), 73 leukemia (53 AML, 7 CML, 6 ALL, 4 PLL, 3 CLL) and 8 patients with other hematological disorders (3 MDS, 2 MF, 2 aneuploidy, and 1 ATLL) taken at baseline (median 7 days before transplant (range 1-19 days) and 5 weeks post-transplant were analysed retrospectively (male/female ratio 61/39, median age 51 years, range 19-71). FLC kappa and lambda were measured using automated immunoassays nephelometrically and results summated (κFLC+λFLC) to give a combined polyclonal FLC measurement (cFLC). Delta cFLC were characterised as increased, stable or decreased. Intact immunoglobulin (IgG, IgA, IgM) measurements were analysed in a similar fashion. All statistics were performed on SPSS v19.0.
Results: Median cFLC values at baseline and at week 5 post-transplant were 25 mg/L (range 2.21-371) and 15.63 mg/L (range 0.54-56.73), respectively. At week5, the absolute values of cFLC increased in 25/100 (median increase 7.19 mg/L, range 0.18-38.15) and decreased in 75/100 patients (median decrease 13 mg/L, range 0.02-335.51). Overall survival (OS) was better in patients with increased delta cFLC and these patients were more likely to have acute GVHD (aGVHD) compared to patients with decreased delta cFLC (75%ile survival rate: not reached v. 231 days, respectively, P<0.001; 50%ile rate of aGVHD free survival: 55 days v. not reached, respectively, P=0.004). Due to assay analytical variation, subtle changes in the delta cFLC may not represent increase or decrease in cFLC levels. A cut-off of ±8 mg/L was used to define delta cFLC in to 3 groups: 1) increased, 2) stable, or 3) decreased. Patients with increased delta cFLC (n=10) had a better OS compared to patients with stable (n=36) or decreased (n=54) delta cFLC and patients with stable delta cFLC had a better OS compared to patients with decreased delta cFLC (75%ile survival rate: not reached v. 533 days v. 206 days, respectively; P=0.001). In comparison, changes in intact immunoglobulin measurements did not offer significant information on the outcome of these patients (p value for IgG P=0.34; IgA P=0.15; and IgM P=0.057).
Summary and Conclusions: Delta increases in FLC correspond to better OS and an increase in GVHD, suggesting they may be early markers of engraftment and immune reconstitution. Changes in intact immunoglobulins did not offer the same information, possibly reflecting longer serum half-lives which may buffer subtle changes in concentrations.

P887

STUDY OF SOLUBLE HLA-G LEVEL DYNAMICS IN HEMATOLOGICAL PATIENTS UNDERGOING AUTOLOGOUS OR ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Human leukocyte antigen G (HLA-G) is a non-classical major histocompatibility complex (MHC) class I molecule with immune-modulatory properties. HLA-G expression is highly tissue restricted and can be elevated by a variety of inflammatory cytokines including IL-10 and INF-γ, heat shock, radiation, oxidative stress and hypoxia. Aberrant HLA-G expression was found in a variety of pathologic situations, including solid as well as hematologic malignancies (AML, CLL, NHL and HL). Soluble HLA-G (sHLA-G) is generated by proteolytic cleavage of HLA-G1 surface antigen. Increased sHLA-G plasma levels has been suggested as disease specific markers in several solid tumors, with higher levels correlating with an advanced disease status. HLA-G molecules have an inhibitory effect on cells of the immune system including NK, T cells and dendritic cells, suggesting that they may have a role in immune surveillance and transplantation. In hematological malignancies both tumor and the BM stroma may contribute to plasma sHLA-G. We hypothesized that sHLA-G is increased in patients (pts) with hematological disorders and may have a biological significance in patients undergoing either autologous (Auto) or allogeneic (Allo) SCT.
Aims: To study plasma sHLA-G levels and dynamics in pts with hematological disorders undergoing SCT.
Methods: Patients undergoing SCT between 08/2006 and 08/2009 were included in this study. sHLA-G was determined in ACD plasma collected before conditioning and at 2 days intervals until engraftment. Concentrations were determined by ELISA using MEM-G and HRP-antiB2-microglobulin as capture and detection antibodies, respectively, and are expressed as ng/mL. sHLA-G levels determined during conditioning were compared with baseline values and are expressed as fold changes (level at a specific day/baseline).
Results: 106 pts, median age 50 years (range 1-72), F/M 44/62 undergoing either Auto (n=27) or Allo (n=79) SCT were included in the study. Their baseline characteristics are shown in Table 1. Mean sHLA-G level prior to SCT was 40.7 ng/mL (range 5-148) in the entire cohort and was significantly different according to diagnosis (mean±SEM); 71±17 ng/mL in HL, 56±12 ng/mL in ALL, 55±16 ng/mL in severe aplastic anemia, 55±5 ng/mL in CML, 52±9 ng/mL in myelofibrosis, 48±4 ng/mL in CLL, 40±8 ng/mL in MDS, 37±8 ng/mL in NHL, 32±3 ng/mL in AML and 23.50±5 ng/mL in MM (one way ANOVA, P=0.0123). Mean sHLA-G levels were similar in pts transplanted in CR1 or 2 (42±7 ng/mL), active disease (PR+PD) (40±6 ng/mL) and in pts with no prior therapy (39±7ng/mL). sHLA-G levels gradually increased in Allo SCT pts, achieving maximal levels at engraftment±5 days, with mean fold changes from baseline of 2.6±0.4. In contrast, sHLA-G level in Auto SCT pts remained relatively stable with mean fold change at engraftment of 0.8±0.2 compared to baseline levels. Pre-transplant sHLA-G levels in Allo SCT pts developing acute GVHD (n=24, 30%) were not significantly different from pts with no GVHD (43±6 ng/mL and 44±5 ng/mL, respectively), however in AML pts, pre-transplant higher sHLA-G levels were observed in pts with acute GVHD (41±10 ng/mL) compared to those without acute GVHD (31±4 ng/mL), P=0.3.

Table 1. Clinical characteristics of study patients.

	N=106
Age (years) median (range)	50 (1-72)
Gender F / M	44 / 62
Diagnosis N (%) Acute leukemia- AML / ALL Multiple myeloma MDS Lymphoma – HL / NHL Severe aplastic anemia Myelofibrosis CML CLL Unknown	 24 (23%) /10 (9%) 13 (12%) 10 (9%) 6 (6%) / 16 (15%) 6 (6%) 3 (3%) 3 (3%) 5 (5%) 10 (9%)
SCT-N (%)- Allo / Auto	79 (75%) / 27(25%)
Disease status at SCT N (%) CR1 / CR2 PR / Progressive disease Untreated Unknown	 11 (10%) / 13 (12%) 20 (19%) / 18 (17%) 18 (17%) 26 (25%)

Summary and Conclusions: sHLA-G levels in patients with hematological disorders are quite variable with the highest values found in HL pts. Serial plasma sHLA-G measurements in SCT pts demonstrate different dynamics in Allo SCT compared to Auto SCT. Further investigation into the clinical significance of these findings is warranted.

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PHASE I/II STUDY OF PANOBINOSTAT FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH HIGH RISK MDS OR AML: INITIAL RESULTS FROM THE PHASE I PART OF THE PANOBEST TRIAL

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Background: After an allogeneic hematopoietic stem cell transplantation (HSCT), leukemic relapse and graft-*versus*-host disease (GvHD) remain major obstacles. Deacetylase inhibitors (DACi) possess both anti-leukemic activity and immunomodulatory effects. Conceptually, post-HSCT maintenance therapy with the DACi panobinostat may benefit patients (pts) with high risk acute myeloid leukemia (AML), but its tolerability and efficacy in this setting have not been investigated.
Aims: This phase I study was designed to determine the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) of the investigational drug panobinostat in adult pts with high risk AML or myelodysplastic syndromes (MDS) in complete hematologic remission (CR) after a reduced-intensity conditioning HSCT.
Methods: Panobinostat was administered in two schedules, either thrice weekly every week (A) or every other week (B) which were examined sequentially. Treatment is initiated between day +60 and +150 after HSCT and given for a maximum of one year. This interim analysis describes the results of schedule A, in which panobinostat was started at a dose of 10 mg TIW and escalated to 20 and 30 mg TIW using a 3+3 design. Eligibility criteria included: ANC ≥1,000/μL, platelets ≥75,000/μL, adequate organ function and no severe GvHD. All pts gave written informed consent. DLT was defined as prolonged grade 4 hematologic toxicity or any non-hematologic toxicity ≥grade 3 unrelated to disease progression or intercurrent illness within 28 days of the first panobinostat dose.
Results: A total of 12 pts (11 AML, 1 MDS) with a median age of 52 years (range, 21-62) have been enrolled (3, 6 and 3 pts at dose levels1, 2 and 3) and are evaluable for MTD. Cytogenetics were classified as intermediate-1 (n=3), intermediate-2 (n=4) and adverse risk (n=5) according to ELN criteria. Study start was at a median of 73 days (range, 60-126) after HSCT from a matched related (n=5) or unrelated donor (n=7) which was performed in active disease (n=11, median bone marrow blasts 22%, range, 8-63) or in CR2 (n=1). The MTD was determined to be 20 mg TIW due to one DLT (fatigue grade 3) at 20 mg and two DLTs (colitis and nausea/emesis grade 3 each) at 30 mg. As of February 2013, 68 adverse events (AEs) grades 2 (n=33), 3 (n=29) and 4 (n=5) were reported. Grade 3 and 4 toxicities included thrombocytopenia (n=7), leukopenia/neutropenia (n=5), anemia (n=1), gastrointestinal symptoms (n=5), sepsis, pneumocystis jirovecii pneumonia, pulmonary infection (n=1 each), herpes stomatitis (n=2), elevated lipase (n=3) or GGT (n=1), hyperuricemia, diabetes mellitus, syncope, deep vein thrombosis and pulmonary embolism (n=1 each). Toxicity was reversible and required at least one panobinostat dose reduction in 3 pts. Acute GvHD grade 2 (n=1) and 3 (n=3) was steroid responsive in 3 pts. One pt required salvage therapy and eventually developed moderate chronic GvHD. Mild chronic GvHD was observed in 2 additional pts. To date, 3 pts have completed one year of panobinostat and 4 pts remain on treatment (days 71-275). Five pts discontinued treatment prematurely after 10-217 days due to grade 3 toxicities (n=2), acute GvHD grade 3 (n=2) or AML relapse (n=1). One MDS pt relapsed after stopping panobinostat on day 10. With a median follow up of 362 days (range, 71-736), 11/12 pts are alive and 10/12 in continuous CR after HSCT. Immunophenotyping revealed no impact of panobinostat on regulatory T cells, other lymphocyte subsets or dendritic cells in the peripheral blood.
Summary and Conclusions: Panobinostat maintenance following HSCT is feasible with a recommended phase II dose of 20 mg TIW weekly. Fatigue and gastrointestinal toxicities were the DLTs. AEs were fully and rapidly reversible after interruption and/or dose reduction. While follow-up is short, the relapse rate is remarkably low considering the high risk patient population.

P889

CONTROLLED EPSTEIN-BARR VIRUS REACTIVATION AFTER ALLOGENIC TRANSPLANTATION IS ASSOCIATED WITH IMPROVED SURVIVAL ASSOCIATED TO AN EXPANSION OF NATURAL KILLER CELLS (NK)

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Background: T- and Natural Killer (NK)-lymphocytes play a main role in immune surveillance of EBV infection. EBV-reactivation (R) frequently occurs in patients having allogeneic transplantation (AT).
Aims: We evaluated the impact of controlled EBV-R on survival of allografted adult patients with haematological malignancies and analyzed the different circulating lymphocyte populations and NK cytotoxicity.
Methods: 190 consecutive adult patients were included in this study. They received fludarabine, busulfan, ATG for reduced intensity conditioning regimen (CR) or cyclophosphamide plus TBI for myeloablative CR. Prophylaxis of acute GVHD was standard. EBV viral load (EBV-VL) was monitored in PB after AT, weekly for the first 6 months (m), then monthly using standard PCR technique. EBV-R was defined by an EBV-VL ³500 copies/mL, increasing 1 week later. This level of EBV-VL was used to decide therapy, first reducing immunosuppression followed by rituximab if no EBV-VL decrease (375mg/m^2, 4 I.V. weekly). CMV co-infection was defined if occurring 1m before or after the EBV-R. Lymphocyte phenotyping (CD3,4,8,3+56+,3-56+,19,25,DR) was performed weekly for 3m, then monthly. Cytotoxicity assays were performed in 8 patients compared to healthy donors using standard technique against Jurkat, CO30 (EBV lymphoblastoid cells) and primary tumor cells.
Results: There were 105 acute leukaemias (26 ALL, 71 AML) and 8 myelodysplastic syndromes, 71 lymphoproliferative disorders (17 lymphomas, 8 CLL and 46 MM), 5 aplastic anaemia and 9 CML. There were 76 females and 114 males (median age: 51y, range 18-69). 86 (45%) patients presented EBV-R, with 74 patients having EBV-VL>1000 copies/mL. There was no statistical difference between the 2 groups (EBV-R and no EBV-R), for clinical characteristics, therapy, type of graft and number of CD34+ cells infused. Patients with EBV-R received more frequently ATG (P=0.004). Median time of EBV-R detection was 73 days (IQR: 40-210) after AT. 69/86 patients received rituximab, and the other 17 patients received acyclovir. Patients with no EBV-R did not receive rituximab. Within 3m after rituximab, GVHD intensity was decreased, particularly for acute GVHD. Median follow up was 36.6m (95%IC 31.5-45.7). Patients with EBV-R had a longer PFS than those with no EBV-R (at 2 years 51% *vs.* 69%, at 5 years 47% *vs* 38%) (P<.04). OS was longer in patients with EBV-R than that observed for patients with no EBV-R (at 2 years 76% *vs.* 64% and at 5 years 63% *vs.* 47%, P<.001). Among the patients with EBV-R, OS and PFS were not significantly different for patients receiving or not rituximab (respectively P=0.67 and P=0.88). OS and PFS were not different between patients according to the cancer (respectively, P=.46 and P=.49). Median duration of lymphocyte measurement was 94 days (IQR 59-248) after AT. B- and particularly T-lymphocytes (CD4+,CD8+) were not significantly different between the 2 groups, respectively P=.29 and P=.48. Only circulating NK cells were significantly different, with a median increased by 22% (P=.03) in patients with EBV-R. CMV co-infection did not impact NK cell counts (P=.058). NK cell count was not different according the use of rituximab (P=.22). The use of rituximab did not modify NK cell counts at 3 and 6m after stopping treatment (both P=.99).
Summary and Conclusions: Controlled EBV-R in allografted cancer patients is associated with better OS and PFS, a significant increase in circulating NK cells and enhanced NK cell cytotoxicity. A prospective study is ongoing for analyzing different subpopulations of NK cells.

P890

MYELOABLATIVE FLUDARABINE PLUS BUSULFAN IV (FB4) REGIMEN COMPARES FAVOURABLY WITH CYCLOPHOSPHAMIDE PLUS BUSULFAN (BUCY) IN PATIENTS UNDERGOING ALLO-SCT FOR MYELOID MALIGNANCY

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Background: The use of the "so called" FB4 regimen associating Fludarabine (40 mg/m^2/days × 4 days) and Busulfan IV (130 mg/m^2/days × 4 days) has been dramatically increased in order to replace the hitherto standard Cyclophosphamide (50 mg/kg/day × 4 days) plus Busulfan (3.2 mg/kg/day × 4 days) regimen (CyBu). Few studies, however, have addressed the impact of such a reduced toxicity conditioning on outcome. In addition, interpretation of the data is confounded by the inclusion of myeloid and lymphoid malignancies.
Aims: The aim of the study was to compare the two myeloablative conditioning CyBu and FB4 in a large-scale cohort of 103 consecutive patients who received allogeneic stem cell transplantation (allo-SCT) for myeloid disease.
Methods: Between January 2007 and December 2012, one hundred and three patients received either CyBu (CyBu-group; n=41) or FB4 (FB4-group, n=62) according to the on-going policy in our unit. Allo-SCT indications were AML (n=77), myelodysplastic syndrome (n=18) and myeloproliferative disorder (n=8). Transplantation modalities were made as homogenous as possible using the following inclusion/exclusion criteria: (i) Patients older than 18 years referred for first allo-SCT.(ii) source of stem cell was marrow or blood from either a sibling or an HLA-matched unrelated donor at allele level. Patients who received allo-SCT from a cord blood or T-cell-depleted graft, and those with malignancies other than myeloid were excluded.
Results: The two groups were comparable in terms of patient's characteristics except for recipient CMV serostatus. Patients of FB4-group received more often ATG and PBSC than those of CyBu-group with P=0.003 and, 0.005, respectively. As February 20th, 2013, median follow-up was of 24.3 months (range, 2.1-74.0). Two years OS, EFS, cumulative incidence (CI) of relapse and NRM were 77%, 73%, 13%, 13%, respectively. All patients engrafted but full donor-type chimerism were less often recorded in FB4-group than in CyBu-group with 54% *vs* 83% (P=.005); 64% *vs* 88% (P=.009) and 65% *vs* 87% (P=.048) at days 30, 60 and 100 post–transplant, respectively. Patients of FB4-group developed more often oral mucositis (92% *vs* 76%; P=.021) and chronic GVHD (46% *vs* 21%; P=.006) than those belonging to CyBu-group. Of note, patients with full-donor chimerism at day 60 developed more often a grade II-IV acute GVHD but chimerism status had no impact on patient's outcome particularly on relapse. In multivariate analysis adjusted to ATG, CMV serostatus and source of stem cells (BM *vs* PBSC), conditioning type (FB4 and CyBu) had no impact on 2-year OS, EFS, relapse and NRM.
Summary and Conclusions: In this large-scale homogenous cohort, results show that FB4 as a reduced toxicity myeloablative conditioning regimen gives similar results as the standard CyBu. Longer follow-up is, however, needed to evaluate the impact of delayed full-donor chimerism on patients relapse.

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LONG-TERM OUTCOME (BEYOND 10 YEARS) AFTER ALLOGENEIC STEM-CELL TRANSPLANTATION WITH REDUCED-INTENSITY COMPARED TO MYELOABLATIVE CONDITIONING; SIMILAR SURVIVAL BUT DIFFERENT PATTERN OF LATE EVENTS

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Background: Allogenic stem-cell transplantation (SCT) with reduced-intensity conditioning (RIC) has been increasingly used over the last 15 years. Comparative studies have generally shown that RIC is associated with higher relapse rate and lower toxicity compared with myeloablative conditioning (MAC) resulting in similar short-intermediate term survival. However, due to shorter follow-up, so far, there is only limited data on the long-term outcome (beyond 10 years) after RIC in comparison with MAC and on the pattern of late events.
Aims: To compare long-term outcome and late events between RIC and MAC SCT recipients.
Methods: We retrospectively analyzed all consecutive allogeneic transplants performed in a single institution between 1/2000 and 3/2003, such that the minimal follow-up for surviving patients is more than 10 years. We analyzed late outcome and late events after SCT.
Results: The analysis included 142 patients, median age 47 years (16-66). Diagnoses included AML (n=50), ALL (n=14), MDS (n=4), CML (n=14), lymphoma (n=29), myeloma (n=23), others (n=8). The donor was an HLA-matched sibling (n=99), matched unrelated (n=36) and mismatched-related (n=7). Disease status at SCT was early (n=33), intermediate (n=24) and advanced (n=85) according to CIBMTR criteria. In all, 59 patients were given MAC and 83 RIC according to institutional criteria. As expected from eligibility criteria, RIC recipients were older (median age 51 years compared to 37 years in MAC recipients, P=0.001). More RIC recipients had lymphoproliferative disorders (54% *vs.* 17%, respectively) and less had acute leukemia/MDS (30% *vs.* 73%, P=0.001). More RIC recipients had advanced disease (63% *vs.* 54%, P=0.07) and more patients had a prior autologous SCT (49% *vs.* 0%, P=0.001). Complete follow-up is available for 139 patients (98%). The median follow-up is 11.4 years (range, 10-13.2 years). Forty-two patients are alive with a 10 year overall survival (OS) of 32% (95%CI, 25-40), 20/58 MAC recipients, 10-year OS 38% (95% CI, 25-50) and 22/81 RIC recipients, 10-year OS 28% (95%, 19-39, P=0.28). Multivariate analysis identified intermediate and advanced disease status, HR 2.5 (P=0.03) and 4.0 (P=0.0004), respectively, as the only independent adverse prognostic factors for OS. Fifty-four patients were alive 5 years after SCT, 12 patients died over the next years, 3 of them beyond 10 years from SCT. Late mortality occurred in 7 of 28 MAC recipients alive 5 years after SCT and was attributed to late relapse (n=2, both AML), chronic GVHD (n=4, 2 of them with lung disease), and MI (n=1). Late mortality occurred in 5 of 26 RIC recipients alive 5 years after SCT due to progressive disease (n=2, lymphatic malignancies, relapse occurred before 5 years) and second malignancies (n=3). Death due to chronic GVHD was more common after MAC (P=0.04) and death due to second malignancies was more common after RIC (P=0.06). The probability of patients surviving 5 years after SCT to survive for the next 5 years was 79% and 88% after MAC and RIC, respectively (P=0.25). Ten years after SCT, 32% of surviving MAC recipients and 4% of surviving RIC recipients were still on immune suppressive therapy (P=0.02).
Summary and Conclusions: Both MAC and RIC regimens allow long-term OS beyond 10 years after SCT with a similar rate. Late events continue to compromise cure beyond 5 years. The pattern of late events is different between the regimens. More events are related to late relapse and chronic GVHD (and in particular lung disease) after MAC, while second malignancies are more common after RIC. MAC is also associated with more protracted requirement for immune suppressive therapy.

P892

HOW MANY AML PATIENTS DO RECEIVE EARLY ALLOGENEIC BONE MARROW TRANSPLANT AND WHICH TRANSPLANT IS PERFORMED? REAL LIFE DATA FROM GENOVA ACUTE LEUKEMIA REGISTRY (REGAL).

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Background: Unlike other haematopoietic malignancies, the therapeutic armamentarium for AML has not changed in the last thirty years. Thereported increase in survival of youngerpatients is mainly a consequenceof theimprovedmanagement of infectionsand complicationsof BMT. In the last years new sources of allogeneic stem cells have been explored, and new transplant procedures have been designed that allow the utilization of haploidentical donors with acceptable transplant related mortality.
Aims: We report a retrospective review of AML treatment in 132 patients enrolled in a regional Italian acute leukemia registry with the purpose of evaluating transplant rate in first CR.
Methods: From October 2010 to January 2013, 132 AML patients have been reported in the Genova Acute Leukemia Registry (REGAL). Median age was 66 years (range 16-87), males were 75 (57%), females 57 (43%). Ninety-two patients had denovo disease (69%); in 40 patients (31%) AML was secondary to MDS (n. 24), chemo radiotherapy (n.9), and chronic myeloproliferative disorder (n.7). Fifteen patients (11%) had acute promyelocytic leukaemia. Relevant comorbidities were present in 75 patients (57%), mainly diabetes mellitus (n. 16), heart disease (n. 11), hypertension (n.18), liver (n.10), lung (n. 10) and other (n.33) diseases. ECOG performance status was 0 in 41 patients (31%), 1 in 51 (38%), 2 in 29 (22%), 3 in 10 (7%) and 4 in 1 (2%). Karyotype could be evaluated in 115 patients (87%) and was favourable in 17 patients (15%), intermediate in 80 (69%), unfavourable in 18 (16%). A molecular profile including study of FLT3 ITD, NPM1 gene mutations and expression of WT1 and BAALC genes was performed in 106 patients (80%).
Results: Five patients received supportive care only (4%), 33 low intensity chemotherapy (25%), 94 were eligible for intensive conventional chemotherapy (71%). In this last group 6 patients had an induction related death (6%), 58 achieved CR (62%). Induction related deaths were 2 (4%) and 4 (9%) in patients younger and older than 60 years, respectively. Complete remission rates were 78% and 43% in patients younger and older than 60 years, respectively. After prognostic work up at diagnosis 40 patients were considered eligible for early BMT (age <65 years and unfavourable cytogenetic or intermediate cytogenetic plus high risk molecular profile or secondary AML), 12 were actually transplanted in first CR (30%) and 9 are disease free and waiting for BMT (22%). Three more patients were transplanted in second CR. Reasons for not receiving early BMT were infections (n. 6), refractory or relapsed disease (n. 12) and unavailable donor (n.1). Stem cell donors have been HLA identical siblings (n. 2, 17%) and haploidentical siblings (n.10, 83%). No patients transplanted in first CR died for transplant related reasons.
Summary and Conclusions: ReGAL data show that 71% of newly diagnosed AML were treated with intensive chemotherapy and 13% of these were transplanted in first CR. BMT in first CR was performed in 30% of eligible patients mainly using haploidentical donors (83%). With the increased utilization of transplant regimens specifically designed for haploidentical donors, transplant related mortality has not changed and lack of donor is not anymore a reason for not receiving an early BMT.

P893

THE MANAGEMENTS OF EBV-ASSOCIATED POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER WITH CENTRAL NERVOUS SYSTEM INVOLVEMENT AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Epstein–Barr virus (EBV)-associated post-transplant lymphoproliferative disorder (PTLD) is a life-threatening complication that follows solid organ transplantation or allogeneic hematopoietic stem cell transplantation (allo-HSCT). The managements of systemic PTLD, including EBV molecular monitoring to identify high-risk patients developing PTLD, diagnosis, preemptive therapy and treatments, have achieved certain consensus. However, there are no uniform approaches to the managements of PTLD with central nervous system (CNS) involvement.
Aims: To explore the managements of EBV-associated CNS-PTLD after allo-HSCT.
Methods: Thirty-six patients with EBV-associated diseases (21 PTLD, 7 EBV fever, 4 encephalitis/myelitis, 3 pneumonia and 1 hepatitis) and 8 patients with unexplainable CNS manifestations from 257 patients undergoing allo-HSCT between August1, 2008 and May 31, 2012 were enrolled in this prospective study. Moreover, 10 patients with EBV-DNA-emia but without EBV-associated diseases and 5 patients who were EBV-DNA negative in blood volunteered to have their CSF monitored as controls (platelet >50×10^9/L).The EBV-DNA of blood and cerebrospinal fluid (CSF) were monitored regularly by real-time quantitative polymerase chain reaction. The rituximab-based treatments followed by adoptive cellular therapy were administered in patients with EBV-associated CNS-PTLD.
Results: Eleven patients were diagnosed as EBV-associated CNS-PTLD, including 8 systemic PTLD accompanying CNS involvement and 3 isolated CNS-PTLD. Ten patients initially presented with fever and 8 patients had CNS symptoms and signs at disease onset, 2 appeared CNS manifestations in the progression of disease and 1 did not present CNS manifestations during CNS-PTLD. Except one, all patients were EBV-DNA positive in blood. The EBV-DNA loads of CSF were significantly higher than those of blood (52571±34993 *vs.* 23083±28080, P=0.028). Of the 9 patients obtaining the immunophenotypic analysis of CSF cells, the results revealed monoclonality in 7 and polyclonality in 2 cases. The 4 patients without rituximab therapy all died of PTLD progression, whereas the 7 patients with rituximab-based treatments all obtained complete response (CR), including the 4 patients who were unresponsive to intravenous rituximab-based treatments finally obtained CR after intrathecal rituximab. The EBV-DNA loads of CSF were positively related to treatment response (P<0.001, r=0.889). With a median follow up of 261 (range 4 to 886) days after CNS-PTLD, 4 patients survived and 7 died. The causes of death were PTLD progressing (n=4), PTLD relapse (n=1), GVHD (n=1) and CMV pneumonia (n=1).
Summary and Conclusions: EBV-DNA detection in CSF might be more specific and sensitive than that in blood for the diagnosis, development and therapeutic evaluation of CNS-PTLD. The immunophenotypic analysis of CSF cells is helpful in the diagnosis of CNS-PTLD. The intrathecal rituximab is an effective method for CNS-PTLD patients who had failed to the intravenous rituximab.

P894

ASSOCIATION OF HLA POLYMORPHISMS WITH EPSTEIN-BARR VIRUS INFECTION AFTER ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Epstein-Barr virus (EBV) infection/reactivation following allogeneic haematopoietic stem cell transplantation (allo-HSCT) can cause deadly post-transplant lymphoproliferative disorders and other EBV-associated diseases. The mechanism for development of EBV-associated diseases remains unclear, but likely involves immunological control of viral infection. Human leukocyte antigen (HLA) molecules are responsible for antigen processing and presentation to the immune system, and therefore have the potential to be associated with development of EBV infection/reactivation and EBV-associated diseases.
Aims: In this study, the association between HLA polymorphisms and development of EBV infection/reactivation or EBV-associated diseases in recipients undergoing allo-HSCT was evaluated.
Methods: HLA data of 280 recipients, who were from south China and received allo-HSCT between July 2008 to December 2012, and their donors were analyzed. The EBV-DNA levels of blood were monitored regularly by quantitative real-time polymerase chain reaction (RQ-PCR).
Results: With a median follow-up of 363 days post-transplantation (range, 17 to 1670 days), 82 cases developed EBV infection/reactivation and 31 developed EBV-associated diseases including 22 EBV-PTLD and 9 other EBV-associated diseases. The 3-year cumulative incidence of EBV infection/reactivation and EBV-associated diseases were 31.5%±3.0% and 12.7%±2.2%, respectively. Both recipient and donor HLA-A11 were protective against EBV infection/reactivation (recipient A11: odds ratio [OR] 0.53, *P*=0.017; donor A11: OR 0.59, *P*=0.044). Recipients with HLA-DR9 had a higher incidence of EBV infection/reactivation than those without (OR 1.80, *P*=0.032). In multivariate analysis, recipient and donor HLA-A11 were confirmed to be negatively associated with EBV infection/reactivation (recipient A11: OR 0.45, 95% confidence interval [CI] 0.241-0.833, *P*=0.011; donor A11: OR 0.50, 95%CI 0.27-0.92, *P*=0.026). Both univariate and multivariate analysis showed donor HLA-A33 (OR 2.92, 95%CI: 1.18-7.23, *P*=0.021) and recipient HLA-B44 (OR 16.60, 95%CI: 1.91-144.03, *P*=0.011) were risk factors for development of EBV-associated diseases.
Summary and Conclusions: Our data suggest that HLA polymorphisms affect development of EBV infection/reactivation and EBV-associated diseases. These findings may be useful in risk stratification and development of prophylaxis strategies.

P895

PERFORMANCE OF FECAL CALPROTECTIN FOR THE DIAGNOSIS AND FOLLOW UP OF ACUTE GASTRO-INTESTINAL GRAFT VERSUS HOST DISEASE

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Background: Although multiple biomarkers of acute intestinal GVHD (GI-GVHD) have been identified, the translation of these from the bench to the bedside remains complex. It's known that fecal calprotectin (FC) correlates very well with disease activity in patients with inflammatory bowel disease as well as in patients with intestinal graft rejection after small bowel transplantation.
Aims: The aim of this study was to evaluate FC as a single biomarker for the diagnosis and follow up of GI-GVHD in adult patients with haematological malignancies who underwent allogeneic stem cell transplantation (SCT) at our center.
Methods: From January 2012, 30 consecutive SCT patients have been monitored for FC (normal range: 0-50 mg/kg) with at least 2 samples/patient (median number3, range 2-13). Fecal samples were collected at 2 week intervals until day 90 from SCT or at the onset of symptoms of GI-GVHD, even after day 90. The diagnosis of GI-GVHD was based on standard clinical criteria, together with other microbiological and histopathological tests.
Results: Eight of 30 tested patients (27%) were diagnosed with GI-GVHD. In this group FC median value was 429 mg/kg (range 29-5000) at the onset of symptoms; only one of these 8 patients presented FC levels in the normal ranges at the diagnosis and during follow-up (<50mg/kg). In one of the 30 patients included in the study the clinical diagnosis of GI-GVHD was not supported by the endoscopic and histological findings and in this patient, the FC median value was 60 mg/kg (range6,25-120). FC median value in all other 21/30 patients (70%) without GI-GVHD was 49 mg/kg (range 6-177). For a cut-off point value of 200 mg/kg, sensitivity for the test was 87,5%, specificity 100%, positive predictive value 100% and negative predictive value 95,6%. We couldn't observe a correlation between FC levels at the onset of GI-GVHD and severity of clinical symptoms. In fact FC median value was 467 mg/kg (range: 29-5000) in patients in stage 1 GVHD compared with 391mg/kg (range: 288-3121) in patients in stage 2-4. We observed that patients achieving stable FC values under 200 mg/kg at the follow-up (in 4 of the 7 patients with abnormal FC findings at the onset of GVHD–true positive) had a more favourable outcome and prognosis.
Summary and Conclusions: Our data confirm that FC is specific, inexpensive, non-invasive test and easy to perform. This biomarker can improve the diagnosis of acute GI-GVHD with an optimal cut-off level of 200 mg/kg. This test seems to be promising also for the evaluation of the activity of GI-GVHD during follow-up and it could be used as a potential decisional tool for anti GVHD therapy modulation.

P896

IMMUNOPHENOTYPIC RESPONSE AFTER ALLOGRAFTING IN MULTIPLE MYELOMA

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Background: Recent studies suggest that immunophenotypic remission (IR) may be a relevant prognostic factor in patients with multiple myeloma (MM). However, data on this role after allografting are lacking.
Aims: To evaluate the impact of IR and compare it to conventional complete remission (CR) in myeloma patients treated with an allograft.
Methods: Sixty-six consecutive patients, median age 54 years (35-66), who underwent an allograft between January 2000 and December 2011 with a follow-up of at least 3 months were included. Disease response was evaluated by serum and urine electrophoresis, and bone marrow aspirate at3,6,12, 18, 24 months after transplant and yearly thereafter. Skeletal survey or MRI were performed yearly or as clinically indicated (overt relapse or complaints of bone pain). Bone marrow aspirates had to contain at least 13000 cells/mL for flow cytometry studies and IR was defined as absence of monoclonal plasmacells detected by 4 or 6-colour staining with the following antibodies: CD38, CD138, CD56, CD19, CD45, cyKappa, cyLambda. CR was defined according to standard criteria (Leukemia. 2006;20:1467-73).
Results: Conditioning regimen was non-myeloablative 2Gy TBI-based in 55 patients, reduced intensity (fludarabine-melphalan-based) in 10 and myeloablative in 1 patient. Post-grafting immunosuppression consisted of cyclosporine with mycophenolate mofetil or methotrexate. Donors were HLA identical siblings in 58 patients and unrelated in 8. Only 1 patient received bone marrow as source of stem cells. Thirty-five/66 (53%) received the allograft as part of the first line treatment, whereas the others (31/66, (47%) were transplanted after disease relapse. Among patients surviving at least 3 months, treatment related mortality was 10.6% at 3 years. After a median follow-up of 69 months (range 19-147), the incidence of acute and chronic graft-*versus*-host disease was 45.6% and 49.3%, without significant difference between responsive and non-responsive patients. At follow-up, 24 patients achieved CR and IR (CR/IR), 22 achieved IR but not CR because of persistence of urine/serum M-component (noCR/IR), and 20 did not achieve either CR or IR (noCR/noIR). Interestingly, none achieved CR without IR. Median overall (OS) and event-free survivals (EFS) were not reached and 59 months in the CR/IR group, 77 and 15 months in the noCR/IR, and 30 and 5 months in the noCR/noIR respectively (P<0.001 for both OS and EFS). Being in the CR/IR group was the only significant predictor for prolonged OS and EFS (P<0.001). Of note, the cumulative incidence of extramedullary disease at first relapse after the allograft was 4.4% in the CR/IR, 31.8% in the noCR/IR and 15.0% in the noCR/noIR groups respectively(P<0.001). Receiving the allograft as first line therapy or later during the disease course did not significantly impact on OS and EFS.
Summary and Conclusions: The achievement of IR showed a significant impact on clinical outcomes including patients who did not clear the M-component. Discrepancies between IR and CR, observed in the noCR/IR group (some 30% of our patient cohort) along with a higher incidence of extramedullary relapse, suggest that myeloma cells may escape immune control outside the bone marrow. In this group, imaging studies such as positron emission tomography may be indicated to allow detection of early relapse.

P897

ALLOGENEIC STEM CELL TRANSPLANTATION FOR ACUTE LEUKEMIA IN INFANTS - EXPERIENCE OF A SINGLE CENTER

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Background: Infant acute leukemia (AL) has distinct clinical and biological characteristics that differentiate it from other childhood AL and has a dismal prognosis. Allogeneic hematopoietic stem cell transplantation (alloHSCT) is the only potential curative therapeutic in the majority of infants with AL. Presently few patients (pts) have a human leukocyte antigen (HLA)-matched sibling, but with the increasing number of unrelated (UR) donors the probability of finding a suitable donor turned this modality of stem cell transplantation (SCT) more frequent.
Aims: To evaluate the outcome of all alloHSCT performed in our center in pts with AL diagnosed in the first year of life.
Methods: Retrospective analysis of the medical records of infant AL identified in the database of SCT of our center over a period of 22 years (June 1989 until December 2012). Statistical analysis was performed with SPSS®v21.
Results: In the 22 years referred above we performed 854 alloHSCT in 772 pts, 187 of whom were <18 years old and we indentified nine children who were allografted for infant AL, the first transplanted in August 1998 and the last in December 2011. Median age at diagnosis was 5 (1-11) months; five were female and 4 male; median time diagnosis to alloHSCT: 9 (8-38) months; median age at transplant was 14 (13-47) months. All children had high-risk AL: 5 acute lymphoblastic leukemia of B lineage (B-ALL), 2 unclassified ALL and 2 megakaryoblastic acute myeloid leukemia (AML-M7, FAB). At diagnosis, CNS involvement was present in 3 pts, all with B-ALL; hyperleukocytosis >50×10^9/L was observed in 6 of 7 pts with ALL. MLL gene rearrangements were present in 5 of 7 pts with ALL; the 2 cases of AML-M7 had complex karyotype. Status of disease at transplant was: first complete remission (CR): 5; second CR: 2; third CR: 2. The donor was unrelated in all pts; stem cell source was: umbilical cord blood (UCB) in 7 (all non-HLA identical), peripheral blood (PB) in 1 (HLA identical: 10/10) and bone marrow (BM) in 1 (HLA identical: 9/10); HLA was typed in high resolution in BM and PB donors and DRB1 in UCB donors; HLA–A and B were typed serologically in UCB. Busulfan based+cyclophosphamide myeloablative regimens were used in all; *anti-thymocyte globulin (*ATG) was used in 8; in 5 ALL pts melphalan was added to the conditioning regimen. Graft-*versus*-host-disease (GVHD) prophylaxis was based in tacrolimus (n=8) or cyclosporine (n=1) associated to methotrexate (n=5) or mycophenolate mofetil (n=4). Acute GVHD grade ≥2 occurred in 7 of 8 evaluable pts. Chronic extensive GVHD occurred in one patient with almost complete resolution after 2 years of immunosuppression. Engraftment occurred in all; in UCB transplants the median time to neutrophils >0.5×10^9/L and platelets >20×10^9/L was 19 and 39 days, respectively. With a median follow-up after transplantation of 49 (31-86) months, 6 children are alive and disease-free. There were 3 deaths, all in ALL pts: 2 (day+135, day+418) due to relapse of the disease and 1 at day+59 due to multiorgan dysfunction. At 3 years the overall survival was 66,7% (±15,7%).
Summary and Conclusions: There are few reports of outcome in leukemias diagnosed in this age group. Despite the poor prognosis features of our pts 67% are disease-free three years after alloHSCT. The search of a suitable donor, related or unrelated, must be performed actively. The results of UCB transplants in this group appear promising. Multi-institutional cooperative trials are needed to evaluate the better treatment strategy in these high-risk pts.

P898

THREE DAYS BUSULFAN ASSOCIATED WITH FLUDARABINE AND ANTITHYMOGLOBULINE CONDITIONING: IS IT SAFE?

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Background: Allogeneic transplantation (HSCT) is the unique alternative to cure patients with advanced disease. Non-myeloablative conditioning has been developed for patients unfit or older than 50-55 years. Conditioning with Fludarabine, Busulfan and antithymoglobulines (ATG) has been described with two days of Busulfan. The excellent tolerance of this association and the need of stabilizing the underlined disease before having a graft *versus* hematologic dis-

ease have suggested that increasing doses of Busulfan may have higher potential of curability, particularly in acute myeloid leukemia (AML). Some reports demonstrating the interest of this conditioning were published recently. If the tolerance of two days of Busulfan (FB2) seems excellent with 2 years non relapse mortality ranging from 6 to 30%, little is known about increased dose of three days Busulfan (FB3).

Aims: The aim of this study was to assess the safety and efficacy of the FB3 regimen.

Methods: Between 2008 and 2012, 51 patients received HSCT with the FB3 regimen conditioning at our institution. Patients received Fludarabine 30 mg/m2 daily on days -6 to -2, IV Busulfan 3.2mg/kg daily on days -6 to -4, and ATG 2.5 mg/kg for 2 days. Nine patients received prophylactic DLI (17.6%).

Results: Median follow-up was 20.53 months. Hematological malignancies were AML/MDS (n=24), lymphoma (n=16), multiple myeloma (n=5), others (n=6). The median age was 58 years (range, 27-66). Thirteen patients (25%) were autografted before allogeneic stem cell transplantation. Median time to transplantation was 12.9 months (range, 0.6-210 months). Twenty patients (39.2%) had a sibling donor, 19 (37.3%) a matched unrelated donor and 12 (23.5%) a mismatched unrelated donor. All patients engrafted with a median platelets and neutrophils recovery of 12 (range, 9-125) and 16 (range, 11-37) days and full donor chimerism was observed in 88.9% at d90. Median CD34 infused was5,7 (range, 2-10.4). One patient (1.9%) experienced graft rejection at day 100. One-year overall, disease-free survivals, cumulative incidences of relapse (CIR) and non-relapse mortality (NRM) were 77.4%, 73.2%, 21.5% and 20.9% respectively (Figure 1). One hepatic veno-occlusive disease (1.9%) was observed in a patient with previously autologous stem cell transplantation. Liver toxicity occurred in 17 patients (33.3%), grade I-II in 7 patients (13.2%), grade III-IV in 10 patients (19.5%). Eight patients (15.7%) developed acute graft-*versus* host disease (aGVHD): grade II-IV (n=5, 9.8%), grade III-IV (n=1, 1.9%) and 16 (31.4%) had chronic GVHD. The survival of the 51 patients was in line with survival observed in RIC conditioning and chimerism was excellent. The NRM described in FB2 RIC regimen was variable, ranging from 6 to 30% at 2 years. It was higher in our study with FB3 regimen (26% at 2 years) than that observed with FLU-TBI. The CIR was lower than that described with RIC regimen. With FB3 conditioning, we observed a lower rate of acute GVH disease (9.8% grade II-IV), compared to the 32 to 47% of grade II-IV aGVHD in the FB2 conditioning remaining that GVHD prophylaxis was similar. In our study, age>60 was not a bad prognostic factor as demonstrated in previous RIC FB2 study (Figure 1).

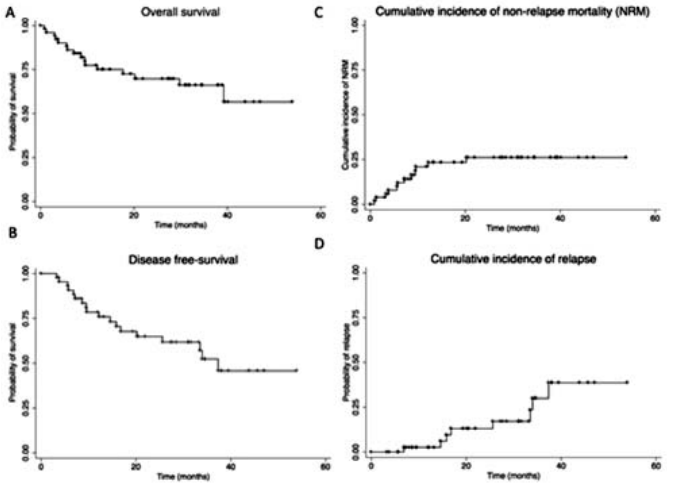

Figure 1.

Summary and Conclusions: The FB3 regimen is associated with about 20% grade III-IV liver toxicity, mostly reversible, and a quite high NRM of about 20%. An additional day of Busulfan that resulted in a low relapse rate, good overall survival and low rate of aGVHD allows its use until 65 years old. Prospective trials comparing FB2 and FB3 regimen are ongoing.

P899

IMPROVED OUTCOME WITH HEMATOPOIETIC STEM CELL TRANSPLANTATION IN A POOR PROGNOSTIC SUBGROUP OF PATIENTS WITH MIXED-LINEAGE-LEUKEMIA–REARRANGED LEUKEMIA: RESULTS FROM A SINGLE-CENTER PROSPECTIVE STUDY

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Background: The role for allogeneic hematopoietic stem cell transplantation (allo-HSCT) in mixed-lineage-leukemia (MLL)–rearranged acute leukemia is poorly understood.

Aims: To determine whether allo-HSCT could decrease relapse rates and improve long-term survival of MLL+ leukemia patients, especially in adults.

Methods: We performed a prospective, single-center cohort study in which 40 consecutive patients diagnosed with MLL–rearranged acute leukemia undergoing allo-HSCT in our institute were enrolled. Informed consent was obtained. The trial was registered at www.chictr.org as # ChiCTR-ONC-12002739.

Results: The incidences of grades II to IVacute graft *versus* host disease (aGVHD) and of grades IIIand IVaGVHD were 32.5% (CI, 16.7%>48.3%), and 7.5% (CI, 0-15.7%), respectively. The cumulative incidences for chronic GVHD (cGVHD) at two years after HSCT were 27.0% (CI, 11.0%>43.0%). After a median follow-up of 12 months, eight patients had relapsed and died from relapse, and twenty-six patients were still alive without disease recurrence. The relapse and NRM rates at two years were 27.0%(CI, 11.6%>42.4%) and 14.3% (CI, 2.3%>26.3%), respectively. The probalities of overall survival and leukemia free survival were 61.6% (CI, 43.2%>80.0%) and 58.6% (CI, 40.0%>77.2%) at two years, respectively. Patients transplanted during their first complete remission (CR1 had a lower relapse rate (20.3% *vs.* 58.3%, P=0.05) and better LFS (66.6% *vs* 20.8%, P=0.11) compared with patients transplanted beyond CR1 (Figure 1).

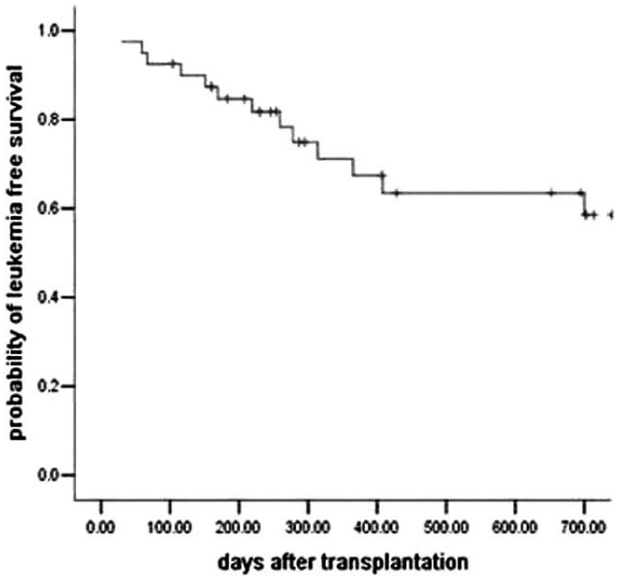

Figure 1. Probability of leukemia free survival in patients with mixed-lineage-leukemia-rearranged leukemia undergoing transplant.

Summary and Conclusions: This study showed that allo-HSCT could be a valuable treatment choice for MLL+ acute leukemia.

P900

PREDICTIVE FACTORS FOR COMPLETE DONOR CHIMERISM AFTER PEDIATRIC UNRELATED CORD BLOOD TRANSPLANTATION (UCBT)

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Background: Umbilical cord blood transplantation (UCBT) is frequently use for the treatment of hematologic malignancies or non-malignant one. Results seems equivalents to matched unrelated donor transplantation. That is why it is used increasingly frequently.

Aims: Our aim was to assess the probability of a complete donor chimerism occurrence (>99,9% donor) after UCBT, taking into account competing risks such as relapse and transplant related mortality (TRM). We wanted to identify its predictive factors and evaluate its impact on post-transplant outcome.

Methods: Our prospective single-center study was conducted between 2001 and 2010. We included all children under 18, who received a first single UCBT after myeloablative conditioning either in the context of malignant disease in complete remission, or of a non-malignant disease. All chimerisms were quantified from peripheral blood using the TAQMAN method. We analysed the occurrence of a complete donor chimerism over time.

Results: Our study included 94 children, 69 who received a transplant for a malignant disease and 25 for a non-malignant one. The mean age of the sub-

jects was 6.6 years old. Overall survival at 5 year was 68%±5%. Cumulative incidence of full donor chimerism at 1 year post-transplantation was 62%. We found 3 predictive factors of chimerism in both univariate and multivariate analysis. Firstly, age at UCBT: older was the patient, higher was the probability to reach a complete donor chimerism ($P=5,5.10^{-3}$). Moreover there was a significant threshold between the first quartile of age (<2,21 years old) and the older patients. The second factor is the HLA disparity between donor and recipient ($P=8,7.10^{-4}$). Lower compatibility (4/6 *vs* 5-6/6) resulted in a higher probability of complete donor chimerism, perhaps in part because of the increased allogenic reactivity of a graft with more HLA disparities. Finally, the third factor was the initial disease: complete donor chimersim was more frequent in children with malignancy (P=0,03). In the multivariate analysis, we did not found any effect of graft cell dose on chimerism. Early complete donor chimerism (*i.e.* >99.9% donor chimerism on D15-D30 post transplant) appeared useful to predict engraftment ($P=3.10^{-3}$) and its absence protected patients from acute Graft Versus Host Disease (GVHD)>2 ($P<1.10^{-3}$), acute GVHD >3 ($P=6.10^{-3}$) and chronic GVHD (P=0,05). We did not detect a significant effect on relapse risk for children with malignancies.

Summary and Conclusions: This study highlights some predictive factors for complete donor chimerism among children receiving UCBT: older age at transplantation, more HLA disparity between recipient and cord blood and a malignant disease. We also demonstrate that early chimerism can predict very accurately engraftment, acute GVHD as well as chronic GVHD and may be a very useful diagnosis tool.

P901

BUSULFAN, MELPHALAN AND ETOPOSIDE FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION ON PATIENTS WITH NON-HODGKIN'S LYMPHOMA: MULTICENTER STUDY FROM CONSORTIUM FOR IMPROVING SURVIVAL OF LYMPHOMA (CISL)

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Background: High dose chemotherapy followed by autologous stem cell transplantation (ASCT) has become the standard approach for relapsed or high risk non-Hodgkin's lymphoma (NHL). Several different high dose therapy (HDT) conditioning regimens have been used for non-Hodgkin's lymphoma (NHL), such as BEAM (carmustine, etoposide, cytosine arabinoside, melphalan), BEAC (carmustine, etoposide, cytosine arabinoside, cyclophosphamide), and CBV (cyclophosphamide, carmustine, etoposide). Carmustine is an active drug in the HDT of NHL but the supply of carmustine is limited in some countries including Korea. Intravenous busulfan containing regimens as conditioining regimen have been used for both allogeneic and autologous stem cell transplantation in patients with hematologic and non –hematologic malignancies.

Aims: The purpose of this prospective multicenter phase II study was evaluate the efficacy and safety of iv busulfan/melphalan/etoposide regimen as a conditioining regimen for high dose chemotherapy in the patients with relapsed or high risk NHL.

Methods: Patients with relapsed or primary refractory NHL or chemosensitive high risk NHL underwent high dose chemotherapy followed by ASCT at 12 centers in Korea. The conditioning regimen consisted of iv busulfan 3.2mg/kg/day i.v. on days -8, -7 and -6, etoposide 400mg/m2/day i.v. on days -5 and -4 and melphalan 50mg/m2/day i.v. on days -3 and -2.

Results: Fifty one patients were enrolled onto the study. Main subgroups were DLBCL (n=25, 49%) and T cell lymphoma (n=19, 37%). At the time of ASCT, the disease status of patients was as follows: 13 patients were high risk in remission, 16 were primarily refractory to inducton therapy, 15 patients were in chemosensitive relapse. All patients had successful stem cell engraftment with a median time to neutrophil recovery of more than 500/mm3 of 10 days (range, 2 to 30 days). Platelet recovery of more than 20,000/mm3 was seen after a median of 10 days (range, 2 to 51 days) with delayed recovery in one patient. Treatment related toxicities included nausea/vomiting in 28 patients (55%), diarrhea in 28 patients (55%) and mucositis in 33 patients (65%), which were grade I or II in the majority of cases. Grade I/II hepatic toxicities occurred in 24% (n=12) and grade III in 6% (n=3). There were no VOD and treatment related death. The median duration of hospitalization for ASCT was 30 days (range, 12 to 80 days). Forty one patients (80%) achieved a complete response 1 month after ASCT, while three patients showed progressive disease. At a median follow up of 27 months, 31(60%) patients exhibited a relapse or progression, while 19 patients had died of disease and one patient had died of heart failure. The estimated 2-year overall and progression free survival for all patients was 58% and 39%.

Summary and Conclusions: This analysis suggests that conditioning regimen of i.v. busulfan/melphalan/etoposide would be well tolerated and effective in patients with relapsed or high risk NHL. Accordingly, this regimen may be regarded as an important treatment option to substitute for BEAM regimen.

P902

MOBILIZATION AND ENGRAFTMENT OF PERIPHERAL BLOOD STEM CELLS IN RELATED HEALTHY DONORS OLDER THAN 55 YEARS

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Background: The increasing scarcity of related donors has led to the use of older donors for allogeneic hematopoietic stem cell (HSC) transplantation.

Aims: The objective of this study was to analyse the influence of age on mobilization and apheresis of peripheral blood stem cells (PBSC) in healthy donors, as well as on the engraftment in recipients.

Methods: Retrospective analysis of the results of mobilization and apheresis of PBSC from related healthy donors referred for HSC collection for allogeneic transplantation between 2001 and 2012 in one single centre.

Results: One hundred twenty-four related donors were included. Median age was 50 years (range 4-77), 70 (53%) were males and 44 (33%) were older than 55 years. The diseases for which HSCT was required were comparable in both groups. Donors were mobilized with G-CSF: doses of 10µg/kg/bid for 5 days if donor's weight was lower than patients' weight (30 cases [24%]), and 5µg/kg/bid for 5 days if donor's weight was higher. Mobilization and engraftment results are summarized in Table 1.

Table 1.

Pre-apheresis data	Donors ≤55 yr. (n=80)	Donors >55 yr. (n=44)	p
WBC ($x10^9$/L)	52.45 (9.4 – 98.3)	50.1 (30.7 – 87.6)	612
CD34+ cells /µL	90.5 (18 – 240)	72 (20 – 172.5)	8
Apheresis data	**Donors ≤55 yr.**	**Donors >55 yr.**	**p**
CD34+ cells($x10^6$) collected	579.3 (135.14 – 1557.24)	513.69 (149.81 – 1290)	844
Side effects	30	8	72
– Paresthesias	25	7	
– Tetany	2	0	
– Other	3	1	
Volume processed (mL)	16131 (4424 – 36906)	18653 (10003 – 26261)	2
Mobilization failure	0	1	331
Days apheresis			690
– 1	87	41	
– 2	2	1	
Engraftment data	**Recipients from donors ≤55 yr.**	**Recipients from donors >55 yr.**	**p**
Days to neutrophils >0.5x10^9/L	14 (10 – 40)	15 (10 – 26)	566
Days to platelets >20x10^9/L	13 (8 – 103)	13 (1 – 49)	856
Transfusion procedures			
– Red blood cells	2 (0 – 32)	3 (0 – 82)	0.944
– Platelets	2 (0 – 52)	2.5 (0 – 82)	
GVHD			
– Acute grade III-IV	18	8	0.862
– Chronic	27	8	0.196
Outcomes of HSCT	**Recipients from donors ≤55 yr.**	**Recipients from donors >55 yr.**	**p**
OS probability (10 years)	44±16%	47±16%	659
NRM probability (10 years)	39±11%	31±15%	568
Relapse incidence	31±11%	32±16%	749

Summary and Conclusions: Although a higher number of peripheral CD34 positive cells after mobilization was obtained in younger donors, a similar number of CD34 positive cells was finally collected. On comparison of the results of HSCT according to donor age, there were no differences in time to engraftment, GVHD incidence,NRM, relapse incidence and OS. These results indicate that the age of the donor should not be a limitation for allogeneic HSCT.

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P903

EFFICACY OF ORAL BUDESONIDE FOR GASTROINTESTINAL GRAFT VERSUS HOST DISEASE IN HAEMATOPOIETIC STEM CELL TRANSPLANT PATIENTS

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Background: Gastrointestinal (GI) graft-*versus*-host disease (GVHD) is one of the most common complications after allogeneic haematopoietic stem cell transplantation (HSCT). Glucocorticosteroids are the mainstay of therapy but are limited by their side effects. Non-absorbable corticosteroids such as oral budesonide (BUD) have been studied for the treatment of GI GVHD to minimize systemic steroid use. In Singapore, BUD is available as a hard capsule of gastro-resistant pellets, hence crushing of the pellets is required for the drug to reach its intended site of action in patients with upper GI GVHD.
Aims: This study aims to evaluate the efficacy of oral BUD as monotherapy in the initial treatment of mild to moderate GI GVHD as well as the side effect profile of oral BUD.
Methods: A single centre, retrospective review was carried out in patients who underwent allogeneic HSCT in Singapore General Hospital during the period of June 2009 to June 2011. Primary outcome was disease status at day 28 after initiating oral BUD monotherapy. Secondary outcomes were duration of oral BUD use,time to initiation of systemic steroids in patients who did not respond or progress on oral BUD, rate of recurrence after discontinuation of oral BUD, duration of response, GI GVHD-related deaths at 6 months and 12 months post diagnosis of GI GVHD, and frequency of side effects attributable to oral BUD.
Results: A total of 28 patients were studied. Median age was 39 years, 53.6% of patients were male. Upper GI GVHD=75%, lower GI GVHD=3.6%, both upper and lower GI GVHD=21.4%. Median onset of GI GVHD was 40 days post HSCT. Dose of oral BUD ranged from 3mg once a day to 3mg four times daily. Doses were titrated according to clinical signs and symptoms. At day 28 post initiation of oral BUD, 57.1% achieved CR and 32.2% achieved PR. The overall duration of oral BUD monotherapy was 35 days. There was a higher rate of CR achieved 28 days post oral BUD therapy in patients diagnosed with isolated upper GI GVHD as compared to patients with lower +/- upper GI GVHD symptoms (61.9% *vs.* 42.9%). Among those patients who eventually achieved CR, 9 patients (34.6%) recurred, although 6 patients reached second CR after a second course of BUD. Twenty-four patients (85.7%) did not require systemic steroids for the treatment of GI GVHD. Minimal toxicities attributed to BUD were reported. Overall survival at 6 months and 12 months were 89.3% and 85.7% respectively.
Summary and Conclusions: This retrospective study showed that oral BUD seemed effective in treating mild to moderate GI GVHD, as a form of local therapy avoiding systemic side effects. The results also indicate that the postulation of destroying the enteric coat of BUD pellets to facilitate immediate release of the drug in an acidic environment like the upper GI for local treatment of GVHD might hold true. Multiple courses of oral non-absorbable corticosteroids may be necessary to achieve or maintain response in some patients. However, the optimal dose and duration of oral BUD to be used in this setting has yet to be determined. Updated results will be presented at the meeting.

P904

PLERIXAFOR 'ON DEMAND' AFTER CHEMOTHERAPY+GRANULOCYTE-COLONY-STIMULATING FACTOR: RESULTS OF A STRATEGY BASED ON PERIPHERAL BLOOD CD34+ CELLS AND AN ALGORITHM FOR THE PRE-EMPTIVE US

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Background: Plerixafor is a CXCR4-inhibitor that improves peripheral-blood-stem-cell (PBSC) mobilization when combined with granulocyte-colony-stimulating factor (G-CSF). Plerixafor 'on demand' after chemotherapy+G-CSF may be efficient and cost-effective, but timing of administration and criteria for patient selection are under investigation.
Aims: Our study was aimed to assess the feasibility and efficacy of plerixafor 'on demand' with chemotherapy+G-CSF and to design an algorithm for the 'on demand' use after high-dose cyclophosphamide+G-CSF.
Methods: Twenty-eight lymphoma (n=25) and myeloma (n=3) patients were treated with chemotherapy+G-CSF and plerixafor: 20 patients had failed a previous mobilization with chemotherapy+G-CSF, 8 received plerixafor as first-line mobilizing therapy. Median age was 53 years (range, 24-68). Median number of chemotherapy lines was 2 (range, 1-4), 10 patients were treated with radiotherapy. The decision of adding plerixafor was based on circulating CD34+ cells at hematopoietic recovery. To devise an algorithm for the 'on demand' use of plerixafor after a first mobilization attempt we reviewed the data of 107 patients treated with cyclophosphamide+G-CSF: 51 myeloma patients received 3-4 gr/ms and 56 lymphoma patients received 6-7 gr/ms. Leukocytes and CD34+ cells were analyzed by a multivariate logistic regression model.
Results: Twenty-seven patients treated with plerixafor 'on demand' collected a median of4,9 × 10^6/kg CD34+ cells (range, 0,8-13) after a median of one plerixafor injection and one leukapheresis (range, 1-3). Twenty patients collected >=4 × 10^6 CD34+/kg. The median fold increase of CD34+ cells after plerixafor was4,7 (range, 1-52). The median time from chemotherapy to plerixafor administration was 15 days (range, 12-25). In our retrospective analysis on 107 patients treated with cyclophosphamide+G-CSF, having a CD34+ cell count <10/µl on the day of leukocyte recovery (>1000/µl) or leukocyte count <1000/µl after day +12 in myeloma and day +14 in lymphoma patients predicted the risk of failing PBSC collection by 2.7 and 2.8 times (P=0.001 and P=0.02) with a sensitivity of 89% and specificity of 88%, respectively.
Summary and Conclusions: Our results show that plerixafor 'on demand' can induce the collection of PBSC in >90% of patients treated with chemotherapy+G-CSF. The retrospective analysis on 107 patients treated with cyclophosphamide+G-CSF showed that those with delayed hematopoietic recovery and CD34+ cells <10/µl at recovery may deserve plerixafor 'on demand', as first-line mobilization therapy.

P905

MYELOABLATIVE CONDITIONING REGIMEN WITHOUT ATG AND TBI IN SINGLE UNIT UNRELATED UMBILICAL CORD BLOOD TRANSPLANTATION WITH HEMATOLOGIC MALIGNANCIES

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Background: Engraft failure and serious infections are still the obstacles of cord blood transplantation (UCBT). As antithymocyte globulin (ATG) and other strong immunosuppressive agent can delayed immune reconstitution and increased the risk of severe infection. Additionally, children and some adults with poor physical condition may not tolerance TBI.
Aims: Therefore, we retrospectively analyze the safety and efficacy of the conditioning regimen which without ATG and TBI in unrelated cord blood transplantation (UCBT) for 21 patients with hematologic malignancies.
Methods: The myeloablative conditioning regimen consisted of fludarabine (FLU), busulfan (BU) and cyclophosphamide (CY). FLU 30mg/m2 was administered intravenously as a single daily dose on days -9 and -8, BU 3.2 mg/kg was also administered intravenously as a single daily dose on days -7 to -4, and CY was administered intravenously over 2 hours at a dose of 60 mg/kg once daily on days -3 and -2 (total dose 120 mg/kg). 24 hours after the completion of conditioning, patients received the UCBT.Graft *versus* host disease (GVHD) prophylaxis was cyclosporin A (CSA) and mycophenolate mofetil (MMF).
Results: With this conditioning regimen we had achieved high engraftment rate (95.2%) and rapid hematopoietic reconstitution.neutrophil engraftment at a median time of 18 (12~30) days and platelet engraftment at a median time of 37(14~83) days. The cumulative incidence of acute GVHD was similar with other reports while pre-engraftment syndrome (PES) and III~IV acute GVHD was slightly higher. Thirteen cases (61.9%) of patients occurred PES. Acute GVHD occurred in 9 cases of the 20 engraftment patients (45.0%) and 5 cases (25.0%) were of grade III~IV. But the chronic GVHD only occurred in one of 19 evaluable patients (5.3%) which were much lower and localized. Post-transplant infection rate declined may associate with the absence of ATG and rapid recovery of the neutrophil. Sixteen patients (76.2%) got infection and two cases (9.5%) died of severe infection. CMV reactivation occurred in 71.4% (15 of 21) of the patients at the time between 18 and 139 days after UCBT. But there were no CMV diseases occurred and no patients died of CMV infection. The cumulative incidence of relapse (9.5%) was significantly lower and no ALL patients relapsed. Three-year overall survival (OS) and event-free survival (EFS) were 66.7% and 61.9% respectively of total patients and these data were similar between high-risk and standard-risk patients (Figure 1).

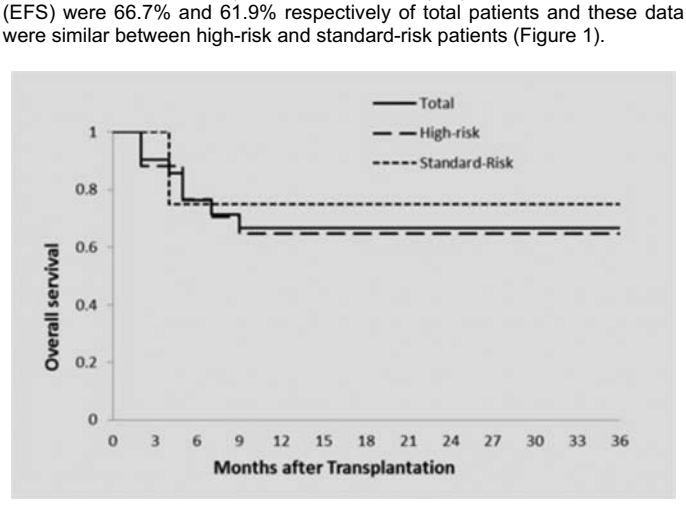

Figure 1. Standard-risk vs. high-risk P=0.76. Overall survival (OS).

Summary and Conclusions: In summary, we found the prominent feature of this conditioning regimen was that the same high engraftment rate, rapid myeloid reconstruction and low incidence of infection. Low relapse rate and good survival is also its significant features. Although the PES and acute GHVD occurred slightly higher, chronic GVHD was significantly reduced so that the patient's quality of life had been improved. More cases needed to be studied to confirm these findings further.

P906

EFFECTS OF HUMANIZED ANTI-CC CHEMOKINE RECEPTOR 4 MONOCLONAL ANTIBODY ON REGULATORY T CELLS AND GVHD AROUND HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ADULT T-CELL LEUKEMIA LYMPHOMA

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Background: Adult T-cell leukemia lymphoma (ATL) is an aggressive peripheral T-cell neoplasm caused by human T-cell lymphotropic virus type I with unique characteristics of resistance to conventional chemotherapy. Allogenic stem cell transplantation is an effective treatment in selected patients with ATL, but high treatment-related mortality and high relapse rate after transplantation are still remained. Recently combination of additional target therapy, a humanized anti-CC chemokine receptor 4 monoclonal antibody(anti CCR4 antibody) with HSCT is expected to improve survival of patients with aggressive ATL. Anti-CCR4 antibody enhances antibody-dependent cellular cytotoxicity not only for CCR4 expressing ATL cells but also T regulatory (T reg) cells expressing CCR4 which are supposed to regulate GVHD after HSCT. The effects of anti-CCR4 antibody on T reg cells and GVHD in the case of HSCT are not elucidated yet.
Aims: We investigated the number of peripheral T reg cells and the characteristic of GVHD in 3 cases of aggressive ATL patients treated with anti-CCR4 antibody before or after HSCT.
Methods: 3 patients with ATL received anti-CCR4 antibody once per week for 6~8 times at a dose of 1.0mg/kg within three months before or after HSCT. We investigated T-cell subset distribution during and/or after anti-CCR4 antibody treatment. And we evaluated clinical characteristics of GVHD after HSCT.
Results: In all cases, regulatory T cells were extremely reduced after anti-CCR4 antibody treatment and the reduction was lasted for more than 3 months. The number of T reg cells at 3 months after final anti-CCR4 antibody was 0.32±0.33/μl. (Normal control: 54.12±13.73 /μl).All patients showed Grade III-IV skin GVHD and 2 of them were steroid resistant. And liver dysfunction or liver GVHD was seen in two cases. No gut GVHD was seen in all cases.
Summary and Conclusions: T reg cells were severely depleted after anti-CCR4 antibody treatment with HSCT and the depletion period of T reg cells were longer in these patients with HSCT than in the ATL patients without HSCT. It was possible that the suppression of T reg cells induced severe skin GVHD.

P907

IMPACT OF INAPPROPRIATE MICAFUNGIN PROPHYLAXIS IN A BONE MARROW TRANSPLANTATION (BMT) UNIT ON THE OUTCOME OF CANDIDIASIS AND ON THE EMERGENCE OF ECHINOCANDIN-RESISTANT CANDIDA KRUSEI ISOLATES

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Background: Despite the increasing use of echinocandin antifungal drugs, its resistance in Candida species remains uncommon. Between 2004 and 2010, the French Mycoses Study Group reported 20 infections caused by echinocandin-resistant (ER) Candida but only two were due to resistant Candida krusei. The first acquired ER Candida krusei isolate, with mutation in the FKS1 subunit, was described in 2007 and no more than ten cases since then.
Aims: We report a single-center experience of an increased incidence of candidemia following exposure to prophylactic micafungin in bone marrow transplant (BMT) patients, comparing to a retrospective cohort exposed to fluconazole prophylaxis. We describe the characteristics of candidemia with acquired ER Candida krusei isolates following exposure to micafungin and caspofungin.
Methods: This was a monocentric retrospective study of BMT patients in University Hospital of Saint-Etienne, France. Between January 1st, 2009 and April 1st, 2011, prophylaxis in patients with hematopoietic stem cell transplantation was based on fluconazole (400 mg/day) whereas after April 1st, prophylaxis was based on micafungin (50 mg/day). Species identification of Candida isolates was achieved by standard mycologic procedures and routinely tested for susceptibility to antifungal drugs. Three Candida krusei isolates were referred to the NRCMA (French National Reference Center for Mycoses and Antifungals). E-test for the susceptibility profile was confirmed using the EUCAST microdilution methods.
Results: We observed no candidemia for the 75 patients exposed to fluconazole prophylaxis while three cases of Candida krusei fungemia were reported in the 37 patients exposed to micafungin prophylaxis. All of three strains were initially susceptible to echinocandin.
A 48-year-old man developed candidemia caused by Candida krusei at Day 6 after cord blood transplant for CLL. He was treated with Caspofungin (70 mg/day) until an ER Candida krusei was isolated in stool cultures, two weeks later. He finally received a liposomal amphotericin B treatment with success.
The second case occurred in a 56-year-old woman who exhibited candidemia at day 11 after pheno-identical BMT for multiple myeloma. Mycafungin prophylactic treatment was switched to caspofungin (70 mg/day) and the patient was admitted to the ICU for respiratory distress. Six weeks later, ER Candida krusei isolates were identified in stool and mouth cultures. Caspofungin was changed to liposomal amphotericin B. The patient died ten days later of respiratory distress escalation with septic presentation.
The third case concerned a 38-year-old man who presented a candidemia due to a Candida krusei on day 5 after pheno-identical BMT for CLL. Liposomal amphotericin B was administered with success. Two weeks later, numerous isolates of Candida krusei were found in the digestive tract. Interestingly, these isolates remained susceptible to echinocandin.
Summary and Conclusions: We observed the outcome of candidemia in patients who received BMT under micafungin prophylaxis. This study underlines the importance of appropriate management of antifungal therapy, both in prophylactic and in curative strategy. All cases of candidemia were due to Candida krusei with uncommon acquired echinocandin resistance in two isolates in stool and mouth cultures. Resistant strains are now being studied at the NRCMA for FKS gene mutations. It will be interesting to investigate whether resistance appeared during treatment or is due to other pre-existing resistant strain expanding under treatment pressure.

Stem cell transplantation - Clinical 4

P908

PLERIXAFOR IS SUPERIOR TO CONVENTIONAL CHEMOTHERAPY FOR FIRST LINE STEM CELL MOBILISATION, AND IS EFFECTIVE EVEN IN HEAVILY PRETREATED PATIENTS

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Background: Plerixafor is an effective 2nd line agent for mobilising haemopoietic stem cells for stem cell transplantation (SCT), and it is increasingly used pre-emptively in patients mobilising poorly. However, its use as a first line agent has not been well studied.
Aims: Assess whether stem cell mobilisation using first line plerixafor and filgrastim is superior to stem cell mobilisation using conventional chemotherapy and filgrastim.
Methods: The Liverpool PHANTASTIC trial (clinicaltrials.gov identifier: NCT01186224) has recruited 100 lymphoma or myeloma patients destined for SCT, between April 2010 and June 2012. Mobilisation comprised filgrastim for 5-8 days, and plerixafor daily from day 4 for up to 4 doses. Stem cells were harvested on day 5 and daily thereafter, until either >4×10^6 $CD34^+$ cells /kg recipient weight were collected or 4 aphereses had been performed. Results were compared with 151 immediately preceding historical controls mobilised by chemotherapy (50% cyclophosphamide 1.5gm/m^2; 50% lymphoma salvage) and filgrastim.
Results: Two patients failed screening (1 renal failure; 1 relapse). Seventy (71%) of 98 plerixafor mobilised patients passed the primary endpoint of >4 × 10^6 $CD34^+$ cells/kg in 1 or 2 aphereses, with no evidence of neutropenia. This is superior to the 48 (32%) of 151 control patients who passed this endpoint (P<0.001). Ninety-four (96%) plerixafor mobilised patients achieved a 'bare minimum' of >2 × 10^6 $CD34^+$ cells/kg, which was significantly more than in control patients (114 (75%); P<0.001). Serious adverse events were not seen in any plerixafor mobilised patient, compared with 14 (9%) control patients, of which 10 were admissions for neutropenic sepsis. Details of prior treatment were available in 97 (99%) plerixafor mobilised patients and in 142 (94%) control patients. These were summarised for each patient using an update of a previously reported score (Clark & Brammer, BMT 1998; 22: 859-863), and a novel simplified score, whereby individual chemotherapy regimes were allocated a score of1, 2 or 3 according to their myelotoxicity. Plerixafor mobilised and control patients were well matched for the amount of prior treatment. Control patients mobilising >2.0 × 10^6 $CD34^+$ cells/kg had significantly lower scores than those who did not achieve this level (P=0.002) and similar findings were seen when using a cut off of 4.0 × 10^6 $CD34^+$ cells/kg. Furthermore, 32 of 36 (89%) control patients with scores in the lowest quartile mobilised >2.0 × 10^6 $CD34^+$ cells/kg, compared with only 25 (69%) with scores in the highest quartile. In contrast, for plerixafor mobilised patients, although those with higher treatment scores tended to mobilise less well, this relationship was not statistically significant, and 24 (100%) and 22 (92%) of 24 patients in the lowest and highest score quartiles mobilised >2.0 × 10^6 $CD34^+$ cells/kg. Eighty-five (87%) plerixafor mobilised patients underwent SCT, compared with 119 (80%) of control patients. With plerixafor mobilisation, only 3 (4%) required additional harvest rounds to achieve 2.0 × 10^6 $CD34^+$ cells/kg, whereas 17 (14%) control patients required additional harvest rounds to achieve this. There is currently no significant difference in the 12-month overall survival between plerixafor mobilised patients (86.2%) and control patients (83.7%).
Summary and Conclusions: Subject to the problems of historical control comparisons, we conclude that plerixafor is a more effective first line stem cell mobilisation than conventional chemotherapy (especially in heavily pretreated patients) and has lower toxicity, with no evidence of an inferior subsequent outcome. Plerixafor merits consideration as the first line standard of care for stem cell mobilisation.

P909

SIMILAR OUTCOMES BETWEEN YOUNGER AND OLDER AGE GROUP IN ADULT PATIENTS WITH SEVERE APLASTIC ANEMIA WHO RECEIVED HLA-MATCHED SIBLING TRANSPLANTS WITH FLUDARABINE-BASED CONDITIONING

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Background: Older age has been a major limitation for HLA-identical sibling allogeneic stem cell transplant (SCT) for severe aplastic anemia (SAA) because of increased transplant-related mortality. Recently, a less toxic conditioning regimen comprised of fludarabine (Flu), reduced dose cyclophosphamide (CY), and ATG (Flu/CY/ATG) was introduced in HLA-identical sibling allogeneic SCT for the patients with SAA.
Aims: We analyzed the transplant outcomes of adult patients with SAA conditioned with Flu/CY/ATG to compare younger age group (<40 years) and older age group (≥40 years).
Methods: Ninety-five consecutive adult patients transplanted from March 2002 to May 2012 at our center, followed by conditioning with Flu (30 mg/m^2/day × 6 days), CY (50 mg/kg/day × 2 days), and rabbit ATG (2.5 mg/kg/day × 4 days) were analyzed. All patients received CsA plus short course of methotrexate as GVHD prophylaxis.
Results: The median age of enrolled patients was 38 years (range, 15-58 years). The median ages of younger age group (n=50) and older age group (n=45) were 29 (range, 15-39) and 47 (range, 40-58) years, respectively. Thirty-four (35.8%) patients had very SAA at the time of allogeneic SCT. Stem cell sources were bone marrow (BM) in 69 patients, peripheral blood stem cell (PBSC) in 14 patients, BM with immune-magnetically selected CD34+ PBSC in 12 patients. The conditioning regimen was well tolerated, which showed low early mortality (5.3%). All patients achieved neutrophil engraftment with median time of 12 days (range, 5-20). The 100-day incidence of acute GVHD of grade ≥2 was 6.4% (95% CI, 2.6-12.6) and 2-year incidence of chronic GVHD was 9.3% (95% CI, 4.3-16.6). With a median follow-up of 3 years of the survivors, overall survival (OS) was 93.7% (95% CI, 86.4-97.1). Seven patients (7.4%) experienced secondary graft failure and 6 patients of them have successful durable engraftment after second allogeneic SCT. Seven patients died of sinusoidal obstruction syndrome (n=1), cytomegalovirus pneumonia (n=2), sepsis (n=2), and clonal evolution to AML (n=1) and MDS (n=1). There was no significant difference of OS between the younger age group and the older age group (96.0% *vs.* 91.1%; P=0.218). When we classified the patients into 4 age groups; <30 years (n=27), 30-39 years (n=23), 40-49 years (n=30), and ≥50 years (n=15), there was also no significant difference in OS between them (96.3%, 95.7%, 93.3% and 86.7%, respectively; P=0.578) (Figure 1).

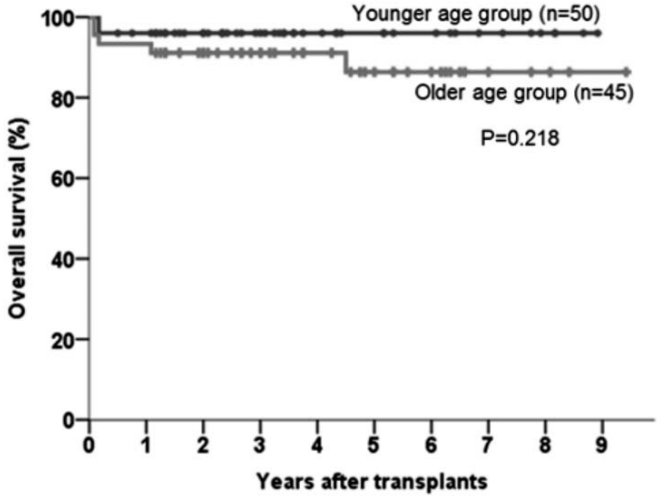

Figure 1.

Summary and Conclusions: Our study suggests the HLA-identical sibling transplants conditioned with Flu/CY/ATG for the patients with SAA showed the similar outcomes irrespective of age groups. It indicates the patients aged more than 40 years can be suitable candidates for HLA-identical sibling transplants.

P910

POORER OUTCOME AFTER CORD BLOOD THAN AFTER MATCHED RELATED OR UNRELATED DONOR ALLOGENEIC STEM CELL TRANSPLANTATION PREPARED WITH REDUCED TOXICITY CONDITIONING FOR HIGH RISK ACUTE MYELOID LEUKEMIA

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Background: When patients present a high risk acute myeloid leukemia (AML), allogeneic hematopoietic stem cell transplantation (Allo-SCT) is recommended. In many situations, a matched related (MRD) or matched unrelated donor (MUD) is lacking. Many reports showed that unrelated cord blood (UCB) represents a valuable alternative, but to our knowledge, no study specifically focused on high

risk AML transplanted after reduced toxicity conditioning (RTC) regimen.
Aims: The objective of this study is to evaluate the efficacy and the toxicity of UCB in the setting of high risk AML patients who lack a MRD or MUD.
Methods: We retrospectively analyzed patients undergoing RTC Allo-SCT in our institute from 2000 and 2012 for a high risk AML (patient not in complete remission (CR) at the time of Allo-SCT; second CR and beyond; adverse karyotype abnormalities (AdvK); First CR achieved after more than one induction course; CNS involvement; granulocytic sarcoma). We then compared outcome of patients who received graft from MRD or MUD *vs.* those who received UCB when no donor was found.
Results: One hundred sixty two consecutive high-risk AML patients treated in a single center were included and compared according to donor source (MRD or MUD (N=87+37) *vs.* UCB (N=38)). Median age was 51 years [range: 18-70], 131 patients (81%) were transplanted in CR, 57 patients (36%) had AdvK and the median follow up was 46 months [range: 7-35], without significant difference between MRD/MUD and UCB groups. In the UCB group, most of patients (34/38, 89%) received conditioning regimen containing cyclophosphamide, fludarabine and 2 Gray total body irradiation. In the MRD/MUD group, most of patients (93/124, 75%) received RTC regimen containing fludarabine, busulfan and antithymocyte globulin. Cyclosporine A (CsA) alone was used as graft-*versus*-host disease (GVHD) prophylaxis for 91/124 MRD/MUD patients (73%) while all UCB patients and 33/124 MRD/MUD patients (27%) received CsA plus mycophenolate mofetil (MMF) for GVHD prophylaxis.
Grade II/IV acute GVHD was 23% *vs.* 42% (P=0.026) and extensive chronic GVHD was 23% *vs.* 8% (P=0.038) in MRD/MUD and UCB groups respectively. Early-NMR at day 100 was lower in the MRD/MUD group than in the UCB group (4% *vs.* 16% respectively, P=0.013) (Figure 1 A). However, there were more delayed non-relapse deaths in the MRD/MUD group (N=17) than in the UCB group (N=2), leading to similar overall NRM between the MRD/MUD and UCB patients (at 2 years: 16% *vs.* 21% respectively, P=0.580). The cumulative incidence of relapse at 2 years was 28% *vs.* 49% in the MRD/MUD and UCB groups respectively (P=0.049) (Figure 1 B). By multivariate analyses of progression free survival (PFS) and overall survival (OS), the use of UCB (HR for PFS: 2.24 [1.39-3.59]; HR for OS: 1.83 [1.10-3.05]), CR at the time of Allo-SCT (HR for PFS: 0.31 [0.19-0.50]; HR: 0.34 [0.20-0.57]) and AdvK (HR for PFS: 1.69 [1.10-2.59]; HR for OS: 1.71 [1.08-2.71]) were independent significant factors influencing survivals.

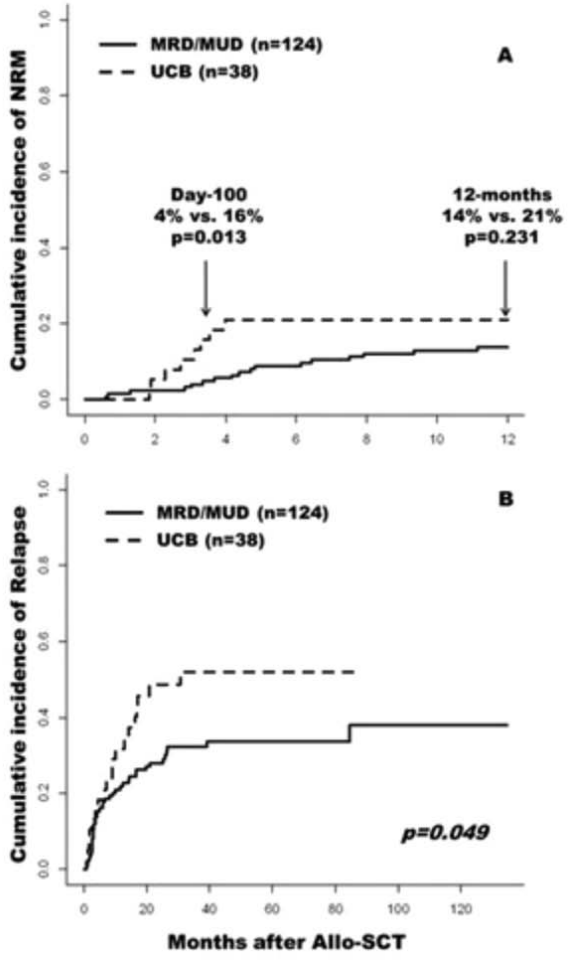

Figure 1.

Summary and Conclusions: We conclude that even if UBC allows extending Allo-SCT to patients who lack MRD/MUD, outcome of high risk AML transplanted from UCB remained poor in the context of RIC. Indeed, early NRM remains a major issue and about half of high risk AML patients relapsed after UCB Allo-SCT leading to shorter survivals compared with MRD/MUD. These results need to be prospectively confirmed; nevertheless, new strategies to improve anti leukemic effect in the setting of UCB Allo-SCT are needed as well as the investigation of other alternative donors such as haploidentical donors.

P911

OLDER AGE IS NOT A CONTRAINDICATION FOR UNMANIPULATED HAPLOIDENTICAL GRAFTS, WITH HIGH DOSE POST-TRANSPLANT CYCLOPHOSPHAMIDE

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Background: We have recently shown that hematologic malignancies can be successfully grafted with unmanipulated haploidentical bone marrow (HAPLO) and post-transplant high dose cyclophosphamide (PT-CY), following a myeloablative conditioning regimen (Raiola *et al.*, BBMT 2013). However, older age is known to be a negative predictor of survival after allogeneic transplants
Aims: In this study we wanted to test whether older age is a contraindication for HAPLO grafts with PT-CY.
Methods: Patients. Eligible for this study were 108 consecutive HAPLO transplants, grafted between September 2009 and september 2012. Second allogeneic transplants were excluded. The median age was 46 years (17-74); The majority of patients (47%) had acute leukemia, the second more frequent diagnosis was lymphma (23%) and myelodisplasia (12%). Fifty patients were in remission (CR1+CR2) and 58 had active disease at transplant. Graft *versus* host disease (GvHD) prophylaxis consisted in PT-CY 50 mg/kg, on day+3 and +5, cyclosporine (from day 0), and mycophenolate (from day +1). The conditioning regimen was myeloablative (MA) in 86 patients and non myeloablative (NMA) in 22. The median follow up for surviving patients of 354 days (149-1102). Patients were divided in young/old according to the median age (46 years).
Results: Univariate analysis. The cumulative incidence of grade II-III acute GvHD was 21% *vs* 4% for younger/older patients respectively (P=0.003), and of moderate chronic GvHD 14% *vs* 22% (P=0.1); the cumulative incidence of transplant related mortality (TRM) for younger/older patients was 9% *vs* 23% (P=0.03), and the actuarial 3 year survival 63% *vs* 42% (P=0.03). The crude survival for patients aged <30 (n=32), 31-40 (n=15), 41-50 (n=19), 51-60 (n=25) and >60 (n=17) is 78%, 86%, 78%, 60%, 53% (P=0.1)
Multivariate analysis. When age was entered in a multivariate analysis on survival, together with disease phase (remission/advanced), interval diagnosis transplant, female donor in male recipient and intensity of the conditioning (MA/NMA), there was no effect of patient age (P=0.9) and the only predictor was disease phase at transplant (remission/relapse). Indeed the older patient population included more patients with relapsed disease (64%) as compared to 44% in the younger patients (P=0.03).
Summary and Conclusions: When stratified for patient and disease variables, older patients with hematologic malignancies have outcome comparable to younger patients. Our data indicate that older age–in our experience up to 70 years- should not be a contraindication for HAPLO transplants with PT-CY.

P912

KIR-LIGAND INCOMPATIBILITY IN THE GRAFT-VERSUS-HOST DIRECTION DID NOT AFFECT OUTCOMES OF SINGLE UMBILICAL CORD BLOOD TRANSPLANTATION WITHOUT ATG FOR ACUTE LEUKEMIA IN COMPLETE REMISSION

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Background: Killer cell immunoglobulin-like receptor (KIR)–ligand incompati-

bility may have some important roles in transplantation outcomes such as leukemia relapse and leukemia-free survival. It was reported that cord blood transplantation (CBT) for acute leukemia in complete remission (CR) from KIR-ligand incompatible donors in the graft-*versus*-host direction was associated with decreased relapse and improved survival. In the other report, KIR-ligand mismatch was associated with development of severe acute graft-*versus*-host disease (GVHD) and risk of death after double cord blood transplantation with reduced intensity conditioning (RIC) regimen. Therefore, the role of KIR-ligand incompatibility in CBT is not clear and controversial.
Aims: In order to clarify the effect of KIR-ligand incompatibility in the graft-*versus*-host direction on the outcome of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) patients in CR after single cord blood transplantation, we assessed the outcome of CBT registered in the Japan Society for Hematopoietic Cell Transplantation (JSHCT) database between 2001 and 2010 (A Study From the HLA Working Group of the JSHCT).
Methods: 642 acute leukemia (356 AML and 286 ALL) patients in CR and donors whose HLA-A, -B, -C, -DR alleles were determined by DNA typing. Patients and donors were categorized according to their KIR-ligand incompatibility in the GVHD direction by determining whether or not they expressed HLA-C group 1 or2, Bw4 or A3/A11. All these patients did not receive ATG as a preparative regimen. We compared overall survival (OS), disease free survival (DFS), relapse incidence, non-relapse mortality (NRM) and GVHD between KIR-ligand incompatible and compatible group using the JSHCT database.
Results: 128 patient –donor pairs (AML 69, ALL 59) were HLA-A, -B or –C KIR-ligand incompatible (A3/A11 25, Bw4 68, C 61) and 514 compatible. Reduced intensity conditioning (RIC) regimen
(187, AML 129, ALL 58) defined basically as the use of fludarabine plus low-dose busulfan or melphalan with or without low-dose total body irradiation. Univariate analysis showed no significant differences between KIR-ligand incompatible and compatible group in both AML and ALL patients of OS, DFS, relapse incidence, NRM and acute GVHD (P=0.674, P=0.688, P=0.353, P=0.766, P=0.569 for AML, P=0.628, P=0.352, P=0.693, P=0.492, P=0.691 for ALL, respectively). Also, there were no significant difference in OS, DFS, relapse incidence, NRM and acute GVHD between KIR-ligand incompatible and compatible group in both AML and ALL patients by multivariate analysis. The conditioning regimens (RIC and myeloablative conditioning) also did not affect these results.
Summary and Conclusions: There were not positive and negative effects of KIR-ligand incompatibility in GVHD direction on single cord blood transplantation outcome in both AML and ALL patients without ATG in Japan. Administration of ATG as a preparative regimen may be important to obtain positive effect of KIR-ligand incompatibility in GVHD direction on cord blood transplantation outcomes such as survival and relapse in especially AML patients.

P913

ASSESSMENT OF LIVER FIBROSIS IN PATIENTS WITH B-THALASSEMIA MAJOR UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION USING TRANSIENT ELASTOGRAPHY

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Background: Transient Elastography (TE) is an efficient non-invasive technique for evaluation of liver fibrosis which has been recently used much more widely.
Aims: This study attempts to estimatethe diagnostic accuracy of TE in patients with β-thalassemia major (TM) undergoing Hematopoietic Stem Cell Transplantation (HSCT).
Methods: This is a prospective study in which 58 TM patients withmedian age=8 years (range: 2.6-20 years) enrolled. For all patients, we measured liver stiffness using both TE in kilopascals (kPa) and liver biopsy based on Ishak score. The diagnostic accuracy of TE and liver biopsy were evaluated using linear discriminated analysis (the area under the receiver operating characteristic curves (AUROCs)).
Results: Of 58 patients, there were 26 patients (44.8%) with mild fibrosis, 12 (20.7%) with moderate fibrosis and 2 (3.4%) with severe fibrosis. The correlation of TE values with thalassemia classification (P<0.001), iron deposition (P<0.037) and fibrosis stage (P=0.008) were statistically significant. The median TE values in patients with severe fibrosis (stage 3-5) and mild or no-fibrosis groups were 4.5 (range, 3.0-13.0) kPa and 4.0 (range, 2.5-9.0) kPa, respectively. Prediction of high fibrosis stages (stage >=3) using TE with cut-off of 4.35 kPa, AUROC was 0.670 (95% confidence interval [CI]: 0.508-0.833) with 76.9% sensitivity (95% CI: 70.8-81.8) and 57.8% specificity (95% CI: 53.3-60.3).
Summary and Conclusions: TE has presented itself as a new, non-invasive method for assessment of fibrosis stage in TM patients undergoing HSCT and maybe useful as an alternative to liver biopsy. Noticeable, TE also can be utilized as an invaluable technique for following up the liver status in TM patients after transplantation.

P914

PROGNOSTIC IMPACT OF CHRONIC GRAFT VERSUS HOST DISEASE IN PATIENTS WITH MYELOID NEOPLASMS UNDERGOING REDUCED INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION–A SINGLE INSTITUTION SERIES

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Background: Allogeneic stem cell transplant (allo-SCT) is often the only curative option for patients with myeloid neoplasms. Patients with acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN) and overlap syndromes have successfully been transplanted with myeloablative strategies. The advent of reduced intensity conditioning (RIC) has not only decreased the non-relapse mortality (NRM), but has also made allo-SCT a viable option for older patients and patients with comorbidities. RIC relies on the graft *versus* leukemia (GVL) effect to eradicate the neoplastic clone. While this is difficult to measure, a surrogate marker is often the presence of graft *versus* host disease (GVHD).
Aims: To analyze single institution data on survival and transplant related outcomes for patients with myeloid neoplasms that have undergone RIC allo-SCT. To validate the survival benefit associated with the development of chronic GVHD.
Methods: After due IRB approval, 159 successive patients with myeloid neoplasms that had undergone RIC Allo-SCT at the Mayo Clinic were included in the study. The myeloid neoplasms were diagnosed and risk stratified according to World Health Organization (WHO) criteria. RIC conditioning was initiated at our institution in 2000 and patients in this study were included up to January 2013. All data was retrospectively abstracted and analyzed to compute transplant related outcomes such as; NRM, risk of disease relapse, overall-survival (OS), disease free survival (DFS), acute and chronic GVHD. The two major RIC used were; fludarabine and melphalan (n=138) and fludarabine and IV targeted busulfan (n=21). GVHD prophylaxis and liver injury prophylaxis were according to institutional standards. GVHD was evaluated and graded according to WHO and NIH scales.
Results: Of the 159 study patients, 99 (62%) were males, with a median age at transplant of 60 years (range, 14-71 years). There were 92 (58%) patients with AML [50 (54%) CR1, 19 (21%) CR2, 3 (3%) ≥CR3, 10 (11%) PIF, 10 (11%) untreated], 24 (15%) with MDS with excess blasts [12 (48%) CR1, 6 (24%) PIF, 7 (28%) untreated], 24 (15%) with MDS without excess blasts, 13 (8%) with myelofibrosis and 6 (4%) with CML [4 (67%) accelerated phase, 2 (33%) chronic phase]. At last follow-up, 70 (44%) deaths were recorded, with the median follow-up for the study being 12 months [range, 0–109 months] (23 (14%) from 2000-2005, 74 (47%) from 2005-2010, and 62 (39%) from 2010 onwards]. At last follow up, 118 (75%) patients remained in CR, while 28 (17%) had relapsed. In patients transplanted up to 2010, OS and DFS were 17 months (range 0–109 months) and 13 months (range 0–98 months), respectively. The 10 year NRM was 37%. Ninety-four (60%) patients developed acute GVHD [17 (11%) grade1, 42 (26%) grade2, 26 (16%) grade 3 and 9 (6%) grade 4]. Ninety-one (57%) developed chronic GVHD [16 (10%) mild, 34 (21%) moderate and 41 (26%) severe]. There was no difference in OS (P=0.06) and DFS (P=0.07) between the two conditioning regimens. In univariate analysis, factors adversely influencing OS included low cell dose (P=0.03), graft failure (P<0.0001), grade 4 acute GVHD (P=0.02) and absence of chronic GVHD (<0.0001). On a multivariable analysis; low cell dose (P<0.0001), graft failure (P<0.0001) and absence of chronic GVHD (P<0.0001) retained independent significance.
Summary and Conclusions: The development of chronic GVHD strongly correlates with improved OS and DFS in patients with myeloid neoplasms undergoing RIC allo-SCT, strongly supporting the role for an active graft *versus* leukemia effect.

P915

***IN VIVO* T-CELL DEPLETION WITH LOW-DOSE ANTITHYMOCYTE GLOBULIN TO REDUCE ACUTE GVHD IN UNRELATED DONOR STEM CELL TRANSPLANT FOR PATIENTS WITH SEVERE APLASTIC ANEMIA**

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Background: For the patients with severe aplastic anemia (SAA) who do not have a sibling donor and cannot acquire an adequate response to immunosuppressive therapy, allogeneic stem cell transplantation (SCT) from an unrelated donor is used. However, higher incidence of graft-*versus*-host disease (GVHD) in the unrelated SCT setting remains an obstacle. We have reported that allele-mismatched unrelated grafts and peripheral blood stem cells (PBSCs) were risk factors for GVHD (Lee JW, *et al.*, Biol Blood Marrow Transplant 17: 101-108, 2011).
Aims: This study is performed to evaluate the effects of *in vivo* T-cell depletion with low-dose antithymocyte globulin (ATG) for reducing GVHD in SAA

patients who received not only transplants from mismatched unrelated donors, but also PBSCs from some matched unrelated donors.
Methods: Seventy-five patients with SAA (n=61) or VSAA (n=14) had received SCT from unrelated donor. Conditioning regimen consisted of fractionated total body irradiation (TBI, 800 cGy) and cyclophosphamide (100-120 mg/kg) from 2000 March to 2009 July.From 2009 August ATG (1.25 mg/kg/day; Genzyme, Cambridge, MA, USA) for 2 days was added as a part of conditioning to reduce GVHD in patients receiving HLA mismatched grafts or PBSCs. Patients were classified into the following three subgroups: (1) patients who had HLA matched (8/8), BM donors (group 1; n=33); (2) of patients who had HLA mismatched or PBSCs donors, those who did not received ATG (group2, referred to as historic controls; n=28); (3) those who received ATG (group 3; n=14).
Results: Seventy-five patients including 37 men and 38 women were assessed. Their median age was 29 years (range, 15-59 years) and the median interval from the diagnosis to transplantation was 48 months (range; 2-323 months). The majority of patients received multiple transfusions (median; 75 units, range: 4-405) prior to SCT. After a median follow-up of 55.5 months, the 4-year estimated OS rates were 87.9, 92.9, and 85.7 for group1,2, and3, respectively and there was no statistical difference (P=0.727). All patients achieved primary engraftment, and only one patient in the group 2 suffered from secondary engraftment failure. The cumulative incidence (CI) of acute GVHD (aGVHD, ≥grade II) was 36.4%, 64.3%, and 21.4% in each group. Multivariate analysis showed that the CI of aGVHD in the group 3 was comparable with those of the group 1 (RR of 0.53, *P*=0.320), whereas the patients without ATG (group 2) had a higher incidence of aGVHD (RR of 2.23, *P*=0.028). The CI of chronic GVHD (cGVHD) showed the no difference (43.0% in group1, 57.1% in group2, 30.6% in group3, *P*=0.247). Interestingly, decreased occurrence of aGVHD translated into lower rate of CMV reactivation (55.9% in group1, 85.2% in group2, 35.7% in group3, P=0.003).
Summary and Conclusions: This study suggests that the use of a low dose of ATG can reduce aGVHD and CMV reactivation, with comparable rates of successful engraftment in the unrelated transplantation setting from HLA mismatched or PBSCs donor.

P916

IMPACT OF ABO INCOMPATIBILITY ON UNRELATED CORD BLOOD TRANSPLANTATION
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Background: Umbilical cord blood transplantation (UCBT) has now become a more common treatment for patients with hematologic malignancies who lack matched related or unrelated donors. however, few reports have addressed the impact of ABO incompatibility on the clinical outcome such as engraftment, transfusion requirements and survival after UCBT.
Aims: we retrospectively analyzed the impact of ABO mismatching on clinical outcome of 121 patients including 51 ABO-identical, 23 minor, 39 major, and 8 bidirectional ABO-incompatible recipients after UCBT.
Results: With a median follow-up of 11 months(range, 5-151months), disease free survival (DFS) rate among the ABO-identical, minor, major, bidirectional ABO-incompatible group were 71.7%, 60.0%, 37.1%, 71.4% (P=0.014) while OS did not differ significantly between the four groups(76.1%,65.0%, 48.6%,71.4%, P=0.078). DFS(68.2%, 42.9%,P=0.009) and OS estimates (72.7%, 52.4%, P=0.031) of the ABO identical/minor incompatible group also differ significantly from the ABO major/bidirectional incompatible group. These results were confirmed in multivariate analysis. No significant difference in engraftment times, transfusion requirements, GVHD, relapse and none relapse mortality (NRM) between groups was noted. Severe immune hemolysis or pure red cell aplasia did not occurred among these patients.
Summary and Conclusions: DFS and OS are better with ABO identical/minor incompatible group in UCBT.

P917

SECONDARY ANTIFUNGAL PROPHYLAXIS BASED ON RESPONSE TO INITIAL ANTIFUNGAL THERAPY FOR PATIENTS WITH A HISTORY OF INVASIVE PULMONARY ASPERGILLOSIS IN ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background: Patient with a history of invasive fungal infection (IFI) is in considerable risk of relapse and death after allogeneic haematopoietic stem cell transplantation (allo-HSCT). Prophylaxis with broad-spectrum antifungal agents has significantly reduced morbidity and mortality of IFI after transplants. However, the optimal agents for secondary antifungal prophylaxis (SAP) are not well defined.
Aims: In this study, the choices of prophylactic agents were made based on treatment response to initial antifungal therapy and the efficacy and safety of SAP were evaluated in recipients undergoing allo-HSCT.
Methods: A total of 88 patients undergoing allo-HSCT with prior invasive pulmonary aspergillosis (IPA) were enrolled in this prospective, multicentre study. Sixty-one patients had stable IPA and 27 had active IPA at the time of transplants. SAP was given from the start of the conditioning until 90 days post-transplantation for patients with stable IPA or until eradication or stability of residual foci for patients with active IPA. The same agent was given for prophylaxis if patients developed acute graft-*versus*-host disease (aGVHD) and were treated with corticosteroids and/or monoclonal anti-T cell antibody. The agents of SAP included itraconazole in 19, voriconazole in 44, caspofungin in 19 and liposomal amphotericin B in 6 cases.In addition, surgery was performed in 4 patients who had the cavity lesion or nodule in lung with more than 2cm in diameter before transplants.
Results: The median time of SAP was 96 days (range, 13 to 183 days).The success rate of SAP was 89.8%. Nine patients (10.2%) developed breakthrough IFI and none discontinued prophylaxis due to study drug toxicity. The incidence of breakthrough IFI was not different between different prophylactic antifungal agents (P=0.708). The incidence of breakthrough IFI was not different between patients with active and stable IPA (P=0.126). Within a median follow up of 403 days post-transplants (range, 13-2060 days), 26 patients developed IFI, including 9 within the period of prophylaxis and 17 after the end of prophylaxis. The 3-year cumulative incidence of IFI was 36.7%±5.9% and the 3-year cumulative incidence of IPA relapse was 31.0%±5.8%. The incidence of relapse in the patients with stable IPA was lower than that in the patients with active IPA (20.6%±5.9% *vs.* 55.7%±11.8%,P=0.002). Multivariate analyses showed that active IPA at the time of transplants (hazard ratio [HR]=4.328, 95% confidence interval [CI] 1.801-10.399, P=0.001), relapse of underlying primary disease (HR=6.195, 95% CI 2.157-17.793, P=0.001) and chronic GVHD (HR=3.241, 95% CI 1.077-9.750, P=0.036) were risk factors for IPA relapse. All patients who developed IFI after transplants received salvage therapy except for one who quit because of concomitant primary disease relapse. Of the 24 evaluable patients, 11 patients achieved CR, 9 achieved PR and 4 had no response to the treatment. At the end of follow-up, 44 patients were alive and 44 died. The 5-year overall survival (OS) and disease-free survival (DFS) post-transplants were 46.7%±5.7% and 43.1%±5.6%, respectively. The 5-year IFI-related mortality was 5.5%±2.7%.
Summary and Conclusions: Our data indicate that SAP based on the response to initial antifungal therapy has favourable efficacy and safety for the patients undergoing allo-HSCT with a history of IPA. Active IPA might not increase the risk of breakthrough IFI, but increase the rate of IFI relapse.

P918

A MONOCLONAL PROTEIN FOUND IN RELATED STEM CELL DONORS: ARE WE DOING IT RIGHT?
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Background: Increase in the use of non-myeloablative stem cell transplantation has resulted in a higher age of patients and their sibling donors. Although prior to HLA typing we use a questionnaire to exclude morbidity, predonation medical tests may reveal abnormalities resulting in donor rejection. In particular doubt regarding the use of G-CSF may arise if a monoclonal protein (M-protein) is found. After exclusion of multiple myeloma or lymphoproliferative disease, classification as monoclonal gammopathy of undetermined significance (MGUS) or transient reactive M-protein can often not be made because lack of follow-up time.
Aims: We evaluated prevalence and follow-up of M-protein in related stem cell transplantation (SCT) donors.
Methods: We retrospectively studied referral of related SCT donors seen in our department between 2009 and July 2012.
Results: Using immunofixation, we found an M-protein in 16 (13.1%) of 122 related donors (age 40-69) and in 23.3% of donors above 60. In 40% the M-protein concentration was >2 g/L and one donor had 40% plasma cells in the bone marrow; 4 more than 5% (max 20%) plasma cells and 11 showed marrow aspirates within the normal range. Donor follow-up ranged from 2 months to 3 years. In three donors the M-protein disappeared (after2, 6 and 12 months), one donor was diagnosed with a smouldering myeloma; the others were classified as MGUS. Of donors above 60 years 15 (43.3%) were deferred for G-CSF because of various medical reasons, of which 7 (23%) were rejected due to an M-protein. An M-protein occurs more often at older age and is associated with an increased incidence of haematological malignancies during 20 years follow-up after diagnosis. However, adverse consequences of G-CSF used for stem cell mobilisation on the proliferation of clonal cells producing a M-protein, are not known. Recently consensus was reached among Dutch haematologists to allow G-CSF in case of presumed MGUS with a low concentration M-protein if the transplant physician agrees. According to guidelines, subjects with an M-protein require follow-up for their plasma cell dyscrasia. This should be apart from the 10-year follow-up by the donation centre that is line with the present guidelines for unrelated donors that have been treated with G-CSF.

Summary and Conclusions: Potential SCT donors above 60 years show a high deferral rate up 43.5%, in half of these depending on whether M protein is considered as contra-indication for G-CSF. Using sensitive techniques an M-protein occurs more frequently (13%) in siblings of patients with haematological disorders. In particular in donors above 60 years (23.3%) the incidence is much higher compared to 3-5% of the general population above 50 years. Although at present for MGUS with a low concentration of M protein, G-CSF mobilisation is not considered a donor risk, these findings stress the importance of long term follow up of all related donors.

P919

TBI AND CYCLOPHOSPHAMIDE PLUS ATG IS WELL TOLERATED AND EFFECTIVE AS A PREPARATIVE REGIMEN FOR HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION WITHOUT IN VITRO T-CELL DEPLETION

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Background: There is a lack of data for patients with haploidentical HSCT after preparing with TBI/Cy plus ATG.
Aims: This is a retrospectively study to analyze the toxicity and efficacy of total body irradiation (TBI) and cyclophosphamide (TBI/Cy) plus ATG as a conditioning regimen prior to haploidentical hematopoietic stem cell transplantation (HSCT).
Methods: The TBI/Cy plus ATG regimen consisted of TBI (770 cGy, a single dose), Cy (1.8g/m^2, two doses) and ATG (6 or 10mg/kg). Twenty patients (median age: 24 years old) receiving haploidentical HSCT between October 2008 and November 2012 were evaluated. Among them, 15 patients suffered from high-risk leukemia.
Results: There was one patient died before engraftment. The myeloid engraftment and platelet engraftment were obtained at a median of 12 days (range: 10–19) and 16 days (range: 10-29) after HSCT for other 19 patients, respectively. Both incidences of myeloid and platelet engraftment were 95%. A total of 19 patients with myeloid engraftment had completing-donor-type chimerism in peripheral blood by 30 days after transplantation. There was no case of secondary graft failure. The major Grade III and IV regimen-related toxicities were fever, diarrhea, and oral mucositis, which were well managed. Acute graft-*versus*-host disease (aGVHD) developed in 16/19 patients, consisting of Grade I in 12 patients(); II in 4; III and IV in no patient. No patients died of GVHD and infection. The 100-day transplant-related mortality was 5.6%. To reduce relapse, six patients received prophylactic modified donor lymphocyte infuse (mDLI), one patient received chemotherapy plus preemptive mDLI, one patient received chemotherapy plus therapeutic mDLI and 3 patients with Philadelphia chromosome-positive received prophylactic tyrosine kinase inhibitors. With a median follow-up period of 347 days (range: 35–1321 days), 18 of 19 patients had received complete remission after HSCT. The other one patient received remission in bone marrow with extramedullary non-remission. At last 4 patients relapsed and 3 patients died of relapse within one year after HSCT. The one-year incidence of relapse was 34.8%.The one-year overall and leukemia-free survival were 66.5% and 61.9%, respectively.
Summary and Conclusions: TBI/Cy plus ATG might be feasible and effective conditioning regimen for haploidentical HSCT, especially for patients with high-risk leukemia. The long-term observation of the efficacy and antileukemic effect of this regimen and a future prospective study with more patients to compare this regimen with BU/Cy plus ATG are required.

P920

HIGH LEVEL OF VASCULAR ENDOTHELIAL GROWTH FACTOR IS ASSOCIATED WITH INCREASED RELAPSE RATE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: High level of vascular endothelial growth factor (VEGF) is a well-known predictor of worse outcome of chemotherapy in a variety of hematologic malignancies.
Aims: The aim of this study was to evaluate if overproduction of VEGF is also a prognostic factor after allogeneic hematopoietic stem cell transplantation (HSCT).
Methods: The study included 91 patients. 73% had acute leukemia, 10% chronic myeloid leukemia, 8% myelodysplastic syndrome and 9% other hematologic malignancies. 52.7% of patients were grafted without hematologic remission at time of transplant. Median age was 38 years (range 15-60). 30% were grafted from related and 70% from unrelated donor. 24% received myeloablative conditioning, 76%>reduced intensity conditioning. VEGF A was measured by ELISA. The blood sampling points were before conditioning, on day 0 and upon engraftment.
Results: Based on series of univariate and multivariate analyses of VEGF concentrations before conditioning a cut-off value of 50 pg/mL was found for which there was a significant difference for outcomes of HSCT. Patients with VEGF>50 pg/mL had higher 2-year relapse rate (53% *vs* 25%, P=0.008, Figure 1) and decreased 2-year event-free survival (EFS) (20% *vs* 46%, P=0.021). Overall survival was not different between two groups (60% *vs* 53%, P=0.658), as some of the patients were successfully salvaged after relapse. The association between high VEGF concentrations and 2-year relapse rate was still significant for day 0 time point (55% *vs* 26%, P=0.018), but with higher cut-off value of 90 pg/mL. In multivariate analysis for relapse that incorporated absence of remission before HSCT, type of donor and presence of acute graft-*versus*-host disease, VEGF>50 pg/mL before conditioning was an independent risk factor (HR 2.77, P=0.024, HR 95%CI 1.15-6.70). In a multivariate analysis for EFS high VEGF also had a significant negative impact (HR 2.17, P=0.025, HR 95%CI 1.10-4.26).

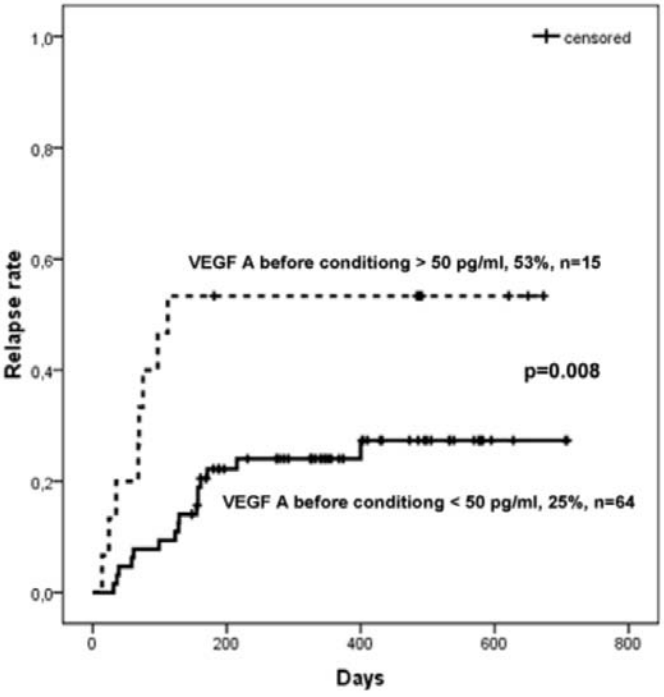

Figure 1.

Summary and Conclusions: In conclusion, this study confirmed that tumor-associated overexpression of VEGF is a negative prognostic factor for hematologic malignancies not only after chemotherapy, but also after HSCT.

P921

CLINICAL IMPACT OF ENGRAFTMENT SYNDROME IN ADULT PATIENTS WHO RECEIVED ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Engraftment syndrome (ES) has been recognized as a complication that occurs during neutrophil recovery following autologous or allogeneic stem cell transplantation (allo-SCT). Although pro-inflammatory cytokine and subsequent cellular and cytokine interactions have been reported to play a major role, the pathogenesis of ES remains unclear. Likewise, the clinical significance of ES has been controversial.
Aims: The aim of this study was to clarify the clinical impact of ES on clinical course and transplant outcome.
Methods: We retrospectively analyzed 132 consecutive adult allo-SCT recipients, who achieved durable neutrophil engraftment, between January 2006 and August 2011 at Gunma University Hospital and Saiseikai Maebashi Hospital in Gunma, Japan. ES was defined according to the diagnostic criteria described by Spitzer. Fisher's exact test was used for comparison of binary variables. The Mann-Whitney U-test was used for comparison of continuous variables. Overall survival (OS) rates were estimated by the Kaplan-Meier method and were compared using the log-rank test. The Cox proportional hazards regression model was used for multivariate analysis of predisposing factors. P<0.05 was considered to be statistically significant.
Results: Of the 132 allo-SCT recipients involved in this study, 78 patients were male, and 54 were female. The median age was 48 years (range, 18–72 years). ES occurred in 34 patients (25.8%). Of the 34 patients with

ES, 34 patients (100%) presented with non-infectious fever, 31 (91%) had skin rash, 30 (88%) experienced weight gain, and 26 (76%) developed non-cardiogenic lung edema. To identify the predisposing factors for ES, patient characteristics, transplant procedure, and donor type were examined; grafts from an unrelated donor and minor ABO-mismatched donor were identified as predisposing factors for ES. Multivariate analysis revealed that these two factors were independent. Regarding the impact of ES on clinical course, patients with ES more frequently developed thrombotic microangiopathy (TMA), grade II-IV acute graft-*versus*-host disease (GVHD), and chronic GVHD when compared with patients without ES (TMA: 29% *vs.* 10%, respectively, P=0.036; acute GVHD: 74% *vs.* 44%, respectively, P=0.027; chronic GVHD: 85% *vs.* 44%, respectively, P=0.012). There was no difference in the incidence of veno-occlusive disease (VOD) between the groups. The 5-year cumulative incidence of underlying disease relapse for patients with ES was higher than for patients without ES (8% *vs.* 29%, respectively, P=0.048). The 5-year cumulative incidence of treatment-related mortality (TRM) for patients with ES tended to be higher than for patients without ES (42% *vs.* 22%, respectively; P=0.151). The 5-year OS rates were comparable between patients with and without ES (57% *vs.* 57%, respectively; P=0.893). Of the 34 patients with ES, eight (23%) received no treatment, and all symptoms spontaneously resolved within 7 days after onset of ES. Of the remaining 26 patients, 14 received 1 mg/kg/day of methylprednisolone (mPSL), and 12 received 2 mg/kg/day or more of mPSL. Steroid administration was effective in all 26 patients independent of steroid dose, and all symptoms were resolved on the day after initiation of treatment. Among the three groups stratified according to treatment strategy, there was no difference in clinical course, including the incidence of VOD, TMA, acute GVHD, and chronic GVHD.

Summary and Conclusions: These findings suggest that ES has two conflicting effects on transplant outcome: increase in TRM and decrease in underlying disease relapse. Regarding treatment strategy, because of the excellent response to steroids, administration of excessive amounts of steroids is considered to cause the increase in TRM. The appropriate steroid dosage for the treatment of ES should therefore be investigated using a prospective study design.

P922

PROGNOSTIC IMPACT OF PRETRANSPLANT SERUM FERRITIN FOR OUTCOMES IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION. EPIDEMIOLOGY OF PROSPECTIVE, MULTICENTER ARGENTINIAN STUDY

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Background: Iron Overload (IOL) is a common problem in red cell transfusion–dependent patients undergoing Hematopoietic Stem Cell Transplant (HSCT). Transplant-related mortality (TRM) seems to be higher in patients with IOL secondary to more incidence in infections, mucositis, hepatic Sinusoidal Obstruction Syndrome (SOS) and Graft Versus Host Disease (GVHD).
Aims: 1)We prospectively evaluated the frequency of transfusional IOL in patients who underwent HSCT. 2)We compared main outcomes between both groups (with and without transfusional IOL).
Methods: Eight Argentinian Bone Marrow Transplants Units studied 109 pediatric and adults patients who received HSCT from july 2010 to july 2012. 99 patients were eligible according to inclusion/exclusion criteria. Median age at HSCT: 36 years (range: 2–69). Male/female ratio: 1.6. Ferremia, Transferrin Saturation (TS) and Serum Ferritin (SF) were analyzed previously to HSCT, at day +7, day+60, day+100 and day+360. Primary Hemosiderosis (PH) was studied by molecular tests. Transfusional IOL were defined when the ferritin levels were more than 1.000 ng/mL. Exclusion criteria were: 1) Active infections during the baseline time-point; 2) Patients wtih previously quelation therapy and 3) PH diagnosis.
Results: According to the source of HSCT: 59 (59.6%) were Autologous, 24 (24.2%) were Allogeneic Related and 16 (16.2%) were Allogeneic not related. Two groups were divided according to ferritin levels: Group 1 (with baseline SF less than 1.000 ng/mL), 44 patients (45%), median SF: 429.9 ng/mL (range: 32-960) and Group 2 (with SF levels more than 1.000 ng/mL), 54 patients (55%), median SF: 2960 ng/mL (range: 1038-12.800). Diagnosis of transfusional IOL according to the source of stem cells: Autologous: 51%, Allogeneic Related: 63% y Allogeneic not Related: 57%. Comparison between both groups were performed related to most important variables (Table 1).

Table 1.

	≥ 20 Transfusions (%)	Grade III/IV Mucositis (%)	Fever between 0 – 100 days (days)	Antibiotics between 0 – 100 days	Hospitalization days
Group 1	2	22	3.5	5.5	26.8
G1 vs G2	**p = 0.001**	**p = 0.008**	**p = 0.001**	**p = 0.001**	**p = 0.001**
Group 2	67	48	6.7	12.7	31.7

	Positives Cultures 0 – 100 days (%)	Sinusoidal Obstruction Symdrome (patients)	Acute GVHD (%)	Neutrophils Engrafment (days)	Platelet Engrafment (days)
Group 1	11.1	0	26.6	19.5	26.9
G1 vs G2	**p = 0.001**	**p = 0.131**	**p = 0.28**	**p = 0.181**	**p = 0.131**
Group 2	40.7	2.6	16	15.5	18.9

	EFS (%)	OS (%)	TRM (%)
Group 1	83	94	2
G1 vs G2	**p = 0.095**	**p = 0.022**	**p = 0.047**
Group 2	70	75	11

Summary and Conclusions: Baseline Serum Ferritin with levels more tan 1.000 ng/mL (Group 2) presented: more tan 20 transfusions, more days of fever, more days of antibiotics, more percentage of positive cultures, more percentage of mucositis grade III/IV and more days of inpatients in the Bone Marrow Transplant Unit. TRM were higher and Overall Survival (OS) were lower in Group 2. At day+100, median SF levels still remains elevated: 3113.2 ng/mL, showing that the body does not have physiologic mechanisms to eliminate the excess of body iron. These results indicate that SF more than 1.000 ng/mL should be considered in prevention transplantation decision-making and also in the post-HSCT period.

P923

LATE LEUKEMIA RELAPSE (LLR) AFTER ALLOGENEIC HEMATOPIETIC STEM CELL TRANSPLANTATION (ALLO-HSCT)

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Background: A LLR may occur in 1-3% of leukemia patients after a post-transplant follow-up period longer than 5 years. As disease recurrence can be determined by recipient's leukemic stem cells re-growth or by donor's HSCs leukemic transformation, conventional cytogenetic/FISH and RT PCR studies play a crucial role in determining the origin of relapse and in activating therapeutic procedures aimed at eradicating the leukemic population.
Aims: The present study was aimed at establishing the incidence and the origin of LLR in a series of 182 patients submitted to allo-HSCT at a single Institution.
Methods: Cytogenetic and FISH studies were carried out as already reported, whereas Quantitative and nested RT PCR assays for Chronic Myeloid Leukemia monitoring was performed according to the Italian LabNet guidelines. Evaluation of chimerism and Minimal Residual Disease (MRD) was performed at day +100, +180, +365 and every six months post-transplant. Conventional cytogenetic studies were performed whenever required.
Results: In our series a LLR occurred in 4 patients (incidence of 2.1%) and was due to recipient's leukemic cells re-growth. They were three females and one male. At the time of allo-HSCT two patients were CML in first chronic phase, one patient an AML in first complete remission (CR) and one patient an AML progressed from a high-risk myelodysplastic syndrome with resistant disease. All patients received a standard BU-CY conditioning, sex-mismatched HSC from a sibling donor and standard GvHD prophylaxis. The two CML patients relapsed as Ph' positive acute lymphoblastic leukemia (ALL) 7 and 16 years post-transplant. On day +100 both patients showed no signs of GvHD, a complete chimera with a normal male karyotype and a negative BCR-ABL nested RT PCR. One month before haematological relapse one patient with no chronic GvHD (cGvHD) was still a complete chimera and MRD negative. The other patient, who on day+329 developed a limited cGvHD, remained a complete chimera without any BCR-ABL transcript until 16 years post-transplant when she complained of otitis and fever. At this time point a marrow examination showed the almost exclusive presence of CD34+, TdT+, CD10+ Ph'+ lymphoblasts and a BCR-ABL/ABL ratio=214,2 (International scale). The t(3;11)(q26;q23) AML in first CR developed an extensive cGvHD on day +183. The patient remained a complete chimera with a normal female karyotype from day +100 to five years post-transplant when he complained of fever. A chest X-ray showed a left pulmonary infection. At this time a marrow examination revealed the almost exclusive presence of blast cells which karyotype was: 50,XY,t(3;11)(q26;q23),+4,+8,t(12;?)(p21;?),-14,+mar1,+mar2, +mar3[13]. The last trisomy 8 positive AML patient with active disease and no signs of GvHD remained a complete chimera with a normal male karyotype until fifteen months post-transplant when she was still on immune-suppressive treatment and devel-

oped an initial first marrow relapse. Upon withdrawal of such a therapy she developed an extensive cGvHD and re-acquired a complete chimera and a normal male karyotype. The complete chimera was maintained until almost ten years post-transplant when she complained of a pain in the left pelvic region. A physical examination revealed a mild splenomegaly and a marrow examination 20% leukemic cells with the following karyotype: 47, XX,+10/48XX, +8,+10. An abdomen CT scan revealed two pelvic masses which biopsy demonstrated leukemic infiltration.
Summary and Conclusions: Our data confirm that LLR is a very rare event potentially caused by a not expected loss of leukemic stem cell quiescence which allows them to escape myeloablative conditioning toxicity. In addition, it raises the question of whether RT PCR is truly the best method to monitor MRD since recent evidence suggests that in CML RT PCR is less sensitive than genomic methods which may identify residual leukemic cells more effectively.

P924

THERAPEUTIC APPROACH TO RELAPSE OF PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Allogeneic stem cell transplantation (allo-SCT) is a potentially curative treatment for patients (pts) with Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). In 1st complete remission (CR1), disease-free survival (DFS) ranges from 21 to 57%. Relapse is the primary cause of treatment failure (±30% of pts in CR1). Several studies have shown improved DFS for pts treated with tyrosine kinase inhibitors (TKI) prior to allo-SCT. Optimal management of post-transplant relapse is still a matter of debate. Role of TKI is being explored in this setting and results appear promising particularly in pts with low tumor burden [positive Minimal Residual Disease (MRD+)].
Aims: To evaluate relapse in the setting of allo-SCT for Ph+ ALL.
Methods: Retrospective analysis of 30 consecutive pts who underwent allo-SCT for Ph+ ALL at our Center from 1998 to 2012. Data analysis was performed using SPSS.
Results: Cohort of 30 pts (male=17) with a median age of 41.5 years (18.0–53.0). Induction regimen consisted of chemotherapy alone (CT; n=5) and combination CT+TKI (n=25). At allo-SCT, 27 pts were in CR1, 13 with MRD+. Median time from diagnosis to allo-SCT: 8.2 months (4.5–15.7).
Conditioning regimen was busulfan-based myeloablative (n=29) and non-myeloablative (n=1). Donors were matched-related (n=19), matched-unrelated (n=5), mismatched-related (n=1) and mismatched-unrelated (n=5). Stem cells source was peripheral blood (n=26), bone marrow (n=3) and umbilical cord (n=1). Graft-*versus*-host disease (GvHD) prophylaxis consisted of calcineurin inhibitor plus methotrexate (n=28)/mycophenolate (n=2). After the procedure all pts achieved CR, but 5 remained *BCR-ABL*+. Post-transplant 2-year OS and DFS were, respectively, 33.1% and 26.9%; median follow-up: 33.1 months (6.9–77.1). Two-year cumulative incidence of hematological relapse (HR) and isolated molecular relapse (MR) was 39.3%(±9.3) and 12.7%(±6.4), respectively. There was 1 isolated extramedullary relapse (EMR) at 26 months. Four of the 5 pts who remained *BCR-ABL*+ experienced HR (1 under imatinib). Median time to relapse: 4.7 months (1.1–26.0). On univariate analysis, chronic GvHD showed a trend to lower frequency of relapse (26.7% *vs.* 46.7%, P=0.07). On multivariate analysis, the only factor associated with relapse was pre-SCT treatment [at 6-month: 60%±19.2 (CT) *vs.* 20.2%±8.0 (CT+TKI), P=0.048]. Treatment approach to relapse was heterogeneous: IS reduction (↓IS; n=1), ↓IS+TKI (n=3), CT+TKI (n=2), CT+TKI+DLI (donor lymphocyte infusion; n=3), TKI+DLI (n=1), orchidectomy+CT+DLI (n=1) and CT+2nd allo-SCT (n=2). Two early deaths occurred after relapse. Four of the 11 pts with HR obtained CR after CT+TKI+DLI (n=3) and TKI+DLI (n=1), yet 2 remained MRD+. The pt with EMR obtained CR after orchidectomy+CT+DLI. All pts with MR achieved complete molecular response (↓IS+TKI=2; ↓IS=1). Five of the 8 pts who responded are still alive and 2 remain disease-free (18.0 and 62.4 months after HR and MR, respectively). Post-relapse 2-year OS and DFS were 36.4% and 21.4%, respectively. Five pts experienced a 2nd relapse (HR=3, MR=1, EMR+HR=1) and 1 pt, who remained *BCR-ABL*+ after HR, had molecular progression 2 months after CR.
Summary and Conclusions: Our study confirms that Ph+ ALL has a high incidence of relapse, even after allo-SCT. CT without TKI before allo-SCT was the only statistically significant factor associated with relapse. Heterogeneity of treatment options in this series reflects the difficulty of managing post-transplant relapse. TKI and immunotherapy (DLI or ↓IS) appear to have a major role in relapse treatment.

P925

IMPACT OF THE 2009 UK UMBILICAL CORD BLOOD TRANSPLANT CONSENSUS STATEMENT ON PRACTICE AND OUTCOMES AT A SINGLE TRANSPLANT CENTRE

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Background: Umbilical cord blood (UCB) transplants have become standard practice in the treatment of malignant and non-malignant hematological disorders. We have performed UCB transplants at the Royal Marsden Hospital (RMH) since 2006; however practice has increased and changed over time due to encouraging international results, two UK clinical trials and publication of a UK consensus statement. The recommendations from the consensus statement were adopted at RMH in 2009.
Aims: Our first aim was to establish whether recommended conditioning regimens have been used consistently in UCB transplants at RMH since 2009. Our second aim was to compare the outcome for recipients of UCB transplant pre and post 2009.
Methods: We performed a retrospective analysis of the records of all UCB transplant patients at RMH between 2006 and 2012. We reviewed a total of 37 records of 24 adult and 13 pediatric patients. We compared results according to the transplant era (pre and post 2009). Incidence of non-relapse mortality, relapse, and acute graft *versus* host disease (aGvHD) were calculated using competing risk analysis.
Results: Eighteen UCB transplants were performed before 2009 and 19 have been performed since. The overall median follow-up time was 28.6 months (95% CI 10.7-46.4). Median follow-up pre 2009 was 44 months (95% CI 25.7-62.2), and post 2009 was 18.6 months (95% CI 15.8-21.3). The overall one year survival rate was 49% (95% CI 32-65%). One year survival among the pre 2009 group was 33% (95% CI 12-55%) *versus* 66% (95% CI 36-97%) in the post 2009 group (P=0.097). Cumulative incidence of one year non-relapse mortality pre 2009 was 39%, and post 2009 was 16% (P=0.186). Cumulative incidence of relapse at one year pre 2009 was 28%, and post 2009 was 24% (P=0.754). Cumulative incidence of acute graft *versus* host disease (aGvHD) was 56% pre 2009 and 47% post 2009 (P=0.699). Neutrophil engraftment by day 42 was achieved in 16 patients (89%) in the pre 2009 group and 17 patients (90%) in the post 2009 group. Epstein-Barr virus (EBV) reactivation requiring treatment (PCR >40,000 copies/mL) occurred in 5 (28%) patients in the pre 2009 group. No patients in the post 2009 group required treatment for EBV reactivation (P=0.017). Before 2009 seven different conditioning regimens were used. Six patients (33%) received regimens that were subsequently recommended in the consensus statement. After 2009, 19 (100%) patients received one of the recommended conditioning regimens. Pre 2009 no patients were treated as part of a UCB transplant clinical trial compared with 6 patients (32%) post 2009.
Summary and Conclusions: Following publication of the 2009 consensus statement, all UCB transplant patients at RMH were treated with one of the four recommended conditioning regimens. Post 2009 there is a trend towards better one year survival. This may be explained by a standardized approach, but the recognized improvement in outcome with centre experience, and the inclusion of more patients in clinical trials may also have contributed. Non-relapse mortality and relapse rates are not significantly different between the two groups. Post 2009 antithymocyte globulin was no longer used in conditioning regimens. Despite this, rates of aGVHD were similar between groups. However, this may have contributed to the significant reduction in EBV reactivation in the post 2009 group. Overall, these results are encouraging, and multi-centre investigation is warranted to see if these findings are borne out in larger patient groups.

P926

CMV-SPECIFIC CD8+ T-CELL IMMUNE RECOVERY AFTER ALLOGENEIC STEM CELL TRANSPLANTATION MONITORED BY HLA-MULTIMERS

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Background: CMV reactivation remains an important problem in allogeneic stem cell transplantation.
Aims: We investigated the use of multimers in monitoring CMV-specifc T-cell immune reconstitution after allogeneic stem cell transplantation
Methods: We present the results of a pilot study using pentamer (PM) and streptamer (ST) multimer complexes for monitoring CMV-specific CD8+ T-cells (CTLs). We analysed 14 patients that underwent allogeneic Stem Cell Transplantation (allo-SCT). All patients and donors were positive for the HLA-A*02:01 allele. PM and ST were directed against the epitope NLVPMVATV (495-503) of the CMV phosphoprotein 65 (pp65). Samples were obtained at 15-day intervals until day +90 and monthly thereafter.
Results: Three patterns were observed. In 2 patients (14%) no CMV-specific-

CTLs could be detected despite several CMV reactivations, requiring prolonged cumulative antiviral therapy (67 and 136 days each). In 6 patients (43%) CMV reactivation occurred at a mean of 36 days (10-74) and triggered a rapid increase of CMV-specific-CTLs with a median of 6.9 × 105/L (range 0.02-279.7). The CMV-PCR became immediately negative and antiviral therapy was stopped promptly after a median of 15.3 days (8.7-23). Finally, 6 patients (43%) showed an early immune reconstitution with CMV-specific-CTLs detected with a median of 1.53 × 105/L (range 0.2-54.72) in the absence of CMV-PCR reactivation at a median of 20.5 days (10-34) post-SCT. No CMV-PCR reactivation was observed in this group with a median follow-up of 8 months (3-14).
Summary and Conclusions: Monitoring CMV-specific-T-cells might be able to distinguish patients at higher risk of recurrent virus reactivation and in need of prolonged antiviral therapy. Patients with increasing CMV-specific-CTLs detectable at the time of CMV-PCR reactivation may only need a short course of antiviral therapy, while those with early CMV-specific-CTLs may be protected from CMV reactivation.

Hematopoiesis

P927

IDENTIFICATION OF THE EARLIEST BRANCH POINT FOR MYELO-ERYTHROID DEVELOPMENT IN ADULT HEMATOPOIESIS

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Background: In murine hematopoiesis, the myeloid *vs.* lymphoid lineage commitment downstream of multipotent stem/progenitor cells was first demonstrated outside of the Lineage$^-$ Sca-1$^+$ c-kit$^+$ (LSK) fraction, where common myeloid progenitor (CMP) and common lymphoid progenitor (CLP) were prospectively isolated (Akashi *et al.*, Nature 2000). Thereafter, by utilizing mice harboring a fluorescent reporter for GATA-1 transcription factor, we proposed that the initial uP-regulation of GATA-1 within LSK fraction marked the earliest progenitor population which fully committed to the myelo-erythroid lineage. (Arinobu *et al.*, Cell Stem Cell 2007). However, the significance of GATA-1$^+$ LSK in normal and malignant hematopoiesis is still elusive because the corresponding population in wild-type mice has not been identified.
Aims: The purpose of present study is to identify the earliest myelo-erythroid progenitor corresponding to GATA-1$^+$ LSK in wild-type mice as well as to evaluate the physiological significance in normal and leukemic hematopoiesis.
Methods: In order to identify the cell surface markers specifically expressed on GATA-1$^+$ LSK, cDNA microarray analyses were conducted. LSK population was subdivided by using these specific surface markers. Lineage read-outs of isolated populations were analyzed *in vitro* cultures and *in vivo* congenic transplantation assays. Gene expression profiling of the normal counterpart of GATA-1$^+$ LSK was analyzed by cDNA microarray and single cell quantitative RT-PCR. Furthermore, the physiological significance of this population in myelopoiesis was evaluated by the systemic bacterial infection model (cecal ligation and puncture) and the bcr-abl transduced myeloproliferative neoplasm model.
Results: Gene expression profiling revealed several candidates for surface antigens specifically expressed on GATA-1$^+$ LSK. Among them, a high level expression of CD41 clearly marked GATA-1$^+$ LSK by FACS analysis. CD41^{hi} LSK purified from wild-type mice gave rise exclusively to GM and MegE colonies at the expense of lymphoid colonies and showed robust GM and MegE differentiation potential *in vivo*, lacking lymphoid read-outs. CD41^{hi} LSK possessed strong and long-lasting reconstitution potential compared to LMPP or CMP, presumably reflecting their immaturity. The head-to-head competitive transplantation assay, in which 500 CD41^{hi} LSK cells were injected together with same numbers of LMPPs or CMPs into an individual recipient, also demonstrated that CD41^{hi} LSK gave rise to 10-fold larger number of mature GM cells compared to the original CMPs or LMPPs. In concordance with their myelo-erythroid lineage restriction, CD41^{hi} LSK expressed both GM- and MegE-affiliated genes but not lymphoid genes. Concerning lineage-instructive transcription factors, each single CD41^{hi} LSK cell expressed either one or both of GATA-1 and PU.1 at a very low level, reflecting the priming status of myeloid *vs.* erythroid fate dicision (Miyamoto *et al.*, Developmental Cell 2002). In order to explore the physiological significance of CD41^{hi} LSK, we generated mouse models of systemic bacterial infection as well as myeloproliferative neoplasm induced by bcr-abl transduction. CD41^{hi} LSK but not LMPP robustly expanded in both infectious and leukemic models, indicating that CD41^{hi} LSK is a critical checkpoint for physiological and malignant myelopoiesis.
Summary and Conclusions: CD41^{hi} LSK represents the earliest branch point of myelo-erythroid development and resides upstream of the conventional CMP which was originally identified outside of LSK. CD41^{hi} LSK is mainly involved in both physiological and malignant myelopoiesis. Based on these data, we propose to redefine the true CMP as CD41^{hi} LSK population.

P928

FUNCTIONAL RELEVANCE OF AUTOTAXIN (ATX) AND LPA RECEPTOR EXPRESSION IN NORMAL HEMATOPOIETIC STEM CELLS AND PRIMARY MESENCHYMAL STROMAL CELLS

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Background: The proliferation and differentiation of hematopoietic stem and progenitor cells (HSPCs) within the human bone marrow is characterized by a tightly regulated micro-environment in the stem cell niche. Besides cell-cell interactions of HSPCs and mesenchymal stromal cells (MSCs) and several cytokines and growth factors, various other soluble factors determine stem cell fate. Among these, small bioactive lipids, *e.g.* sphingosine-1-phosphate (S1P) and lysophosphatidic acid (LPA), have recently attracted attention.
Lysophosphatidic acid (LPA) is mainly generated by the lysophospholipase D-activity of the secreted enzyme autotaxin (ATX). LPA binds to specific G-protein coupled receptors on the cell surface and thereby increases migration and proliferation of various cell types, *e.g.* T-cells. We recently identified for the first time high ATX expression in specific subsets of human leukemic as well as

HSPCs, namely CD34+ cells and could confirm an autocrine function of the protein in these cells (Ortlepp *et al.*, Exp. Haematol, in press).
Aims: To further understand the role of ATX in human hematopoiesis, we characterized expression and activity of ATX and LPA-receptors in HSPCs as well as in MSCs, the dominant cell type within the hematopoietic niche.
Methods: Primary HSPC and MSC were obtained from healthy stem cell and bone marrow donors after informed consent. Expression and enzymatic activity was characterized using RT-PCR, Western blot and specific substrate assays (FS-3). Functional activity was studied using transwell migration assays, the proliferative capacity was tested by MTT assays, whereas the clonogenic potential was investigated using colony-forming assays in the presence of the specific substrate lysophosphatidyl choline (LPC) as well as the specific small-molecule ATX-inhibitor.
Results: To our surprise, ATX expression found in primary human MSCs was 5 to 10-fold higher than in CD34+ and leukemic cells. Inhibition of ATX in MSC resulted in a 50% reduction of MSC-vitality. Although ATX levels expressed in primary HSCs were considerably lower than in MSCs, our data indicate that ATX is an important autocrine factor for migration and vitality of these cells, since addition of LPC and LPA in absence of MSCs increased CD34+ HSC migration by 15 and 30%, respectively. Blocking of ATX decreased vitality to 20%, reduced colony growth and led to aberrant differentiation especially within the erythroid and myeloid lineage. Sorting experiments of different HSPC-populations (CD34+/- and CD38+/-) indicate that high ATX- mRNA expression was found in all CD34-positive subpopulations, whereas CD34-/CD38+ cells showed significantly lower expression. LPA-receptor profiling revealed almost exclusive expression of the LPA1-receptor in MSCs, whereas HSPCs express predominantly the LPA2-receptor, with a 50%>higher expression in CD34-/CD38+ compared CD34+/CD38- cells.
Summary and Conclusions: Taken together, our data indicate that ATX is highly expressed within the bone marrow micro-environment. Our results show that the expression originates not only from stromal cells but also that HSCs can produce ATX in an autocrine fashion, suggesting that interference with its function may have a profound effect on cell migration and differentiation.

P929

STEM CELL ZINC FINGER 1 (SZF1)/ZNF589: A PRIMATE-RESTRICTED GENE, DIFFERENTIALLY EXPRESSED AFTER HYPOXIC STRESS AND REGULATING THE PROLIFERATION OF HEMATOPOIETIC CELLS
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Background: The Stem cell Zinc Finger 1 (SZF1)/ZNF589 protein belongs to the large family of Krüppel-associated box domain (KRAB) zinc finger transcription factors, only present in higher vertebrates, which epigenetically repress transcription by recruiting chromatin modifying complexes to the promoter regions of their target genes. Although the distinct biological functions of most KRAB-zinc finger proteins remain unknown, recent publications demonstrate their implication in fundamental processes, such as cell proliferation and apoptosis, differentiation, development, and tumorigenesis. SZF1/ZNF589 was identified by Liu *et al.* (1999) as a gene with one of two isoforms specifically expressed in CD34+ cells, strongly suggesting a role in epigenetic control of gene expression in hematopoietic stem cells (HSCs). However, the function of SZF1/ZNF589 in hematopoiesis has not yet been studied.
Aims: Structural and functional characterization of the SZF1/ZNF589 stem cell zinc finger protein and the investigation of its role in the hematopoietic system.
Methods: Lentiviral-mediated overexpression of specific shRNAs to induce stable knock-down of the SZF1/ZNF589 protein in hematopoietic cells.
Results: We found that SZF1/ZNF589 is a primate-restricted gene. Interestingly, the human SZF1/ZNF589 gene contains a species-specific evolutionary genomic DNA change introducing a premature stop codon at the place of the second cysteine residue in the 5th zinc finger domain, apparently leading in humans to truncation of the protein with loss of the last 11 zinc finger domains. We confirmed the human-specific sequence change at the genomic and mRNA level in human and monkey cell lines. Interestingly, Western-Blot experiments revealing either endogenous or tagged-overexpressed SZF1/ZNF589 proteins demonstrated that the human SZF1/ZNF589 mRNA encodes two peptides: one of predicted molecular weight of 41 kDa generated by termination at the premature stop codon, and a second one of 86 kDa, like SZF1-2/ZNF589 of the other primates, probably generated by suppression of translational termination at the UGA stop codon. This human-specific sequence change demonstrates the still ongoing evolution of the SZF1/ZNF589 gene and could result in the incorporation in humans of a selenocysteine at the place of a cysteine in other primates. We performed loss-of-function studies in the K562 and MV4-11 hematopoietic cell lines by lentiviral gene transfer to induce stable SZF1/ZNF589 knockdown. Two different SZF1/ZNF589 specific shRNAs decreasing 2 to 4 fold the expression of the SZF1/ZNF589 transcript and protein both resulted in the same phenotype reducing the proliferation of hematopoietic cells to about 60%. Treatment of cells with $CoCl_2$ 300µM to mimic hypoxia conditions demonstrated the induction of differential isoform expression with disappearance of the small isoform of SZF1/ZNF589, as observed by Western-Blot. This suggests that the balance between the two SZF1/ZNF589 isoforms is regulated by hypoxia.
Summary and Conclusions: In summary, our results demonstrate that SZF1/ZNF589 is a primate-restricted gene, with a human-specific DNA-change conferring potential species-specific DNA-binding properties. It regulates proliferation of hematopoietic cells and is differentially expressed under hypoxic stress conditions. Hypoxia is a typical feature of the bone marrow HSC niche and belongs to the micro-environment components controlling HSC quiescence and mobilization. Thus, SZF1/ZNF589 might be implicated in regulatory pathways involved in maintenance, proliferation and differentiation of HSCs.

P930

COMBINED ECTOPIC OVEREXPRESSION OF GABP SUBUNITS IMPAIRS CELLULAR PROLIFERATION AND VIABILITY, AND INDUCES MYELOID DIFFERENTIATION IN VITRO
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Background: The heteromeric transcription factor complex GA-binding protein (GABP) consists of two subunits, the alpha subunit (GABPA) carrying the DNA-binding ETS domain, and the beta subunit (GABPB1) harboring the transcriptional activation domain (TAD). Using murine knock-out models, GABP was shown to be involved in the self-renewal of hematopoietic stem cells and the differentiation of myeloid and lymphoid lineages. Moreover, recent studies displayed an impaired BCR-ABL-mediated transformation in GABP knock-out mice.
Aims: To further elucidate the molecular function of GABP in human hematopoietic cells as well as to investigate its putative role in leukemia, we studied its effects on proliferation, viability and myeloid differentiation by ectopic overexpression of GABP subunits either alone or in various combinations.
Methods: Human GABPA and GABPB1 cDNAs were overexpressed in BaF3, 32D, SC-1 and U937 cells using the retroviral expression vectors pRSF91.IRES.eGFP.pre* or pRSF91.IRES.dTomato.pre*. Directed deletion mutants targeting the ETS domain, the heterodimerization domain of GABPA, and the TAD of GABPB1 were used as functional controls. To modulate ectopic expression, subunits were fused to a degradation domain derived from an FKBP1A mutant cDNA that led to rapid degradation of the fusion proteins. By the addition of Shield1 (Takara/Clontech) these fusion proteins were stabilized and offer a tunable system for studying GABP dose effects.
Results: Combined overexpression of GABPA and GABPB1 (isoform β2), the predominant isoform in human hematopoietic cell lines, impairs the proliferation of myeloid and lymphoid cell lines, an effect that was not observed when GABPA or GABPB1 were overexpressed alone. When combining deletion mutants of one subunit with the wild-type protein of the respective other subunit, proliferation inhibition was drastically reduced, thereby demonstrating that functional GABP is critical for the observed proliferation inhibition that was confirmed in the murine pro-B cell line BaF3. In addition, combined overexpression of wild-type GABP subunits in lineage-negative primary bone marrow cells from C57BL/6J mice led to an almost complete loss of colony-forming capacity. Besides this proliferation block, a reduction of cellular viability was observed in U937 cells when metabolically active cells were monitored using WST-1. In U937 cells, frequently used as a human myeloid differentiation model, further analyses displayed a dose and GABPB1 isoform-dependent induction of CD11b by flow cytometry. Vice versa, shRNA-mediated knock-down of GABPA impairs PMA-directed monocyte-like differentiation of U937. Prolonged culturing of U937 cells co-expressing both GABP subunits finally led to the expression of CD14 and a monocyte-like morphology seen in Wright-Giemsa stained cytospins. Concordantly, combined overexpression of alpha and beta subunits led to the expression of Gr-1 in the myeloblast-like cell line 32D.
Summary and Conclusions: Ectopic overexpression is a feasible tool to elucidate the molecular function of GABP in hematopoietic cells. Moreover, the established tunable protein stabilization system can be used for more precise investigation of dosage effects. Concerning the impaired cellular viability, the proliferation block and cellular differentiation, observed even in an immortalized leukemia cell line, our results support the critical and powerful role of GABP in hematopoietic network maintenance and cellular differentiation. Further investigations are underway to study the effects of combined ectopic overexpression in more detail, and to learn for example (i) whether the impaired cellular viability is the result of actively induced apoptosis, (ii) how the proliferation block is achieved, and (iii) whether *CD11B* is a direct target gene of GABP.

P931

DECREASED EXPRESSION OF DICER1 IN BONE MARROW MESENCHYMAL STROMAL CELLS FROM PATIENTS WITH HEMATOLOGICAL MALIGNANCIES
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Background: Bone marrow-derived mesenchymal stromal cells (BM-MSC) are the stromal cells that may interact with the cancer cells of leukemia and myelodysplastic syndrome (MDS). Recently, a study conducted by Raaijmakers *et al.* showing primary defect (*Dicer1* deletion) in BM stromal cells is sufficient to induce MDS/AML in a murine model (Nature 2011). It is not known whether this model can be applied to the real world patients of MDS/AML and other hematological malignancies.
Aims: To elucidate any potential relationship between *Dicer1* expression in BM-MSCs of normal adults and various hematological malignancies and the prognostic impact on the induction outcome (chemo-sensitive or chemo-resistant). Besides, we also want to see whether the expression level of Dicer would be influenced by the change of disease status (complete remission or relapse).
Methods: 66 samples of BM-MSCs isolated from 62 patients of hematological malignancies were selected for analysis. 44 samples of them were collected from patients at initial diagnosis of disease, including AML (17), MDS (8), ALL (7), myeloma (8), and Philadelphia-chromosome positive CML (Ph-CML, 4). 8 samples of them were collected from normal healthy adults. Another 14 samples of them were collected from patients of AML after receiving standard chemotherapy, 6 in a status of complete remission and 8 in relapse. "3 pairs" of BM-MSCs from 3 AML patients at different clinical status were also examined to see the influence of disease status on the expression of *Dicer1*. Quantification of expression of *Dicer1* was done by using the Real-Time PCR System. The expressions of *Dicer1* were normalized against that of housekeeping gene *GAPDH*. A fold change in expression was calculated using the $2^{-\Delta\Delta Ct}$ formula.
Results: The expression of *Dicer1* (expressed as $2^{-\Delta\Delta Ct}$, mean±standard deviation) in BM-MSCs is significant lower in patients of AML (2.029±1.815), MDS (2.112±1.161), CML (1.678±1.060), ALL (1.348±1.405), and tend to be lower in patients of MM (2.469±1.540) comparing to that of normal adults (3.782±1.327; *P*=0.010, 0.012, 0.027, 0.004, and 0.074 respectively, Mann-Whitney U test; Figure 1). After adjust for the effect of age, gender, and cytogenetic status by using ANCOVA, the expression of *Dicer1* remains significantly low in patients with hematological malignancies (F=7.601, *P*=0.008). For AML patients at initial diagnosis, the expression of *Dicer1* in BM-MSCs do not differ significantly between patients who responded to induction (n=7; 2.234±2.051) and patients who refractory to induction (n=4; 1.469±1.449; P=1.000, Mann-Whitney U test). For AML patients who had received standard chemotherapy, however, the expression of *Dicer1 in* BM-MSCs was significantly lower for patients in refractory or relapsed status (n=8; 2.421±0.885) than that of patients in complete remission (n=6; 4.941±3.096; *P*=0.020, Mann-Whitney U test, Figure 1). For the "3 pairs" of BM-MSCs from 3 AML patients, the expression of Dicer1 was much higher when disease was in remission comparing to that in diagnosis or in relapse.

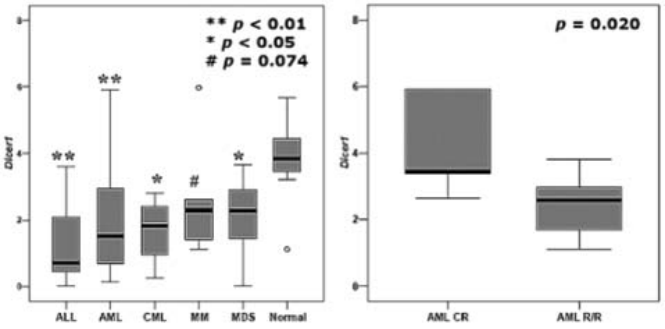

Figure 1.

Summary and Conclusions: Down-regulation of *Dicer1* in BM-MSCs is not a unique feature for MDS/AML, it can also be seen in other hematological malignancies, including disease with clear leukemogenic mechanism like Ph-CML. Decreased expression of *Dicer1* in BM-MSCs may therefore not be the "primary event" in leukemogenesis. Rather, our data clearly showed the expression level would be different when the disease status was altered, indicates the expression of *Dicer1* in BM-MSCs could be modulated by the neighboring hematological cancer cells. Future studies are needed to elucidate how the expression of *Dicer1* is modulated, and what's the physiological impact in real world patients.

P932

FUNCTIONS OF JARID1B IN THE REGULATION OF HEMATOPOIESIS AND LEUKEMOGENESIS

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Background: One of the mechanisms involved in the control of development is through epigenetic regulation of histone proteins. Of these, lysine methylation on histone proteins is considered an important epigenetic modification in transcriptional regulation during hematopoiesis and leukemogenesis. Whilst JARID1B, a member of the JARID1 histone 3 lysine 4 (H3K4) demethylases, was found essential for the self-renewal of embryonic stem cell and melanoma stem-like cell and involved in transcriptional regulation of cell cycle, such as mouse *Egr1*, *Bmi-1*and *p27*, during embryo development. In addition, JARID1B is involved in the terminal differentiation of neural cells and the expression of oncogenic microRNAs during macrophage differentiation. However, the function of JARID1B in hematopoiesis and leukemogenesis has not been systematically studied. We believe that JARID1B has the potential to play crucial role in hematopoietic stem cell self-renewal and differentiation, while its dysregulation is associated with the deregulation of leukemia gene transcription program.
Aims: To identify expression profiles of JARID1B in leukemia and hematopoietic cells, and the effect of knock down on cellular proliferation, differentiation and self-renewal properties in leukemic cell lines and hematopoietic stem cells.
Methods: Gene expression patterns of JARID1B were examined by qPCR analysis in 28 human leukemia cell lines, 20 whole white blood cell samples from CML (Chronic Myeloid leukemia) patients, and normal mouse/human hematopoietic cell populations–this include hematopoietic stem cell, common myeloid progenitor, common lymphoid progenitor, T cell, B cell, NK cell, monocyte, neutrophil, dendritic cell. Lentivirus based shRNA gene knock-down approach was used to study the cellular functions of JARID1B in K562 leukemic cell line and in mouse hematopoietic stem cells (Lin-/Sca-1+/c-Kit+). Proliferation, differentiation and self-renewal abilities were examined by PI (Propidium Iodide) staining, cell counting and colony formation assay.
Results: The expression profile of *JARID1B* in a panel of normal hematopoietic cells demonstrated that it was more than 3-fold uP-regulated in differentiated hematopoietic cells, when compared with the hematopoietic stem cells and progenitor cells, suggesting that the enhanced cellular level of JARID1B is associated with the loss of cell self-renewal properties and the initiation of hematopoietic lineage commitment. In contrast, JARID1B expression in most of the leukemia cell lines and CML patient samples were about 3-fold down-regulated. Although knockdown of JARID1B in K562 leukemic cell line and in normal mouse hematopoietic stem/progenitor cells showed no significant changes in cell proliferation and cell-cycle pattern, we observed a reduction on total colony formation number in serial re-plating assays, suggesting a basal cellular level of JARID1B is required to maintain self-renewal property.
Summary and Conclusions: Our results show the levels of expression of JARID1B are low in leukemic cells lines and with lower expression levels seen in progenitor and stem cells compared to mature hematopoietic cells. This pattern of expression together with knock out of JARID1B causing a reduction in self-renewal properties in leukemic cell lines and hematopoietic stem cells suggest a role for JARD1B in the control of hematopoiesis and in leukemogenesis.

P933

ROLE OF BMI-1 IN NORMAL MOUSE HEMATOPOIETIC STEM CELLS AND LEUKEMIC CELLS

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Background: Bmi-1, as a key component in polycomb repressive complex1, plays an indispensable role in the regulation of cell proliferation, self-renewal and multi-lineage differentiation of hematopoietic stem cells (HSCs). Previously, Bmi-1 was reported as a negative regulator of *Ink4a-Arf* tumor suppressor gene locus and *Ikaros* lymphoid priming gene in HSCs. However, little is known about the other downstream cellular targets of Bmi-1 in the control of self-renewal and multipotency in HSCs. Besides, aberrant expression of BMI-1 was observed in human leukemias such as acute myeloid leukemia, chronic myeloid leukemia, and B-cell non-Hodgkin lymphoma, suggesting the deregulation of BMI-1 is highly associated with leukemogenesis, yet its function remains unclear.
Aims: To study the epigenetic regulatory mechanism of *Bmi-1* in proliferation and self-renewal of hematopoietic stem cells and to find out how *Bmi-1* regulates the leukemogenic features of SEM leukemic cells.
Methods: In normal hematopoiesis, hematopoietic stem cells with Lin$^-$Sca-1$^+$c-Kit$^+$ phenotype were isolated from mouse bone marrow and were subjected to *Bmi-1* gene manipulation. Fsunctional assays were performed in *Bmi-1* knockdown or over-expressed LSK cells, including (a) colony forming assay to assess the self-renewal property, (b) cell cycle pattern analysis by PI staining, (c) qRT-PCR to examine the expression of potential *Bmi-1* target genes. In leukemia condition, *BMI-1* was over-expressed in SEM cells and analysed the leukemic properties by the above functional assays.
Results: Q-RT-PCR analysis demonstrated that knockdown of *Bmi-1* gene in HSCs leads to de-repression of $p16^{Ink4a}$ and $p19^{Arf}$genes, without alteration of other cell cycle regulators and hematopoietic lineage genes, suggesting that the reactivation of *Ikaros* lymphoid priming gene may represent a late differentiation event. Interestingly, cell cycle analysis showed that depletion of *Bmi-1* drives cells from G0/G1 phase to S phase, which opposites to the effect of the elevated $p16^{Ink4a}$ and $p19^{ARF}$ expression in blocking the entry to S phase, suggesting that an p16/p19-independent regulatory pathway may exist in HSCs. Colony forming assay also demonstrated reduced colony forming ability in *Bmi-1* knockdown HSCs, which indicates defects in the self-renewal ability, whereas overexpression of Bmi-1 maintains cell proliferation and promotes colony forming ability. In BMI-1 over-expressing leukemic SEM cells, we observed reduced cell proliferation and loss of colony forming ability, demonstrating a negative regulatory role of BMI-1 in the leukemogenic features of SEM cells. Interestingly, $p14^{ARF}$

and *p19INK4A* gene expression was independent of BMI-1 regulation in SEM cells, suggesting an altered function of BMI-1 in leukemia.
Summary and Conclusions: Bmi-1 plays a vital role in sustaining cell proliferation and self-renewal of normal hematopoietic stem cells, which is in contrast to its inhibitory function under leukemic condition.

P934

CONNECTIVE TISSUE GROWTH FACTOR (CTGF) CONFERS CELL ADHESION-MEDIATED DRUG-RESISTANCE (CAM-DR) IN ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is a heterogeneous disorder of the hematopoietic progenitor cell characterised by a differentiation block and uncontrolled proliferation. Although several mechanisms of leukemic transformation have been identified, the prognosis of AML patients is still poor. There is increasing evidence that leukemic stem cells are protected against cytotoxic stress in the bone marrow niche (cell adhesion-mediated drug–resistance, CAM-DR). Targeting the interaction of stromal and leukemic cells seems to represent a promising therapeutic approach and is currently investigated in several clinical trials (*e.g.* CXCR4-inhibitors). Recent data suggest that the CCN family of cysteine-rich secreted proteins is an important mediator of cell adhesion and extracellular matrix (ECM) modulation in physiological as well as in malignant processes. CCN1 (cysteine-rich protein 61, Cyr61) and CCN2 (connective tissue growth factor, CTGF) are major targets of the transcriptional cofactors YAP/TAZ which act as the key downstream mediators of the mechano-sensitive hippo signalling pathway. YAP and TAZ were found to confer stem cell capacities and drug resistance in different solid cancers. In AML the hippo pathway and the function of CTGF is only poorly understood.
Aims: The purpose or this study was to identify and validate new factors involved in niche–leukemia interaction and CAM-DR.
Methods: We used a well-established coculture system comprised of the leukemic cell line MOLM14 and the stromal cell line MS5. To identify target genes within the leukemic niche, we performed a qRT-PCR array covering 84 known ECM and adhesion genes using cDNA of MOLM14 and MS5 maintained in co- or monoculture for 72 h. Differentially regulated genes were confirmed using RQ-PCR and functionally validated upon shRNA-mediated knockdown.
Results: In our qRT-PCR array, we identified a 12-fold upregulation of CTGF in cocultured MOLM14 cells. Independent experiments confirmed increased CTGF expression in MOLM14, OCI-AML and MV4;11 cells when cultured with stroma. Further, we also detected a substantial upregulation of Cyr61 in these cell lines. Similar results were found upon culture on fibronectin or 293FT cells. To mimic conditions in the bone marrow niche more accurately, we repeated the experiments under hypoxic conditions. Again, we found upregulation of CTGF and Cyr61. Finally, we observed only minor upregulation when MOLM14 cells were separated from stroma by transwells. These date suggest that upregulation of CTGF and Cyr61 is a common phenomenon in AML cells, is independent on the used feeder layer but requires direct contact. As CTGF and Cyr61 are targets of the transcriptional cofactors YAP/TAZ, we investigated the expression of TAZ upon coculture on MS5 cells. Protein levels of TAZ became readily detectable in MOLM14 cells upon coculture but not in monoculture. As we did not observe any changes in mRNA levels, we assume posttranslational modifications causing stabilization of TAZ. To investigate whether TAZ is indeed involved in the regulation of CTGF we performed shRNA-mediated knockdown of TAZ in MOLM14 cells. This resulted in reduced expression of CTGF and Cyr61 upon coculture. To functionally examine the role of CTGF in CAM-DR, we performed CTGF knockdown-experiments. MOLM14 cells, transduced with CTGF-shRNA were cultured on MS5 cells and treated with Ara-C for 72 h. Knockdown of CTGF caused a significant (**) inhibition of the observed CAM-DR in MOLM14 cells (Figure 1).

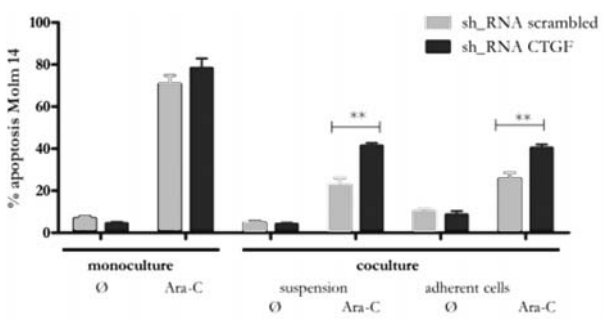

Figure 1. CTGF is crucial for rescue effect in coculture.

Summary and Conclusions: In this study we identified two members of the CCN family, CTGF and Cyr61, to be upregulated in cocultered AML cell lines. Our data suggest that the hippo pathway is the upstream-regulatory mechanism. Knockdown of CTGF enhances apoptosis in coculture, indicating an important function in CAM-DR. Therefore, targeting CTGF might be a promising therapeutic approach in AML.

P935

HUMAN BONE MARROW ADIPOCYTES MAINTAIN THE SURVIVAL AND DIFFERENTIATION OF HEMATOPOIETIC STEM CELLS

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Background: Active hematopoietic bone marrow declines with age and is gradually converted to fatty marrow, that fills the entire marrow cavity. So, adipocytes are abundant cells in the human bone marrow, where they are implicated in metabolic functions and in complex interrelationships with other stromal cells. The role of fat in this specific microenvironment is largely unknown.
Aims: We characterized bone marrow adipocytes (BM-A) molecularly and functionally to understand their functions in the hematopoietic microenvironment.
Methods: Healthy bone marrow was harvested from the iliac crest of bone marrow donors (n=9, median age 45, range 29-55). BM-A were isolated after collagenase digestion and filtration and cultured *in vitro*. We characterized these cells for their structural and molecular features, studying their morphology, immunophenotype and the gene expression profile of genes involved in white and brown adipogenic lineage. In particular, we investigated BM-A role in supporting hematopoiesis by a long term co-culture system with CD34+ cells. Moreover, we analyzed a cytokines secretion profile of BM-A, studying molecules that influence hematopoietic stem cells by increasing their proliferation or retaining the cells in a functionally immature state.
Results: Primary cultures of BM-A exhibited a typical unilocular white adipocytic morphology, maintaining their shape without noticeable lipolysis even after 30 days of culture time. Data displayed the expression of many white adipogenic genes, such as PPARg and its target genes (GPDH, LPL, Adiponectin, aP2 and LEP). Moreover, BM-A expressed some regulatory genes of brown adipocytes, like Dio2, CIDEA and PGC1a, suggesting their metabolic function in the bone marrow microenvironment. Then, BM-A were analyzed for the expression of stem cells markers, showing a pattern of surface antigen positively stained for CD90, CD105, CD271, CD117, CD31, CD133 and lowly for CD34. To show the functional properties of BM-A in the bone marrow microenvironment, we studied the interrelationship between BM-A and hematopoietic stem cells. BM-A demonstrated their ability to support the survival and differentiation of hematopoietic stem cells. Results showed that BM-A were stromal cells with the hematopoietic supporting function similar to mesenchymal stem cells, which are known for their hematopoietic supporting capacity. Moreover, we analyzed the amounts of hematopoietic stimulatory and inhibitory cytokines. Data demonstrated that BM-A secreted at high levels cytokines promoting the differentiation of committed hematopoietic progenitors, such as IL-6, IL-8 and G-CSF. At the same time, the negative regulators of hematopoietic microenvironment (IL-10, IL-17, MIP-1a, and TNFa) were not produced at significant levels.
Summary and Conclusions: Collectively, data shown here characterized BM-A at the morphological, phenotypic, molecular and functional levels. BM-A represent a population of white adipocytes, with the expression of typical white adipogenic markers and some regulatory genes of thermogenesis. These data suggested that adipocytes do not play a passive role as space fillers in the bone marrow but rather may provide a localised energy reservoir. BM-A expressed stem cells surface antigens, suggesting a possible plasticity of these cells. Moreover, BM-A are stromal cells with hematopoietic supporting capability that are most likely regulated by the production of specific molecules.

P936

ROLE OF CALORIC RESTRICTION IN TUMORIGENESIS THROUGH STEM CELLS

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Background: Environment and life-style are perceived as critical factors that can influence healthy aging and the risk of cancer and other age related diseases such as chronic degenerative and metabolic diseases. One of such factors is diet, which seem to act as a major antagonistic element to health leading to nutritional disorders, overweight and obesity, increasing the risks of life threatening diseases such as cardiovascular diseases and cancer. Interestingly, caloric restriction (CR), that is reduced intake of calories, has not only proved to delay aging but also to reduce cancer incidence in animal models. As the mechanism of action of CR is still poorly understood, it is currently not possible to apply CR-mim-

icking approaches to prevent diseases. Further, most studies on the mechanism of cancer have focused on deregulation in somatic cells rather than SCs that has recently emerged as the site of origin of cancer. Among all, hematopoietic SCs (HSCs) are well studied and have been shown to deteriorate with age due to accumulation of DNA-damage and loss of epigenetic regulation that potentially increases their genomic instability and disposition for neoplastic transformation. In fact, acute-myeloid leukemia has been shown to derive from HSCs.
Aims: The aim of this study is to investigate the cellular and molecular mechanisms underlying the protective effect of CR on tumorigenesis. In this study, we test the hypothesis that SCs are a critical target of CR using the blood tissue as model system, by analyzing the functional effect of CR on SCs.
Methods: The effect of diet on the proliferation and differentiation of stem cells in bone marrow was examined by combination of methylcellulose-based colony forming assays and fluorescence activated cell sorter analyses, using SC surface and proliferation markers. The effect on SC function was determined by competitive bone marrow transplantation assay. Whole Transcriptome Shotgun Sequencing was used to analyze the effect of CR on global changes of gene expression in lineage negative cells from CR and SD fed C57BL6/J mice.
Results: We have recently found that CR significantly influences the rate of tumour-related deaths of mice carrying null mutations of the tumour suppressor gene p53 (p53-/- mice). We observed that transplantation of bone marrow from p53-/- mice (fed on CR diet) into irradiated mice decreases the tumorigenesis in the recipient mice compared to the bone marrow transplanted from p53-/- mice fed on standard diet. p53 has been shown to regulate adult SC self-renewal suggesting a role for CR and its tumour suppressor activity in controlling the stem cell pool. We have established that 10-14 weeks of CR is sufficient to achieve the molecular adaptation (*e.g.* decreased IGF1 level by 40%) and body weight (~15% weight loss) changes associated with CR by measuring IGF1 levels and weight in mice fed on CR diet. We then measured the proliferation of stem cell population in bone marrow mononuclear cells (BMNC) obtained from mice fed on CR diet and ad-libitum diet, and found that CR decreases the percentage of proliferative stem cells by 20-50%. Gene expression analysis of lineage negative hematopoietic cells by microarray indicates that CR regulates the expression of several genes involved in metabolism, cytokine signaling and cancer pathways.
Summary and Conclusions: Our preliminary data point towards an effect of CR on the cell cycle status of SCs via inhibition of proliferation, which suggests that CR may protect from cancer via its action on SC functions.

P937

ANALYSIS OF HUMAN ENDOSTEAL HSC NICHE; A NOVEL POPULATION WITH HIGH EXPRESSION OF CD34 AND ESAM IS ADJACENT TO TRABECULAR AREAS OF HUMAN BONE MARROW

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Background: In adult bone marrow (BM), it is believed that the specialized microenvironments regulate self-renewal or differentiation of hematopoietic stem/progenitor cells (HSPC). However, our knowledge of those niches is incomplete and there are significant species differences in hematopoiesis between mice and humans. For instance, murine hematopoietic stem cells (HSC) express CD150 and CD38, but not CD34, whereas in humans, a $CD34^+$ $CD38^-$ subset is enriched for HSC. We and others have reported both of human and murine HSC express ESAM (Yokota *et al.* Blood. 113:2914-23, 2009). In mice, at least some HSPC niches are located in trabecular areas of marrow, and HSC obtained from different sites are not identical. However, little is known about the distribution of HSPC within human BM, because BM aspirated from pelvis are mainly used for human hematopoietic studies.
Aims: To study human hematopoietic niches by analyzing bone-associated cells in the trabecular areas of human BM, especially $CD34^{high}$ $ESAM^{high}$ cells released from human femoral heads.
Methods: Bone samples were collected from patients receiving hip bone replacement surgery and cord bloods (CB) were from healthy, full-term neonates after delivery. All participants provided prior informed consent, using protocols approved by the Investigational Review Board of Osaka University Hospital and Oklahoma Medical Research Foundation. For preparation of cells adjacent to bones, trabecular tissues were treated with 2 mg/mL collagenase IV and DNase and gently agitated for 1 hour at 37 C. Mononuclear cells were separated by Ficoll-Paque PLUS centrifugation from CB. Collected cells were tested *in vitro* and *in vivo* assays for their hematopoietic or endothelial differentiation potential. In addition, $CD34^{high}$ cells were sorted using flow cytometer for some experiments.
Results: After collagenase treatment, more mesenchymal stem cells, which have been reported as HSC niche components, and more $CD34^+$ HSPC were collected from human bone specimens compared to samples without treatment. We found there were two distinct populations according to CD34 and ESAM expression levels in $CD34^+CD38^-$ cells. The intensity of ESAM and Thy-1 positive percentage were high in $CD34^{high}$ cells compared to $CD34^{low}$ cells. These $CD34^{high}$ cells also express Tie-2. Not all, but some CB lots had a similar subset. Cell-cycle analysis revealed these cells were dormant. Little or no cells were recovered under several hematopoietic conditions; stromal-cell free cultures for HSC expansion, cocultures with OP9 stromal cells, or CFU assays. $CD34^{high}$ $ESAM^{high}$ cells derived from CB could not reconstitute after transplantation via tail vein in sublethally irradiated immunodeficient mice. However, $CD31^+$ $Flk\text{-}1^+$ endothelial cells were generated after a 5 week long-term culture supporting the differentiation of both hematopoietic and endothelial cell lineages.
Summary and Conclusions: Bone-associated cells included HSPC and mesenchymal lineage cells that presumably reside in specialized niches. The methods we have developed can be used to dissect such microenvironments in human bone marrow. Our initial analysis focused on a quiescent $CD34^{high}$ $ESAM^{high}$ $Thy\text{-}1^+$ $Tie\text{-}2^+$ population. After long-term culture supporting the differentiation of both hematopoietic and endothelial cell lineages, only endothelial cells were generated, while no hematopoietic cells were recovered. The $CD34^{high}$ $ESAM^{high}$ subset adhering to bones seemed to be endothelial stem/progenitors. Interestingly, the numbers of this population varied according to individual CB. Our newly identified subset might be involved in endothelial cell-related events, including reconstitution of grafts after hematopoietic stem cell transplantation or repair of BM injured by irradiation/chemotherapy. Further studies would clarify clinical roles and utility of these unique bone associating cells.

P938

HEAD-TO-HEAD COMPARATIVE STUDY OF WHARTON'S JELLY AND BONE MARROW MESENCHYMAL STEM CELLS

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Background: Mesenchymal stem cells (MSCs) can be easily isolated from the connective tissue surrounding umbilical cord vessels, namely Wharton's jelly (WJ). Although WJ-MSCs display typical MSC characteristics, a head-to-head comparison with Bone Marrow (BM)-MSCs, which represent the most extensively studied population of adult MSCs, is still lacking.
Aims: Provided that *ex vivo* MSC expansion is a prerequisite for clinical MSC-applications, in the present study we seek to comparatively investigate the characteristics of WJ- and BM-MSCs, cultured under identical conditions.
Methods: MSCs were isolated and expanded from consenting healthy donors' BM aspirates (n=4) and from the WJ of full-term neonates (n=4) after written informed consent of the family. MSCs were *in vitro* expanded until passage (P) 6 and phenotypically characterized by flow cytometry (FC). MSCs were induced to differentiate *in vitro* to adipocytes and osteoblasts using appropriate culture media. MSC differentiation was assessed with cytochemical stains (Oil Red-O for adipocytic, and Alizarin Red/Von Kossa for osteoblastic differentiation). MSC growth characteristics were assessed by evaluating the population doubling time and by a methyl triazolyl tetrazolium (MTT)-assay throughout passages. MSC survival was evaluated by FC with 7-Aminoactinomycin D (7-AAD) and MSC senescence was estimated by the percentage of $SA\text{-}b\text{-}gal^+$ cells in cultures. MSC karyotypic stability was assessed with classic G-banding. MSC hematopoietic supportive capacity was evaluated in co-cultures with allogeneic BM $CD34^+$ cells. The co-cultures were kept for a total of 5 weeks. At weekly intervals the non-adherent cells were counted and assayed for clonogenic progenitor cells.
Results: WJ-MSCs displayed a spindle-shape morphology, similar to BM-MSCs. Furthermore WJ- and BM-MSCs displayed identical immmunophenotype, as evidenced by the expression of CD90,CD105,CD44,CD29,CD73 and the lack of expression of CD45,CD14,CD34,CD31. WJ-MSCs displayed superior proliferative potential compared to BM-MSCs throughout passages (P<0.05). There was no significant difference between WJ- and BM-MSCs in the proportion of apoptotic cells ($7\text{-}AAD^{bright/dim}$) at P2 or P6. Senescent ($SA\text{-}b\text{-}gal^+$) cells were significantly fewer in P6 WJ-MSC cultures, compared to BM-MSCs (P<0.05). No chromosomal abnormalities were observed in WJ- or BM-MSCs during *in vitro* expansion. With regards to differentiation capacity, WJ-MSCs required much longer time to differentiate into osteoblasts and adipocytes as compared to BM MSCs. Finally preliminary results from an ongoing series of experiments suggest that WJ-MSCs do not significantly differ from BM MSCs in the ability to support the hematopoietic progenitor cell growth.
Summary and Conclusions: *Ex vivo* expansion did not induce any cytogenetic abnormalities in either WJ- or BM-MSCs. WJ-MSCs exhibited greater proliferative potential and reduced senescence as compared to BM-MSCs, cultured under identical conditions. Cell apoptosis on the other hand did not differ between WJ- and BM-MSCs, at least in early and intermediate passages. WJ-MSCs appeared to be qualitatively different in terms of adipogenc and osteoblastic differentiation as compared to their BM-MSCs Finally our preliminary results suggest that WJ-MSCs may sustain normal hematopoiesis, thereby establishing a potential new model for the *ex vivo* expansion of BM hematopoietic stem cells. Experiments to probe more deeply into the hematopoietic supportive capacity of WJ-MSCs, as well as their growth and senescence characteristics are underway.

P939

ALTERATION IN GENE EXPRESSION PATTERN IN STROMAL PRECURSOR CELLS OF PATIENTS BEFORE AND AFTER ALLOGENEIC HEMATOPOIETIC STEM CELLS TRANSPLANTATION

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Background: Hematopoiesis depends on stromal microenvironment. There is a feedback between stromal precursor cells and quality of hematopoiesis. The alteration in stromal microenvironment was described in patients with hematological malignancies.
Aims: The aim of the study was to investigate the changes in some genes' expression in multipotent mesenchymal stromal cells (MMSCs) of patients with hematological malignancies before and after allogeneic hematopoietic stem cell transplantation (alloHSCT).
Methods: AlloHSCT was performed to 15 patients (11 male, 4 female) with various diagnosis. Conditioning was myeloablative in 10 patients and reduced intensity in 5. After informed consent bone marrow was aspirated from patients before conditioning and 30, 60, 90, 120, 180 and 360 days after alloHSCT and from 50 healthy donors during the aspiration for alloHSCT. MMSCs were cultured in standard conditions. Gene expression level in 1st passage MMSCs was estimated by real-time PCR. The relative expression levels were calculated using the $\Delta\Delta C_t$ method. A Student's t-test was used to evaluate statistical significance; values of $P<0.05$ indicated a significant difference.
Results: There were no differences in relative expression level of genes taking part in growth of MMSCs (BMP-4, FGFR1) between donors and patients. However expression of JAG1 doubled in MMSCs of patients after alloHSCT. The expression level of FGFR2 decreased significantly in patients' MMSCs comparing with donors and correlated with lower cumulative cell production in patients regardless of the amount of time (up to 1 year) that passed after alloHSCT. There was no difference between patients before transplantation and healthy donors in IL-1b expression. After alloHSCT the expression level of IL-1b increased 2.5-fold (P=0.04). It probably relates to stromal activation. There were no changes in IL-1b receptor 1 expression level. There was a tendency to elevation of differentiation markers expression - SPP1 and PPARg. The data suggest the alterations in MMSCs population, which probably consists of more mature precursor cells with lower proliferative potential after alloHSCT. The most profound changes were revealed in some immunomodulating genes. The expression of IL-6 significantly increased 1.5 fold in patients MMSCs before transplantation and 3 fold after comparing with healthy donors. The IDO1 expression level elevated 18 fold in patients MMSCs independently of transplantation procedure. The expression of other immunomodulating genes–PTGES, CSF1 and LGALS1 remain invariable in all groups. All patients were pretreated before alloHSCT, so it is impossible to distinguish between the damage of stromal cells caused by disease and by previous therapy. It is obvious that stromal precursor cells are altered in patients before alloHSCT. The changes include retarded MMSCs growth rate combined with the decrease in FGFR2 expression, and increased expression of IL-6 and IDO1. The procedure of transplantation aggravates all these characteristics and in addition the activation of JAG1 and IL-1b occurs suggesting stromal activation due to process of donors' HSC engraftment.
Summary and Conclusions: These data indicate significant permanent alterations in stromal precursor cells of patients before and following alloHSCT.

P940

ROLE OF BONE MARROW STROMAL CELLS IN THE REGULATION OF HUMAN EARLY LYMPHOPOIESIS

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Background: The mechanism to regulate human early B and T cell differentiation from hematopoietic progenitors remains less defined. We previously reported that telomerized human bone marrow stromal cells support the generation of cyCD79a$^+$CD10$^+$CD19$^+$proB and CD7$^+$CD56$^-$ proT cells from human hematopoietic progenitors (Nakamori *et al.*, Br J Haematol. 157(6):674, 2012).
Aims: Here we examined the role of bone marrow stromal cells in the regulation of early B and T cell differentiation from human hematopoietic progenitors, using this culture system.
Methods: CD34$^+$CD38$^{lo/-}$CD45RA$^-$CD7$^-$CD19$^-$CD10$^-$human hematopoietic progenitors were cocultured with the telomerized stromal cells in the presence of stem cell factor, flt3 ligand, and thrombopoietin (3GF). To elucidate the role of stromal cells, insert plates were used to separate the contact between hematopoietic progenitors and stromal cells. Alternatively, cultures were performed with conditioned medium collected from cultures with stromal cells in the presence of 3GF.
Results: Coculture of hematopoietic progenitors separated from stromal cells in the presence of 3GF for three weeks did not affect the generation of CD7$^+$CD56$^-$ proT cells but prevented the generation of CD7$^-$CD10$^+$CD19$^+$ B cell differentiation from hematopoietic progenitors. Similar data were obtained by incubation of hematopoietic progenitors with conditioned media from cultures with telomerized stromal cells. By time course analysis, CD7$^+$CD10$^-$CD19$^-$ and CD7$^-$CD10$^+$CD19$^-$ cells were developed from hematopoietic progenitors after 7 to 14 days of cultures with stromal cells. Reculture of the generated CD7$^+$CD10$^-$CD19$^-$ cells with stromal cells for another 14 days gave rise to CD7$^+$CD10$^-$CD19$^-$ and CD7$^-$CD10$^+$CD19$^+$ cells, while the generated CD7$^-$CD10$^+$CD19$^-$ cells mainly differentiated to CD7$^-$CD10$^+$CD19$^+$ proB cells. On the other hand, cultures with the conditioned medium without stromal cells inhibited not only the generation of CD7$^-$CD10$^+$CD19$^-$ cells from hematopoietic progenitors but also the generation of CD7$^-$CD10$^+$CD19$^+$ proB cells from CD7$^+$CD10$^-$CD19$^-$ and CD7$^-$CD10$^+$CD19$^-$ cells. These data suggest that human stromal cells support early T cell differentiation by producing soluble factors and enhance B cell differentiation by direct contact with hematopoietic progenitors.
Summary and Conclusions: These data indicate that the direct contact with human stromal cells plays an important role in the regulation of early B and T cell differentiation by enhancing B cell differentiation.

P941

DEREGULATION OF PI3K/AKT SIGNALING IN BONE MARROW MESENCHIMAL STROMAL CELLS FROM PATIENTS WITH *DE NOVO* AND THERAPY-RELATED ACUTE MYELOID LEUKEMIA

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Background: In addition to neoplastic transformation of hematopoietic progenitors, a damage of bone marrow microenvironment can contribute to leukemia development and maintenance. Several functional and morphological abnormalities of bone marrow mesenchimal stromal cells (BM-MSCs) have been described in myeloid neoplasms. Nevertheless molecular bases of differences between MSCs from normal and leukemic bone marrows are still unknown. PI3K/AKT signaling pathway is involved in several MSC functions and deregulation of genes belonging to these pathways have been described in MSCs from different type of cancers.
Aims: To study the expression profile of genes belonging to PI3K/AKT signaling pathway in MSCs from patients with *de novo* and therapy related Acute Myeloid Leukemia (t-AML), using as normal counterpart BM-MSCs isolated from patients with limited stage lymphoma without bone marrow involvement.
Methods: Study population included 5 patients with limited stage diffuse large B cell lymphoma (DLBCL) without bone marrow involvement, as normal control, and 10 patients with AML, including 5 *de novo* and 5 therapy-related cases. Bone marrow mononuclear cells were obtained by Ficoll-gradient centrifugation of bone marrow samples and cultured in Complete Human MesenCult® Medium (Stem Cell Technologies) in 25 cm^2 flask at 37°C. After 24 hours non-adherent cells were removed and adherent cells were cultured up to 70% confluence, then trypsinized and passed to a new flask. Cells at 2nd passage were collected by trypsinization, RNA was extracted using RNase mini kit (Qiagen) and cDNA was synthesized by QuantiTect Reverse Transcription kit (Qiagen). The Human PI3K-AKT PCR array (RT^{2}Profiler™ PCR Array; SABioscience) was used to analyze mRNA levels of 84 key genes involved in PI3K-AKT Signaling Pathway, in a 96-well plate in the CFX96 thermocycler (Bio-Rad). Relative changes in gene expression were calculated using the ΔΔCt method. An average Ct value of five housekeeping genes (GAPDH, βactin, β2-microglobulin, HPRT1 and RPLPO) was used to normalize the gene expression between sample groups. Fold change (FC) variations ≥1.5 in association to statistically significant T-test (P-value≤0.05) were used for the statistical analysis.
Results: Comparison of MSCs from AML samples *versus* normal controls identified three genes significantly down-regulated in leukemic samples, including GSK3B (P=0.0002, FC=-1.56), MTCP1 (P=0.019, FC=-1.62) and RASA1 (P=0.013, FC=-1.58). Stratifying the analysis according to AML subtypes, three genes were significantly down-regulated in t-AML *versus* normal bone marrow, including GSK3B (P=0.0009 and FC=-1.63), PTEN (P=0.004 and FC=-1.50) and SOS1 (P=0.004 and FC=-1.50). Similarly when comparing MSC from *de novo* AML *versus* normal bone marrow, GSK3B, MTCP1 and RASA1 resulted still down-regulated in MSC from leukemic samples (P=0.005 and FC=-1.51; P=0.018 and FC=-1.96; P=0.007 and FC=-1.81, respectively). No differences were found in the expression levels of studied genes between *de novo* and therapy-related AML.
Summary and Conclusions: Deregulation of genes belonging to PI3K/AKT signaling pathway may contribute to MSC dysfunction described in leukemic bone marrows and can affect their ability to interact with leukemic blasts and normal hematopoietic cells, eventually contributing to bone marrow failure and leukemia development. GSK3B was the most significantly and commonly down-regulated gene in MSCs from leukemic samples and codifies for the serine/threonine protein kinase Gsk3β which is also involved in additional signaling pathways, such as Raf/Mek/Erk and Wnt/β-catenin.

P942

LEVELS OF CIRCULATING HEMATOPOIETIC PROGENITOR CELLS ARE SIGNIFICANTLY INCREASED IN PATIENTS WITH PROSTATE AND BREAST CANCER REGARDLESS OF TUMOR STAGE

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Background: The detection of circulating tumor cells has been discussed as a prognostic factor in patients with various malignancies. Recently, investigators have hypothesized that prostate cancer cells infiltrate the bone marrow thereby competing with hematopoietic stem and progenitor cells (HSPC) for the niche environment. This in turn may then lead to a mobilization of HSPC into the peripheral blood. Therefore the detection of circulating progenitor cells (CPC) may be explored as a diagnostic marker with prognostic relevance in patients with solid tumors.
Aims: We tested the hypothesis whether prostate and breast cancer as systemic diseases increase the number of CPC and whether this is related to the stage of the disease.
Methods: Between November 2011 and July 2012, peripheral blood samples of patients with prostate cancer (n=38), breast cancer (n=78) and an age-matched healthy control group (n=39) were collected after informed written consent. 44 out of the 78 breast cancer patients were treatment-naïve and had not received chemotherapy or radiation therapy before the peripheral blood sample was collected. Chemotherapy had been finished in 34 breast cancer patients at least 8 months before inclusion. None of the prostate cancer patients had received prior chemotherapy or radiation therapy. Metastatic disease had occurred in 9 breast cancer patients and 9 prostate cancer patients by the time of sample collection.
All of the included patients were otherwise healthy, hematological or chronic inflammatory diseases were excluding criteria for this observational study.
HSPC were derived from heparinized blood using density centrifugation and washing. Mononuclear cells were seeded at a concentration of $0{,}334 \times 10\times^4$/1mL in MethoCult (Stemcell Technologies). Experiments were done in triplicate. Colony forming units were quantified after 14 days using an inverted microscope. Univariate and multivariate analyses were used for analyzing the influence of tumor stage, age and body mass index (BMI) on the frequency of CPC. All results were expressed as the mean±SEM.
Results: Univariate analysis showed that patients with prostate cancer had a significantly higher overall levels of CPC (109±15) compared to age matched healthy control group (54±10), P=0,036. This effect was mainly due to a significantly elevated level of CFU-GM (CFU-GM prostate cancer group 11±2 *vs.* CFU-GM in healthy control group 5±1, P=0,021). Tumor stage, age or body-mass index (BMI) did not affect the number of CPC in prostate cancer patients. This effect was confirmed by multivariate analysis.
Univariate analysis of the untreated breast cancer patients revealed a significantly higher number of CFU-GM (13±3) compared to age matched healthy control group (5±2), P=0,047. Multivariate analysis suggested an interaction of CPC levels and patient age. The extent oder besser stage of the tumor disease and BMI did not affect CFU-GM number. Interestingly, the average number of CFU-GM decreased after finishing anti-tumor therapy, indicating either a response to systemic therapy with a lower mobilization of CPC or a direct long-lasting suppression of hematopoiesis by the applied regimen.
Summary and Conclusions: This study shows for the first time elevated levels of CPC even in patients with localized prostate and breast cancer, but no direct correlation with the stage of the disease. The mobilization of CPC in cancer patients may either be an indirect effect of a systemic inflammatory milieu or directly linked to circulating tumor cells interfering with the hematopoietic stem cell niche.

P943

UMBILICAL CORD MESENCHYMAL STEM CELLS WITH IMPROVED SUPPORT FOR HEMATOPOIETIC STEM CELL EXPANSION.

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Background: *Ex vivo* expansion of cord blood hematopoietic stem cells (UC-HSC) is a promising research area because it can potentially provide large number of stem cells capable of successful engraftment and hematopoiesis reconstitution after myeloablative therapy. Also it is indispensable for the development of valuable niche models for cell interaction study and *in vitro* drug testing.
Aims: Establish a MSC-based model of HSC microenvironment; study how the Notch ligands expression in stromal cells regulates the UC-HSC expansion in a coculture system.
Methods: Mesenchymal stem cells from umbilical cord Wharton's jelly (UC-MSC) were isolated and transduced with lentiviral particles carrying DLL1 and JAG1. To measure transduction efficiency and monitor ligand expression we created bicistronic lentiviral vectors with genes of interest and eGFP. DLL1 and JAG1 expression was measured by real-time PCR, percent of transduction efficiency was estimated by FACS based on eGFP expression. $CD34^+$ cells from cord blood mononuclear fraction were isolated using magnetic bead sorting and cultured with NOTCH ligands expressing MSC. After two weeks phenotype of cells in suspension was analyzed using FACS analysis.
Results: Mesenchymal stem cells were transduced with lentiviral particles carrying DLL1 and JAG1 (based on FACS analysis, transduction efficiency was 98% and 90% respectively) and were used as feeder layers in coculture experiments with UC-HSC. Immunophenotypic analysis has shown that after two weeks of HSC cultivation, UC-MSC with expression of DLL1 provided 5.7-fold (±1.73) increase of $CD34^+$ cell numbers in suspension fraction compared to cytokine alone conditions and 2.8-fold(±1.23) compared to control UC-MSC. DLL1-MSC was the only population with ability to support $CD34^+CD38^-$ population which is considered more undifferentiated than $CD34^+CD38^+$. Cultivation of CD34+ cord blood HSC with JAG1-expressing MSC and in cell-free conditions led to 0.9- and 0.6-fold decrease of total CD34+ cell population.
Summary and Conclusions: Our data indicates that coculture of HSC and DLL1-expressing UC-MSC led to expansion of undifferentiated $CD34^+$ fraction of hematopoietic stem cells. Thus DLL1-MSC can be used as a supportive feeder layer for HSC expansion experiments *in vitro* and provide better reproducibility and lesser batch to batch variation than bone marrow MSCs. This study is ongoing. Currently, we are modifying lentiviral vectors with antibiotic resistance genes which will allow establishment of cell lines with more stable expression of Notch ligands.

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ENDOTHELIAL CELLS EXPRESSING CONSTITUTIVELY ACTIVE AKT1 ARE CAPABLE OF RECONSTITUTING OF VASCULAR NICHE IN VITRO.

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Background: Role of the endothelial cells (ECs) in establishment of hematopoietic stem cell (HSC) microenvironment is the ultimate question of modern hematology. Study of interaction between ECs and HSCs *in vitro* require removing several obstacles, one of them is the low viability of human umbilical vein endothelial cells (HUVEC) in HSC expansion media. In our study we produce endothelial cells expressing myristoylated AKT1 (AKT1-ECs) using lentiviral transduction and compare them to ECs carrying empty vector (EV-ECs).
Aims: Our long-term goal is to reconstitute the vascular niche *in vitro* by co-culturing hematopoietic stem cells with endothelial cells and to use this system to identify factors that control the fate of HSCs. The specific aim of this study is to generate endothelial cells with constitutively active AKT1, and test their ability to regulate HSCs expansion, self-renewal and differentiation in co-culture system *in vitro*.
Methods: HUVEC were transduced with a myrAKT1 lentiviral vector that additionally contained eGFP reporter gene. Efficiency of transduction was evaluated using FACS-analysis and fluorescence microscopy by detection of eGFP. Expression of AKT1 was measured by quantitative real-time PCR, immunofluorescence and western-blot analysis. Expression of the endothelial cell markers was analyzed by FACS-analysis and immunofluorescence. ECs viability after culturing in HSCs media was measured using the MTT assay. Isolation of CD34+ HSCs from human cord blood was performed using magnetic microbeads and the purity of CD34+ phenotype was confirmed by FACS. HSCs were co-cultured on the monolayer of AKT1-ECs with usual controls. After3,5,7,9 and 11 days of co-culturing the HSCs were collected and analyzed by FACS-analysis.
Results: AKT1 expression level was increased 12-fold after transduction with myrAKT1. AKT1-ECs expressed Akt1 protein at higher levels compared with EV-ECs and non transduced cells. Phosphorylation status of Akt1 was confirmed by western-blot analysis. AKT1-ECs expressed endothelial markers: PECAM (CD31), VE-cadherin (CD144), MCAM (CD146) and von Willebrand factor. However level of their expression was affected by myrAKT1. For instance level of MCAM, VE-cadherin and von Willebrand factor were decreased compared to EV-ECs and control ECs. Reasonable explanation could be that massive AKT1 activation prevented HUVECs from terminal differentiation to the mature endothelial cells. AKT1-ECs have higher viability and could survive in the HSC expansion media up to 7 days without significantly dropping of the viability. In co-culture experiments AKT1-ECs increased number of CD34+ CD38+HSCs (30% *vs* 0.8% in control).
Summary and Conclusions: We have shown that the endothelial cells expressing constitutively active AKT1 are able to support expansion of HSCs *in vitro* and thus can serve as a model of the vascular niche. We intend to use this model further to study the mechanisms of interactions between the two cell types and identify molecular signals that regulate the fate of HSCs.

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CHEMOKINE SIGNALLING REGULATES HAEMOPOIETIC STEM CELL SURVIVAL

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Background: A key property of haemopoietic stem cells (HSC) is their ability

to maintain quiescence, however the regulation of quiescence in this context is not well understood. A previous microarray study by our group reported that the most uP-regulated group of genes in quiescent compared to proliferating human HSC was chemokine ligands, specifically in the CXCL group, however their role was unclear (Graham *et al.* [2007] Stem Cells, 25, 12).
Aims: We aimed to investigate the role of CXCL chemokines in HSC properties, both in the human and mouse systems.
Methods: To determine their biological function in human HSC we used shRNA to reduce chemokine ligand expression and SB-225002 to block receptor signalling, focusing on CXCL1 (the most uP-regulated chemokine) and its receptor CXCR2. To assess which chemokines were expressed in murine HSC populations and to examine their role in HSC functions, we employed single cell quantitative PCR by Fluidigm and used transgenic knock-out and reporter mice.
Results: Protein expression for CXCL1 and CXCR2 was confirmed on primitive HSC (CD34+CD38-) by immunofluorescence. Knock-down of CXCL1 led to an induction of apoptosis and reduction in colony formation (8.5% *versus* 19.7% and 109 *versus*14, for percentage apoptotic cells and colony counts in control *versus* CXCL1 knock-down, respectively, n=1). Similarly, inhibition of CXCR2 on CD34+ cells for 72 hours using SB-225002 induced apoptosis and reduced colony formation (17±0.3% *versus* 34±3% and 64±24 *versus* 25±12 for percentage apoptotic cells and colony counts in untreated *versus* 1μM treated cells, P<0.05). Platelet Factor 4 (*Pf4*; alias *Cxcl4*) and *Cxcr2* were highly expressed in murine HSC populations. Analysis of CXCR2 null mice has revealed extra medullary haemopoiesis with colony forming activity in peripheral blood and spleen and an expansion of LT-HSC in bone marrow. Transplantation assays using WT or CXCR2 null LT-HSC are currently underway to determine their reconstitution potential. PF4-Cre transgenic mice containing a conditional tandem dimer RFP construct under the control of the Rosa26 promoter (PF4-Cre) were used to confirm that PF4 is expressed in both primitive murine LT-HSC and their mature progeny (Calaminus *et al.* [2012] PLOS ONE, 7(12): e51361). Using this model, we sorted RFP positive and negative bone marrow cells and noted reduced colony forming potential in the absence of PF4 expression (73±6 *versus* 44±2 for colony counts in RFP positive *versus* RFP negative cells, P<0.05). We are currently investigating steady state haemopoiesis and transplantation stress in PF4 null mice and postulate that PF4 supports HSC survival and PF4 null HSC will show reduced engraftment capability. Since data for PF4 were not conclusive from the original human microarray, we have now reassessed the relevance of PF4 in the human system and shown that *PF4* is indeed uP-regulated by gene expression analysis in primitive HSC (CD34+CD38-CD90+) compared with proliferative progenitors (CD34+CD38+) (9-fold change ±0.3 in CD34+CD38-CD90+ fraction in comparison to CD34+CD38+ fraction 1±0.1, P<0.001) with future experiments planned to further investigate the role of PF4 in the human system.
Summary and Conclusions: Enhanced understanding of the regulation of stem cell properties is critical for improving our ability to manipulate normal stem cells *in vitro* and *in vivo* and in diseases such as leukaemia in which leukaemic stem cells are less sensitive to drug treatment.

Cytogenetics and molecular diagnostics

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MONOALLILIC MUTATIONS OF FHL-RELATED GENES PREDISPOSE TO MACROPHAGE ACTIVATION SYNDROME

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Background: Macrophage activation syndrome (MAS) is a serious complication of rheumatic diseases, frequently associated with systemic juvenile idiopathic arthritis (sJIA) but also described in others pediatric inflammatory disorders including juvenile systemic lupus erythematosus (LES) and Kawasaki disease. Due to the close resemblance to a group of histiocytic disorders collectively known as hemophagocytic lymphohistiocytosis (HLH) it is currently classified among the secondary or acquired forms of HLH. Recent evidences suggest a correlation between defects in the cytotoxic machinery, as that caused by mutations in the familial hemophagocytic lymphohistiocytosis (FHL)-related genes, and development of MAS.
Aims: To describe clinical, functional and genetic features of MAS.
Methods: We revised and updated the HLH Italian National Registry to select patients with MAS defined as HLH according to the diagnostic criteria established by the Histiocyte Society with confirmed diagnosis of rheumatologic or autoimmune disease. Clinical data were collected. Functional screening of perforin expression and degranulation was performed by flow-citometry. Genetic study by direct sequencing of currently known FHL-related genes was carried out in our laboratory.
Results: Among the 813 patients referred to the Registry, 38 (5%) were diagnosed as MAS. Of them 13 were male and 25 female; 30 Caucasian and 8 Indian. Median age was 94 (quartiles: 37; 94; 136; 708) months. The underlying causes were: sJIA (n=28), LES (n=4); Kawasaki disease (n=1), dermatomyositis (n=1), undefined rheumatologic (n=3) or auto-immune (n=1) disease. The clinical picture showed: fever (n=28/28; 100%), splenomegaly (n=17/28, 61%), neurologic manifestations (n=7/28, 25%), anemia (n=15/25, 60%), thrombocytopenia (n=14/25, 56%), neutropenia (n=3/25, 12%), hypertriglyceridemia (n=14/25, 56%), hypofibrinogenemia (n=7/25, 28%), hyperferritinemia (n=22/23, 96%; quartiles: 2.430, 10.264, 15.953, 96.000 ng/mL), hemophagocytosis (n=9/25, 36%). Four (10.5%) died of progressive disease. Functional screening of FHL was performed in 22 cases: perforin expression was normal in 14 and reduced in 8 (36%); degranulation was defective in 3/18 (17%) and normal in the remaining 15/18 cases. At least one test was defective in 10/23 (43%). Mutation analysis included *PRF1* (n=36), *UNC13d* (n=22), *STX11* (n=33) and *STXBP2* (n=19) and allowed to identify monoallelic mutations in 11 of the 38 patients (29%), as follows: *PRF1* (n=8/36, 22%), *STX11* (1/33, 3%), *STXBP2* (3/19, 16%). Correlation between functional defect and evidence of mutation is shown in Table 1.

Table 1. Functional and genetic data of 11 MAS patients with monoallelic mutations in FHL-related genes.

UPN	AGE (mos)	PRF expression	GRA	VARIANTS	ANALISI *IN SILICO*	HGMD Database	GENE
238	6	ND	NORM	c.272C>T, p.A91V	Probably damaging	YES	*PRF1*
431	53	ND	ND	c.272C>T, p.A91V	Probably damaging	YES	*PRF1*
527	120	NORM	NORM	c.799G>A, p.V267M	Probably damaging	NO	*STX11*
579	95	RID	RID	c.1034C>T, p.T345M	Probably damaging	NO	*STXBP2*
660	ND	NORM	RID	c.272C>T, p.A91V	Probably damaging	YES	*PRF1*
661	6	RID	NORM	c.272C>T, p.A91V	Probably damaging	YES	*PRF1*
717	106	RID	ND	c.1357G>A, p.V453M	Probably damaging	NO	*PRF1*
738	40	ND	ND	c.1681C>T, p.W561R	Probably damaging	NO	*STXBP2*
746	1	NORM	NORM	c.272C>T, p.A91V c.610G>A, p.A204K	Probably damaging Probably damaging	YES NO	*PRF1* *STXBP2*
787	115	RID	NORM	c.755A>G, p.N252S	Benign	YES	*PRF1*
799	0	RID	NORM	c.272C>T, p.A91V	Probably damaging	YES	*PRF1*

Summary and Conclusions: MAS is a life-threatening complication of rheumatologic disease in children. Heterozygous mutation in one the FHL-related genes is observed in at least 29% of patients with MAS. *PRF1* is the most frequently involved gene, and p.A91V the most frequent single mutation. Of the 8 patients with reduced perforin expression at flow-cytometry, 4 were heterozygous for

PRF1 mutation, while 4 were wild-type. Of the 3 patients with defective degranulation, 1 bears *STXBP2* mutation while two were apparently wild-type; yet, both had moderate reduction of NK activity. Moderate reduction of cellular cytotoxicity machinery appears as a frequent predisposing factor in patients who develop MAS as part of a rheumatologic disease. Whether there are further regulatory mechanisms for the FHL-related proteins in these patients remains to be clarified.

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TRACKING OF THE PRELEUKEMIC PERIOD IN SECONDARY ACUTE LEUKEMIAS REVEALS MULTIPOTENT PROGENITOR AS A TARGET OF MLL REARRANGEMENTS

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Background: Secondary leukemias are often characterized by breakpoints in the MLL gene. The MLL gene is an extremely promiscuous translocation partner, as more than 70 MLL partner genes have been identified so far. The most frequent partner genes (AF4, AF9, ENL, AF10, AF6, ELL) account for >80% of MLL translocated leukemias. In some patients presenting with secondary acute leukemia (sAL) after primary leukemia, archived bone marrow (BM) samples collected during the follow-up of the primary disease might be available, thus providing us with material for backtracking of the sAL markers in the prediagnostic period.

Aims: We considered MLL rearranged sAL cases as a suitable model to study presence and dynamics of the (pre-)leukemic cells in the preleukemic period.

Methods: We have collected BM samples from 5 patients monitored for primary ALL (n=3) or AML (n=2), who developed sAL after variably long period of remission (1,25 to5,5 years). The sAL cases presented as ALL (n=2) or AML (n=3). Leukemospecific clonal markers (Ig/TCR and MLL gene rearrangements) were investigated in BM of both original and secondary diagnoses using real time PCR. In addition, to better characterize the (pre)leukemic clone, FISH and SNP array were performed in some cases. In an attempt to backtrack the sAL cells at different timepoints preceding the sAL, methods of qPCR and FISH were employed.

Results: The sAL were characterized by the presence of either typical (2 sAML cases with MLL/AF9 fusion) or rare (2 sALL cases with MLL/MAML2 and MLL/FOXO3A which we described previously and 1 sAML case harboring a novel, so far undescribed MLL/ME2 fusion) MLL rearrangements. In all 3 patients, who presented with the atypical MLL partner, we backtracked the presence of MLL fusion in BM samples which were taken several months prior to the sAL (20, 23 and 9 months in MLL/FOXO3A, MLL/MAML2 and MLL/ME2, respectively). In both ALL patients, the leukemic clone was defined also by an incomplete IgH rearrangement, which seemed to be specific for the overt disease in both cases, as they were not detected in the MLL positive specimens preceding the sAL diagnosis. In these patients the MLL positive cells comprised unexpectedly large proportion of bone marrow in the preleukemic period (approx. 10% in the MLL/MAML2 case and even >90% in MLL/FOXO3A case). Interestingly, the observed high levels of MLL rearranged cells in these two samples (taken 15 and 16 months before the sALL diagnosis) were followed by a remarkable decrease of the MLL positivity levels before the onset of sALL. The high percentage of preleukemic cells (not restricted to lymphoid lineage) during clinically silent period suggests the involvement of a multipotent cell originally affected by MLL translocation. In 2 sAML patients, harboring typical MLL/AF9 translocation, the fusion gene was not backtracked in any of the prediagnostic samples (the last ones taken 11 and 12 months before the diagnosis).

Summary and Conclusions: Our data suggest, that sAL–as well as de-novo leukemia - is a multiple-step process with protracted latency between the first (MLL rearrangement) and finally transforming hit leading to the active disease. Moreover, the preleukemic clone can reach unexpectedly high levels even during a clinically silent period with normal BM morphology. Finally, our data suggest that typical and rare MLL fusions possibly drive different dynamics of the processes preceding the overt disease.

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COMBINED APPLICATION OF MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION AND FLUORESCENCE IN SITU HYBRIDIZATION TO DETECT CYTOGENETIC ABNORMALITIES IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a genetically heterogeneous disease with diverse clinical outcome. Interphase fluorescence *in situ* hybridization (iFISH) is the most commonly used approach to detect recurrent cytogenetic abnormalities in this entity. The high and increasing number of identified biological and prognostic markers makes the introduction of a cost-effective screening strategy reasonable.

Aims: We aimed to assess the performance of the combined application of iFISH and multiple ligation-dependent probe amplification (MLPA) to reveal genetic alterations in MM.

Methods: Diagnostic bone marrow samples from 81 patients with MM were analyzed using a targeted MLPA kit containing a mix of 42 probes to simultaneously detect copy number alterations (CNAs) for the following regions: 1p32-31, 1p21, 1q21.3, 1q23.3, 5q31.3, 12p13.31, 13q14, 16q12, 16q23 and 17p13. All samples were also screened by iFISH for the presence of hyperdiploidy, deletion/monosomy of chromosome13, deletion of the *TP53* gene, disruption of the immunoglobulin heavy-chain gene, t(4;14), t(11;14), t(14;16), t(8;14), gain of 5q and abnormalities of chromosome 1. The study was conducted in accordance with the Declaration of Helsinki.

Results: A total of 245 alterations were detected in 79 cases (98%). Investigating the same aberrations, the two methods showed a congruency of higher than 90% (Fisher's exact test: p≤0.0003). Low proportion of cells harboring the abnormalities, focal CNAs and unmatched probes were responsible for the discrepancies. MLPA was able to simultaneously analyze several loci thus revealed 95 CNAs not detected by iFISH providing additional information in 53 cases (65%). These abnormalities not detected by commercial FISH probes were successfully validated using specifically designed BAC clones. Altogether, 66 cases showed multiple recurrent genetic alterations in 53 different combinations from which 22 were not recognized using any single method only. Investigating the co-segregation of various abnormalities, we found a pattern similar to those presented earlier by comprehensive studies applying expensive whole-genome screening technologies. Mapping the CNAs on chromosome 1 using more than 20 probes, significant heterogeneity in size and location, variable intra-chromosomal and intra-clonal rates of loss 1p and/or gain 1q were found.

Summary and Conclusions: To our knowledge, MLPA investigation in MM has never been published in international journals. Our study proves that MLPA is robust technique to detect multiple unbalanced genetic abnormalities in this incurable disease group and also shows that MLPA might be a cost-efficient alternative to the more time and resource-consuming approaches. IFISH is able to detect both balanced and unbalanced rearrangements which are cryptic for karyotyping but in routine diagnostics, more than three loci are rarely investigated simultaneously using this method. MLPA has the power to identify high number of submicroscopic gains and losses at a higher resolution than iFISH albeit its sensitivity does not reach that of iFISH. Since both CNAs and balanced aberrations have a great impact on the prognostic classification of MM, the presented MLPA and iFISH strategies should be applied in combination in the diagnostic hematopathology work-flow.

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COMPARISON OF MICROARRAY-BASED GENOMIC PROFILING AND MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) FOR THE DETECTION OF CLINICALLY RELEVANT GENETIC LESIONS, INCLUDING IKZF1, IN ALL

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Background: In acute lymphoblastic leukemia (ALL) specific genomic abnormalities provide important clinical information. In most routine clinical diagnostic laboratories conventional karyotyping in conjunction with fluorescence *in situ* hybridization (FISH) is currently considered as the gold standard to detect such abnormalities. Recently, we showed that microarray-based genomic profiling serves as a superior alternative strategy in a routine clinical diagnostic setting to detect gross genomic copy number alterations (CNAs) (Simons *et al.*, 2011, Genes Chromosomes Cancer 50:969-981). In addition, we demonstrated that this technique allows the detection of focal genomic lesions, frequently harboring clinically relevant ALL-related genes, such as *CDKN2A/B*, *ETV6, PAX5* and *IKZF1*, the latter recently shown to be associated with a high relapse rate in patients with ALL (Kuiper *et al.*, 2010, Leukemia 24:1258-1264). The ability to detect focal lesions, however, largely depends on the resolution of the platform used. Whereas targeted assays, such as multiplex ligation-dependent probe amplification (MLPA), have already proven to be suitable for this purpose (Kuiper *et al.*, 2010; Waanders *et al.*, 2011, Leukemia 25:254-258), such targeted tests do not provide genome-wide information, including gross cytogenetic abnormalities.

Aims: We compared the performance of a novel high resolution genome-wide microarray platform with a commercially available MLPA kit for the detection of ALL-specific genomic copy number abnormalities, including *IKZF1* deletions.

Methods: The Cytocan HD platform (2.7 million probes; Affymetrix) and Chromosome Analysis Suite (ChAS; Affymetrix) software was used for genomic profiling of 23 patients with ALL. In addition, the same samples were tested with commercially available multiplex ligation-dependent probe amplification (MLPA) kits (P335 and P202, MRC-Holland). Both techniques were used to reveal the

genomic copy number status of the recurrently affected genes *IKZF1, EBF1, CDKN2A/B, PAX5, ETV6, BTG1* and *RB1*.
Results: In total 161 loci (*i.e.*, 23 cases tested for 7 loci) were evaluated. One-hundred-twenty-one of these loci (75,1%) showed a match between both tests; 68 loci normal, 53 loci aberrant (CNA). Thirty-nine of the tested loci (24,2%) turned out to be uninformative for comparison of the two techniques, all due to no available or uninterpretable data from the MLPA tests, while microarray revealed interpretable results. One locus (0,6%) revealed a likely clinically relevant "mismatch" between the two tests, namely an *IKZF1* promoter deletion was detected with the microarray approach, which was "missed" by the MLPA test, because the promoter region is not covered in the used MLPA kits.
Summary and Conclusions: We conclude that the Cytoscan HD microarray platform is superior to the molecular MLPA test for the detection of clinical relevant genetic lesions in ALL. It has a higher success rate in the detection of focal CNAs and, in addition, provides a genome wide overview of all CNAs and regions of acquired copy neutral loss of heterozygosity (CN-LOH).

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GENOTYPING OF 25 LEUKEMIA-ASSOCIATED GENES IN A SINGLE WORKFLOW BY NEXT-GENERATION SEQUENCING TECHNOLOGY USING LOW AMOUNTS OF INPUT TEMPLATE DNA

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Background: Myeloid neoplasms are characterized by a variety of gene mutations within different molecular pathways involving cell signaling, transcription factors, epigenetic regulators and RNA splicing. Next-generation sequencing (NGS) technology enables analysis of thousands of sequencing reactions simultaneously. However, large-scale molecular analyses are often hampered by lack of sufficient template DNA or weighting the use of precious patient material.
Aims: We sought (i) to establish a robust and sensitive NGS method for genotyping of the 26 most commonly mutated genes in AML, MDS, MPN, and MDS/MPN overlap syndrome (*ASXL1*, *BRAF*, *CBL*, *CEBPA*, *DNMT3A*, *ETV6*, *EZH2*, *FLT3*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *KRAS*, *LNK*, *MPL*, *NPM1*, *NRAS*, *RUNX1*, *SF3B1*, *SRSF2*, *TET2*, *TP53*, *U2AF1*, *UTX*, *WT1,* and *ZRSR2)* in a single workflow and (ii) to optimize the method for low amounts of input template DNA without introducing bias.
Methods: NGS was applied to simultaneously analyze 26 leukemia-associated candidate genes (represented by 184 PCR amplicons) in 10 patients with chronic myelomonocytic leukemia (CMML) using genomic DNA (gDNA). Thereafter, NGS was performed for all candidate genes after whole genome amplification (WGA) was carried out with 20 ng template gDNA from the same 10 CMML patients using different commercially available kits based on multiple displacement amplification. Differences between variant frequencies in gDNA and WGA samples were determined for two different WGA kits.
Results: For each sample, 25 of 26 genes (180 PCR amplicons) were successfully sequenced with a limit-of-detection of approximately 5%. An overall median coverage of 94,935 reads (range: 74,745 to 117,056 reads) was generated for gDNA samples, with a median coverage of 492 reads (range: 308 to 636 reads) per amplicon. In 10 CMML patients 25 distinct mutations were identified in 11 genes (*CBL*, *FLT3*, *IDH2*, *KRAS*, *NRAS*, *RUNX1*, *SRSF2*, *TET2*, *TP53*, *UTX*, *ZRSR2*). Using WGA samples, mutations above the 5% sensitivity limit were reliably detected achieving an overall median coverage of 96,457 reads (range: 75,386 to 101,870) and a median amplicon coverage of 506 reads (range: 256 to 653 reads). Looking at all variants, WGA kits generated differences of variant frequencies that ranged from -28.19% to +9.94% (mean difference -0.2%, SD=4.08) or from -35.03% to +18.67% (mean difference -0.75%, SD=5.12), respectively.
Summary and Conclusions: Our method permits simultaneous and sensitive analysis of the 25 most commonly mutated genes in AML, MDS, MPN and MDS/MPN in a single sequencing run helping researchers to unravel associations between mutations in different pathways. NGS can be performed after WGA of low amounts of input template DNA for reliable variant detection without introducing significant bias.

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IDENTIFYING AND MONITORING TCRG CLONALITY USING MASSIVELY PARALLEL SEQUENCING AND ASSOCIATED BIOINFORMATICS.

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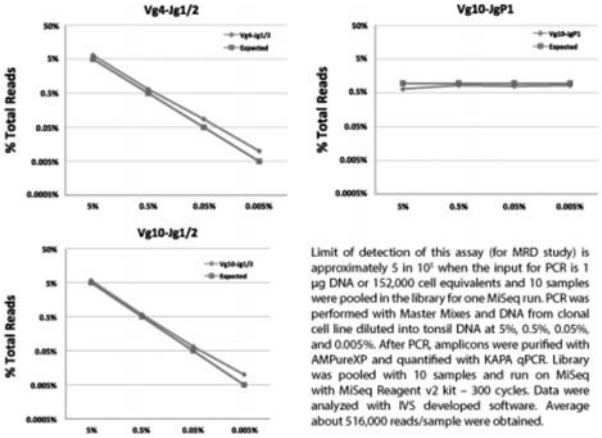
Background: Assays that identify clonal lymphocyte populations in clinical specimens are used on a routine basis to assist in the diagnosis of lymphoproliferative disease. Although detection of clonal lymphocyte populations is not, by itself, diagnostic of malignancy, once a relationship with a malignancy is established, tumor-specific DNA sequences of clonal V-J rearrangements are among the most sensitive patient-specific biomarkers available to monitor minimal residual disease (MRD). In concert with other clinical and diagnostic tools, sensitive detection of clone-specific V-J sequences can help determine therapeutic efficacy and often provide the first indication of clinical relapse. Over the years clonality testing has moved from Southern blot to PCR-based methods. Both these methods employ electrophoresis to fractionate products in order to discriminate between background products and bands that are associated with the presence of clonal populations. However, these methods often do not provide sufficient diagnostic sensitivity and are incapable of identifying the specific V-J DNA sequence data required to track clones in follow up testing. The recent emergence of cost-effective massively parallel sequencing (MPS) platforms and development of targeted assays with associated bioinformatics tools have resulted in powerful new approaches for clonality detection and monitoring.
Aims: To develop a sensitive, robust and reliable, massively parallel sequencing assay, along with associated bioinformatics tools useful for detection and monitoring *TCRG* clonality.
Methods: A single-step PCR targeted all variable (V) and joining (J) region genes of *TCRG* that are rearranged in lymphoid malignancies. The average size of the *TCRG* amplicons generated is compatible with testing fragmented DNA isolated from more challenging samples (*e.g.*, FFPE sections). Multiplexed PCR was followed by amplicon purification using the AMPureXP PCR purification system. Purified amplicons were quantified with KAPA qPCR and pooled into a library. A measured amount of the quantified library was loaded onto the MiSeq massively parallel sequencing system and data was generated using the MiSeq v2 Reagent kit (300 cycles). Data was analyzed using Invivoscribe developed bioinformatics software, which generated frequency distributions, identified V-J DNA sequences, and V-J gene usage.
Results: Data generated with this assay and bioinformatics package identify clonality and DNA sequences of *TCRG* V-J gene rearrangements. This *TCRG* assay has a limit of detection of 5 templates in 10^5 and shows a linear response from 5% down to 0.005%. This assay can be used with genomic DNA from peripheral blood, bone marrow, FFPE, and tissue biopsy specimens. The results obtained compare favorably with those from 454 sequencing and are concordant with clinical diagnosis (Figure 1).



	%IV50022	Total Reads	Vg4-Jg1/2 Length	Vg4-Jg1/2 Reads	Vg4-Jg1/2 %	Vg10-Jg1/2 Length	Vg10-Jg1/2 Reads	Vg10-Jg1/2 %	Vg10-JgP1 Length	Vg10-JgP1 Reads	Vg10-JgP1 %
index1	5	422861	113	27461	6.49	147	24471	5.79	119	2745	0.65
index2	0.5	535086	113	3334	0.62	147	2899	0.54	119	4418	0.83
index3	0.5	477367	113	3264	0.68	147	2969	0.62	119	3899	0.82
index4	0.05	528698	113	348	0.07	147	309	0.06	119	4386	0.83
index5	0.05	521526	113	519	0.1	147	376	0.07	119	4291	0.82
index6	0.05	510438	113	470	0.09	147	355	0.07	119	3585	0.7
index7	0.005	533798	113	35	0.01	147	48	0.01	119	4576	0.86
index8	0.005	560170	113	35	0.01	147	32	0.01	119	4701	0.84
index9	0.005	528563	113	57	0.01	147	33	0.01	119	4019	0.76
index10	tonsil	549100	113	7	0	147	7	0	119	5204	0.95

Figure 1.

Summary and Conclusions: A massively parallel sequencing assay has been developed for the Illumina MiSeq platform that identifies clonal *TCRG* V-J rearrangements, associated patient-specific V-J region DNA sequences, and provides frequency distribution of V region and J region segment utilization. This assay can be used both to detect and monitor lymphoproliferative disease. When coupled with the bioinformatics and visualization software MPS assays can provide robust detection and enhanced data-rich outputs.

P953

MAPPING OF UNIQUE CHROMOSOMAL ABNORMALITIES AS A TOOL FOR SENSITIVE MINIMAL RESIDUAL DISEASE ASSESSMENT IN ACUTE LEUKEMIA PATIENTS

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Background: Acute leukemias (AL) comprise a heterogeneous group of hema-

tologic malignancies, and individual patient responses to treatment can be difficult to predict. Monitoring of minimal residual disease (MRD) is thus very important and holds great potential for improving treatment strategies. Common MRD targets include recurrent cytogenetic abnormalities and mutations in important hematological genes; unfortunately well-characterized targets are lacking in many AL patients.

Aims: Our aim was to develop a flexible strategy for mapping of cytogenetically identified unique clone-specific abnormalities down to the single nucleotide level and, based on the sequence, design a specific real-time PCR assay for MRD assessment in AL patients without any previously described MRD marker.

Methods: Using a combination of molecular cytogenetic techniques (mFISH, mBAND) and molecular biological techniques (next-generation sequencing, long-range PCR, Sanger sequencing) we were able to characterize the DNA sequence flanking unique chromosomal breakpoints. For precise identification of these breakpoints we used fine-needle microdissection of derivative chromosomes followed by next-generation sequencing of the dissected material. Finally, we designed a specific real-time PCR assays for monitoring MRD level during the patients' treatment. To test the applicability of the described approach, a real-time PCR assay based on a unique breakpoint from one patient was compared to quantification based on a well-characterized MRD target present in the same patient.

Results: In conjunction with our previous proof of principle study on the K562 cell line presented at the EHA meeting in 2012, we mapped derivative chromosomes in 4 eligible AL patients [Patient 1–der(4), der(11); patient 2–der(10), der(11); patient 3–der(8); patient 4–der(7)] and performed real-time PCR quantification of unique MRD markers for each patient. A comparison of these newly-designed assays to a standard assay used in clinical practice shows that our technical approach is suitable for the identification of new molecular MRD markers in AL patients (Figure 1).

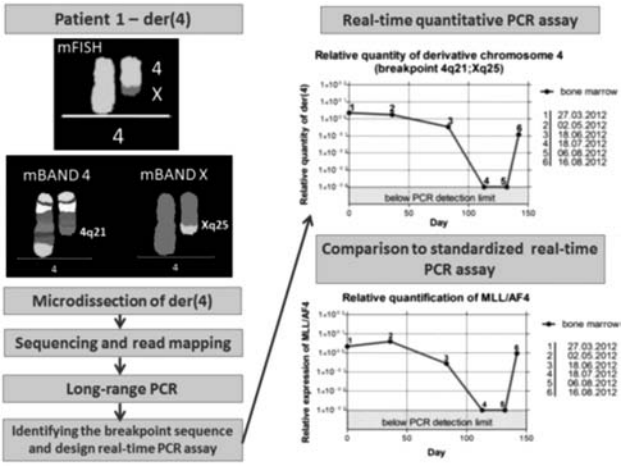

Figure 1.

Summary and Conclusions: The combination of cytogenetic and molecular methods described here enabled us to proceed from the chromosomal level (cytogenetically identified unique marker) to the molecular level (unique DNA sequence). Using this procedure, many chromosomal translocations can be used to identify a target for detecting and quantifying MRD.Our work clearly shows that moving from the chromosomal level to the nucleotide level is feasible and readily applicable for eligible AL patients. The time required for the entire procedure under reasonable standard conditions, from receiving the diagnostic sample to developing the real-time MRD assay, is approximately up to six weeks, allowing its use in clinical practice as a tool for personalized medicine in hemato-oncology.

P954

A MULTIPLEX QPCR ASSAY FOR RAPID DETECTION OF DNMT3A CODON R882 MUTATIONS IN ACUTE MYELOID LEUKEMIA

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Background: Mutations in the *DNMT3A* gene, which encodes DNA methyltransferase 3A, have been reported in approximately 20% of cases with AML and in 8% of cases with MDS. *DNMT3A* mutation is proven to be an independent adverse factor for survival in AML and of possible prognostic value in MDS. A single study has shown improved survival in AML patients with mutated *DNMT3A* treated with high-dose daunorubicin compared with standard-dose daunorubicin. Another study suggests that AML patients with *DNMT3A* codon R882 mutations respond better to treatment with idarubicin compared to standard dose daunorubicin. Mutations in codon 882, substituting Arginine with either Histidine, Cysteine, Proline, Serine or Glycine account for approximately 60% of identified *DNMT3A* mutations in AML.

Aims: To design and validate a multiplex allele-specific qPCR assay targeting the five known codon 882 mutations in *DNMT3A* for rapid detection of these aberrations in AML patients.

Methods: Allele-specific qPCR assays based on TaqMan technology were developed. Forward primers specific for wild type and the five codon 882 mutations were designed and combined with a common reverse primer and a common probe. The five mutation-specific assays were combined in a multiplex assay. The sensitivity of the multiplex assay was 5% of mutant alleles. Cell lines and plasmids encompassing the different codon R882 mutations were used as positive controls. Diagnostic samples from 207 AML patients were analysed using the multiplex qPCR assay.

Results: Twenty-eight of 207 AML patients (13.5%) were found to carry a codon R882 mutation in *DNMT3A* at the time of diagnosis. To identify the specific codon R882 mutations in the individual patients we split out the multiplex assay and performed qPCR for each of the possible *DNMT3A* R882 mutations. Nineteen patients were found to carry the R882H mutation, eight were positive for the R882C mutation, and a single patient was R882P positive. No patient samples with the R882S or R882G mutations were found. All 207 AML patients were previously screened for mutations in additional six genes (Table 1). The *DNMT3A* codon R882 mutation rarely seemed to occur alone. Among the 28 patients with a R882 mutation, 22 patients had concomitant mutations in one or more genes. In line with previous reports by others, statistically significant associations between *DNMT3A and NPM1, IDH1, FLT3-ITD,* and *FLT3-D835* mutations were observed. Eight *DNMT3A* R882 mutated patients had concomitant mutations in both *FLT3-ITD* and *NPM1*. In our cohort of AML patients, we observed no differences in median age and distribution of sex between patients with and without a *DNMT3A* R882 mutation.

Table 1.

Variable	Whole cohort (n = 207)	DNMT3A R882-mutated (n = 28, 13.5%)	DNMT3A R882-wildtype (n = 179, 86.5%)	
Sex, n				
Male	109	16	93	
Female	98	12	86	
Median age, y (range)	64 (19-95)	63.5 (46-95)	64 (19-90)	
No. of mutations, n (%)				
0	98 (47.3)	-	98 (54.7)	
1	53 (25.6)	6 (21.4)	47 (26.3)	
2	30 (14.5)	3 (10.7)	27 (15.1)	
3	23 (11.1)	16 (57.1)	7 (3.9)	
4	3 (1.4)	3 (10.7)	0 (0)	
Mutations, n (%)				
FLT3-ITD	45 (21.7)	11 (39.3)	34 (19)	p<0.05
FLT3-D835	15 (7.2)	5 (17.9)	10 (5.6)	p<0.05
WT1	8 (3.9)	0 (0)	8 (4.5)	ns
CEBPA	14 (6.8)	2 (7.1)	12 (6.7)	ns
NPM1	63 (30.4)	17 (60.7)	46 (25.7)	p<0.001
IDH1	19 (9.2)	8 (28.6)	11 (6.2)	p=0.001
c-kit	2 (1)	1 (3.6)	1 (0.6)	ns

Summary and Conclusions: A specific multiplex assay covering 60% of *DNMT3A* mutations observed in AML was developed. In a retrospective study 207 AML samples were analysed and 28 (13.5%) were found to be mutated in codon R882 in *DNMT3A*. Our observations correspond to previous reports on mutations in codon 882 in *DNMT3A* both with respect to frequency as well as distribution of the different R882 mutations. The observation in two previous studies showing that patients with *DNMT3A* mutations benefit from non-standard induction chemotherapy could increase the demand for a *DNMT3A* mutation screening prior to therapy. The multiplex assay presented here, enables rapid detection of codon R882 mutations in *DNMT3A* within 3 hours after sample receipt in the laboratory. qPCR assays have been proven to be fast and reliable methods that can easily be implemented in diagnostic laboratory settings, thus, the present multiplex assay holds great potential as an easily applicable methodology for the detection of codon R882 mutations.

P955

ARRAY-BASED GENOMIC PROFILING IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) IDENTIFIES HIGHER NUMBER OF PROGNOSTIC RELEVANT GENOMIC ALTERATIONS AND REVEALS NEW RECURRENT GENOMIC ABNORMALITIES

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Background: B-cell chronic lymphocytic leukemia (CLL) is characterized by a highly variable clinical course. Characteristic genomic abnormalities have been shown to provide clinically important prognostic information. The most commonly applied techniques in genetic diagnostics are fluorescence *in situ* hybridization (FISH) and multiplex ligation-dependent probe amplification (MLPA). Recently,

array-based genomic profiling has gained acceptance as a high-resolution new tool for detecting genomic imbalances, which allows genome-wide detection of copy number alterations (CNAs), down to 100 kb in size, and regions of acquired uniparental disomy (UPD).
Aims: The aim of the present study was to compare two genomic array platforms with FISH and MLPA to ascertain whether these routinely used techniques could be substituted by genomic arrays in a diagnostic setting.
Methods: Genomic DNA was extracted from peripheral blood cells of 21 CLL patients. Data on genomic aberrations {del(11q), +12, del(13q) and del(17p)} were obtained by FISH, MLPA or both. To evaluate the sensitivity and specificity of genomic arrays our cohort contained 6 CLL patients in which the genomic abnormality was present in a relatively low percentage of the cells (range 5-21%), and one patient with a focal *TP53* deletion which was missed by FISH. Two different array platforms were used; CytoScan HD Array (Affymetrix) and HumanOmniExpress12v1.0 (Illumina). The following interpretation criteria were applied: (i) the threshold for copy number aberrations was set at >5 Mb, (ii) inclusion of copy number aberrations of segments that coincide with known cancer genes as reported on www.sanger.ac.uk or in literature and that are smaller than 5 Mb and (iii) the threshold for acquired UPD was set at >10 Mb and to telomere.
Results: In 18 of the 21 CLL patients genomic alterations were identified by array-based profiling and FISH or MLPA. In 10 patients array-based profiling was performed on both array platforms, resulting in the identification of the same genomic alterations. All abnormalities observed by FISH were also identified by array-based profiling, including two cases with trisomy12 in 21% of the cells, a case with loss of 17p in 20% of the cells, case with loss of 17p in 16% of the cells, a case with loss of 13q14 in 20% of the cells, and a case with a bi-allelic loss of 13q14 in 5% of the cells. In addition array-based profiling identified a case with focal *TP53* loss which remained undetected by FISH, and a case with an acquired UPD of the short arm of chromosome 17. In this latter case a *TP53* mutation was subsequently established by DNA sequencing. Moreover 14 of the 21 patients (67%) carried additional genetic abnormalities as compared to FISH and/or MLPA, including one patient with the recently identified *MGA* loss and one other patient with chromotripsis.
Summary and Conclusions: Both array platforms have a high sensitivity and CNA present in only about 20% of the cells as determined by FISH and/or MLPA can unambiguously be identified. By applying similar interpretation criteria results obtained from different array platforms are comparable. In addition, we show that microarray-based genomic profiling provides the detection of potential prognostic relevant abnormalities (focal *TP53* deletion, UPD of 17p), novel focal CNAs and acquired UPDs, which would have remained undetected by FISH or MLPA. The prognostic value of novel CNAs and acquired UPDs needs further evaluation in prospective clinical trials.

P956

DEREGULATION OF STEM CELL FACTOR (SCF)-AKT-S6 PATHWAY IN DIAGNOSTIC AML SAMPLES IS ASSOCIATED WITH DISEASE-FREE SURVIVAL: RESULTS OF A VERIFICATION STUDY

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Background: Despite improvements in AML induction therapy, the risk of relapse, especially in older patients (pts), remains high. In addition to older age and high risk karyotype, molecular markers such as FMS-like tyrosine kinase 3 receptor (*FLT3R*) internal tandem duplication (ITD) mutations (*FLT3R* ITD) have been shown to be associated with risk of relapse in pts with AML. However, even within molecularly defined high risk groups substantial heterogeneity in clinical outcomes are observed, suggesting that alternative mechanisms of disease relapse exist and that diagnostic tools to predict disease relapse remain inexact. Single cell network profiling (SCNP) uses multiparameter flow cytometry as a biological tool to conduct a broad functional assessment of intracellular signaling pathways in heterogeneous tissues at the single cell level. We previously identified an SCNP functional signature, SCF-induced phosphorylation of AKT and S6 [SCF:P-AKT/P-S6] that, when present in a subset of AML blasts at diagnosis, was predictive of disease relapse. The SCNP signature demonstrated significant predictive ability when tested in a small independent sample set.
Aims: To verify in a larger sample set the external validity of the original observation and its independence from *FLT3R* mutation status (FMS).
Methods: SCNP was performed on cryopreserved bone marrow mononuclear cell (BMMC) and peripheral blood mononuclear cell (PBMC) samples from 80 pts >55 years of age with non-APL AML who had complete response to cytarabine-based induction therapy on SWOG trials S0112, S0301, S9031, S9333, who were followed for at least three years, and whose leukemic blast cells expressed CD117 (c-KIT). Thawed cells were exposed to modulators, including SCF, for 15 minutes then fixed, permeabilized, and stained using a cocktail of fluorochrome-conjugated antibodies that recognized extracellular lineage markers and intracellular epitopes which correspond to phosphorylated sites on signaling proteins, including AKT and S6.
Results: An association between the SCF:P-AKT/P-S6 signature and relapse-free survival at 3 years (RFS3) was confirmed in this study with an Area Under the Receiver Operating Characteristic Curve (AUROC) of 0.70 (P=0.018). Consistent with previous data, CD117 surface expression levels alone were not predictive of relapse (AUROC=0.48, P=0.6). In addition, and in alignment with the previous observation, the majority of patient samples with a blast subpopulation at diagnosis expressing the SCF:P-AKT/P-S6 signature were FLT3R wild type. In order to assess if the SCF:pAkt/pS6 signature is a significant predictor of RFS3 after accounting for FMS, a logistic regression model of RFS3 was developed to include the SCF:pAkt/pS6 signature, FMS and the interaction between them as predictors. The model showed that the SCF:pAkt/pS6 signature is significantly (P=0.016) associated with RFS3 after accounting for FMS.
Summary and Conclusions: This study confirmed in an independent set of adult non-APL AML pts that the presence of leukemic blasts in PB or BM samples expressing SCF-induced P-AKT/P-S6 at diagnosis is predictive of relapse with 3 years. Together with FMS, this signature provides an opportunity for identifying those pts that may benefit from more intensive post-remission therapy such as stem cell transplant. These data also implicate deregulation of the PI3K pathway as a mechanism for secondary chemo-resistance in at least one subset of elderly AML that could potentially be targeted by tyrosine kinase inhibitors.

P957

HIGH-RESOLUTION GENOMIC PROFILING IN MULTIPLE MYELOMA IDENTIFIES RECURRENT PROGNOSTIC RELEVANT COPY NUMBER ALTERATIONS AND REVEALS NEW RECURRENT GENOMIC ABNORMALITIES

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Background: Multiple myeloma (MM) is a heterogeneous disease with a highly variable clinical course. Genetic abnormalities such as t(4;14)(p16;q32), t(11;14)(q13;q32), t(14;16)(q32;q23), 17p loss, 13q loss, 1q gain, 1p loss and hyperdiploidy have been shown to provide prognostic information (Fonseca *et al.*, Leukemia, 2009). The most common molecular-cytogenetic technique currently used to detect these genetic abnormalities is interphase fluorescence *in situ* hybridization (FISH) on purified plasma cells.
Aims: Although interphase FISH is a well established approach, it is relatively laborious. We, therefore, we aimed to evaluate the sensitivity and detection yield of copy number alterations (CNAs) using microarray-based genomic profiling.
Methods: CD138+ cells (plasma cells) were enriched by an immuno-magnetic cell selection procedure (Stem Cell Technologies) from bone marrow samples of 13 MM patients. Microarray-based genomic profiling and data interpretation were performed as recently reported (Simons *et al.*, Genes Chrom & Cancer, 2011). Interphase FISH on enriched plasma cells was performed using commercially available probes according to the manufacturer's specifications (Abbott Molecular for 13q-, 17P- and hyperdiploidy, and Kreatech Diagnostics for 1q gain and 1p loss).
Results: The microarray-based genomic profiles of all 13 samples disclosed highly rearranged genomes, harboring 3 to 22 distinct CNAs or regions exhibiting acquired uniparental disomy (UPD), both recurrent as well as novel in nature. All recurrent CNAs present in at least 20% of the cells, as determined by FISH, were also detected by microarray-based genomic profiling. In only one patient a minor sub-clone with loss of 17p, as detected by FISH in 13% of tetraploid cells was not identified by microarray analysis. Of interest is that by using interphase FISH a (near)-tetraploid karyotype can easily be misinterpreted as a (high) hyperdiploid Karyotype, while, based on the microarray profiles, a discrimination between these two karyotypes is evident. This notion is of prognostic relevance, especially in cases with loss of 17p. In one patient we also observed a specific form of genomic instability, fulfilling the definition of chromothripsis (Stephens *et al.*, Cell, 2011). Although we performed our microarray-based genomic profiling assays on a relatively small number of patients, recurrent CNAs such as gains of 1q and of chromosomes2,3,5,7,9,11,15, 19, and 21, and losses of 1p and of chromosomes13,14, and 22, and acquired UPDs were readily identified. So far, only limited microarray-based genomic profiling data on enriched plasma cells from patients with MM or MGUS have been reported in the literature, however a remarkable consensus is apparent between these data and our current data. A particular novel finding in the present study is the recurrent occurrence of an acquired UPD of chromosome 12. Clearly, additional data are required to establish the clinical relevance of these observations.
Summary and Conclusions: Our results indicate that microarray-based genomic profiling exhibits a high sensitivity and specificity in identifying MM-associated numerical abnormalities. Although balanced translocations cannot be identified this way, CNAs can be easily identified. In addition, we show that microarray-based genomic profiling allows the detection of novel focal CNAs and acquired UPDs in MM-patients. The prognostic significance of these novel CNAs and acquired UPDs have to be evaluated in prospective clinical trials.

P958

SEMI-AUTOMATIC ULTRA RAPID DETECTION OF THE PML-RARA FUSION TRANSCRIPTS BY RETRO-TRANSCRIPTION LOOP MEDIATED AMPLIFICATION (RT-LAMP) REACTION ON THE LIAISON IAM INSTRUMENT

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Background: After the initial morphologic evaluation the accurate and timely molecular identification of the Acute Promyelocitic Leukemia (APL) associated PML-RARa fusion gene is mandatory to start an appropriate treatment based on all-trans retinoic acid and to reduce the risk of potentially fatal hemorrhagic complications.
Aims: To improve the molecular diagnosis of Acute Promyelocitic Leukemia by introducing an ultra rapid screening test, easy to be performed even in not specialized laboratories, that allows rapid initiation of therapy.
Methods: RT-LAMP is a novel, isothermal, non-PCR method for direct RNA amplification. We applied this technique to the identification of the PML-RARa transcripts in Acute Promyelocytic Leukemia (APL). The system consists in two fluorescent multiplex assays, one specific for the most frequent transcripts (bcr1 and bcr3) and one for the more rare bcr2 starting from 500 and 300ng of total RNA, respectively. To control the extraction procedure, RNA integrity, reaction functionality and absence of inhibitors, both the assays also detect the endogenous GUSb housekeeping RNA as internal control. The reaction is performed in less than 40 minutes into the Liaison IAM instrument (DiaSorin) at constant temperature with a real-time monitoring of fluorescence intensity that decreases proportionally with the amplification progress. The data obtained are directly elaborated by the instrument, that returns results in terms of "positive", "negative" or "Invalid" result.
Results: The analytical sensitivity of the triplex (bcr1-bcr3-GUSb) and duplex (bcr2-GUSb) RT-LAMP assays has been determined on serial dilutions of RNA from the APL derived NB-4 cell line (for bcr1 transcript) or from patients at diagnosis (for bcr2 and bcr3 transcripts) into wild type RNA (from HL-60 cell line). The triplex (bcr1-bcr3-GUSb) assay showed a level of sensitivity of 10^{-3}on both the transcripts, while the duplex (bcr2-GUSb) assay showed a detection limit of 10^{-2} within 37 minutes. The analytical specificity has been established on wild type RNA from several cell lines (HL-60, REH, RS4-11, Kasumi, MV-4, 697, K-562 and TOM-1) for a total of 234 replicates. In all replicates exclusively the GUSb internal control RNA was amplified, demonstrating 100% specificity. The RT-LAMP assays have been finally validated on 96 clinical samples previously analyzed by conventional RT-PCR. Full concordance has also been obtained on negative samples (n=62) and on positive samples at diagnosis (n=34) in which the PML-RARa fluorescent signal was already visible in less than 10 minutes. Moreover, samples were correctly identified as positive for bcr1, bcr2 or bcr3 transcript. This information is required for the next phase of molecular monitoring during follow-up. Interestingly, this assay was also able to amplify a bcr1 transcript variant in which a deletion of PML exon 5 was present.
Summary and Conclusions: The fluorescent RT-LAMP assays for PML-RARa fusion transcripts detection is highly specific, sensitive and rapid. The isothermal single-step format, starting from patients RNA and monitorable in real-time in the Liaison IAM instrument, simplifies the entire reaction set-up. Furthermore, this improvement makes the assay cost-effective and applicable in not highly specialized laboratories. The RT-LAMP could shorter the time to diagnosis for patient affected by APL thus reducing risk of hemorrhagic complications by an early treatment administration.

P959

IMPORTANCE OF MICROARRAY ANALYSIS AS A PROGNOSTIC CLINICAL TOOL IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is a B-cell malignancy with a highly variable clinical course. In addition to the mutational status of the immunoglobulin heavy chain, recurrent genomic abnormalities are among the strongest prognostic markers. Specific aberrations of prognostic significance are usually detected by interphase fluorescence *in situ* hybridization (iFISH). Array-based genomic technologies allow genome-wide screening for genomic alterations. They have proved to be effective in the detection of copy number abnormalities (CNA) and copy-neutral loss of heterozygosity (CN-LOH) and have consequently enabled the identification of new recurrent abnormalities of clinical relevance.
Aims: The aim of this study was to assess the usefulness of array based comparative genomic hybridization (CGH)+single nucleotide polymorphism (SNP) array as an alternative to iFISH for the detection of genomic abnormalities in routine clinical use.
Methods: Peripheral blood samples of 100 CLL patients were analysed both by iFISH and CGH+SNP arrays. The panel used for iFISH analysis included del(13)(q14)(DLEU1, DLEU2), del(11)(q22)(ATM), del(17)(p13)(TP53) and trisomy 12. For array analysis a 180K custom-designed cancer focus array was used from Oxford Gene Technology (OGT). The array was designed for the detection of single exon abnormalities in 17 selected genes (average coverage 1probe/100bp) as well as the simultaneous identification of copy neutral loss of heterozygosity (CNLOH by adding 36000 SNP probes). In addition, the array covers 1500 cancer-associated genes with an average resolution of 2kb, whilst retaining an average backbone resolution of 50kb.
Results: Genomic abnormalities were detected by iFISH in 74 patients and by array in 77 patients. A high degree of concordance was observed (96%) between both methods. A discrepancy was observed in four patients. Low proportions of abnormal cells (<10%) were detected only by iFISH in three patients and, in one patient, a small 13q deletion comprising only the locus DLEU2 was detected solely by array. Furthermore, by array, additional acquired genomic abnormalities not included in the FISH panel were detected in 67 patients. Some were recurrent abnormalities such as loss of 1q, 8p, 10q, 12p, 18p and gain of 2p, 4q, 8q which appear to be of prognostic significance. Large stretches >10 Mb of CNLOH were detected in 13% of the cases and accurate size mapping of clinically relevant genomic markers was obtained in 67.5% of abnormal cases by array analysis. This is particularly important in the case of deletions within 13q14, since larger deleted regions which also include loss of RB1 gene (type II) confer a less favourable prognosis than smaller deletions which comprise loss of the loci DLEU1 and/or DLEU2 only (type I). Overall, array analysis provided additional clinical relevant genomic data in 87% of abnormal cases. In addition, allowing the precise identification of breakpoints, aCGH can determine deleted regions of prognostic significance and identify candidate genes implicated in CLL of potential clinical relevance (Table 1).

Table 1.

			CGH + SNP	FISH
Patients with abnormalities			77	74
Abnormalities (locus FISH)	del(13q) Type I	DLEU1/DLEU2	27	51
		DLEU2	1	-
	del(13q) Type II	RB1/DLEU1/DLEU2	22	-
	del(11q) ATM		12	12
	+12		20	21
	del(17p) TP53		10	10
	others		1	1
	Total		93	95
Patients with additionnal abnormalities			67	-
False negativ	del(13q) Type I	DLEU1/DLEU2	-	2
		DLEU2	1	-
	+12		-	1
	Total		1	3

Summary and Conclusions: CGH+SNP array analysis is a robust, high resolution and sensitive screening method for routine clinical use in CLL and is able to reliably detect abnormalities only present in 10% of the cells. Array analysis shows clearly a higher diagnostic yield than iFISH.

P960

DEVELOPMENT OF BIOINFORMATICS SOFTWARE FOR AUTOMATED ANALYSIS AND VISUALIZATION OF DATA FROM AN MPS TCRG ASSAY

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Background: The commercial availability of massively parallel sequencing (MPS) technology has generated staggering amounts of DNA sequence data from a multitude of applications. Efficient, timely handling and analysis of MPS data is a significant challenge and calls for the development of specialized software. The need for processing software is a critical requirement for the development of MPS assays designed to identify and monitor clonal rearrangements of the antigen receptor loci.
Aims: The specific goal of this work was to develop software for the processing of tens of millions of data sequences generated using the LymphoTrack™ DeepSeq™ TCRG Assay. Software output had to provide an accurate representation of the DNA sequence frequency distribution for the detection and monitoring of *TCRG* gene rearrangements and to provide a visual representation of the resulting analysis to aid in interpretation and tracking.
Methods: The bioinformatics data analysis software package uses Invivoscribe's proprietary primer sequence weight matrix and nucleotide sequence database, as input to the NCBI Nucleotide BLAST program, to analyze LymphoTrack™ DeepSeq™ TCRG Assay data generated from MiSeq™ (in fastq format). The analysis identifies sequence frequency distribution, V-J DNA

sequences, and V-J gene usage. The visualization component uses Microsoft Excel to present the resulting data analysis in a visual format, including a histogram of sequence frequencies.
Results: Analyzed data confirms the DNA sequences from clonal cell lines match published sequences. Good linearity of sequence frequencies was obtained in serial diluted clonal cell lines. Visualization provided an accurate representation of the resulting data. Software was appropriately validated to quantify the sequence frequencies and corresponding identities (Figure 1).

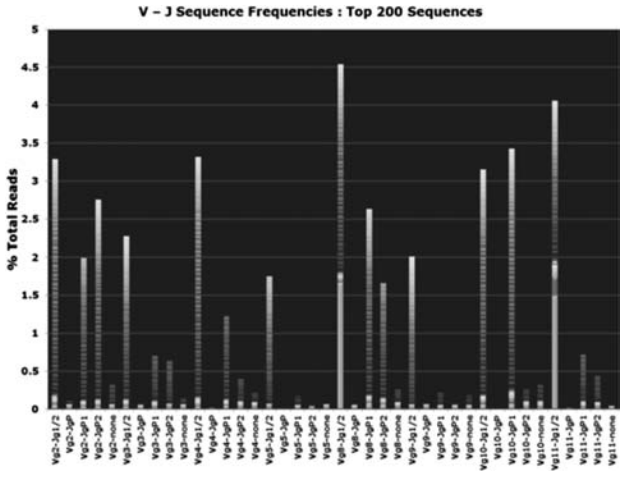

Figure 1.

Summary and Conclusions: This commercially available software package provides fast reliable analysis and visualization output that aids in the interpretation of data generated from this *TCRG* assay.

P961

CHARACTERISTICS OF VARIANT AND ADDITIONAL CYTOGENETIC ABNORMALITIES IN ACUTE MYELOID LEUKEMIA WITH RECURRENT GENETIC ABNORMALITIES
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Background: Acute myeloid leukemias harboring the prognostically favorable t(8;21)(q22;q22), inv(16)(p13q22)/t(16;16)(p13;q22), t(15;17)(q22;q21), and various translocations involving 11q23 form a separate category. Accurate identification of variants of these recurrent genetic abnormalties is important for diagnosis and appropriate treatment. Prognostic aspects of additional cytogenetic abnormalities present are still controversial.
Aims: To investigate the frequencies and patterns of variant and additional cytogenetic abnormalities, the cytogenetic profiles at the initial diagnosis of a total 245 acute myeloid leukemia patients with recurrent genetic abnormalities during the period from 2004 to 2012 referred to Green cross laboratories were analyzed.
Methods: In all, 64 patients with t(8;21)(q22;q22), 104 patients with t(15;17)(q22;q12), 36 patients with inv(16)(p13q22) (n=30) or t(16;16)(p13;q22) (n=6), and 41 patients with 11q23 translocations were investigated for variant translocations and secondary karyotypic abnormalities. To confirm the rearrangement, fluorescence *in situ* hybridization (FISH) and polymerase chain reaction (PCR) tests were performed.
Results: Variant translocations of t(8;21), t(15;17), inv(16)/t(16;16), and 11q23 were found in 6 (9%), 4 (4%), 2 (6%), and 0 (0%) patients, respectively. For variants of t(8;21), 5 patients had three-way translocation involving 4p16, 4q21, 12p12, 19p13.3, and 22p13. One patient had four-way translocation: t(1;21;8;4)(p13;q22;q22;q12). Of the 64 patients, 31 (48%) had additional sex chromosome losses. For variant of t(15;17), 3 patients had three-way translocation, including 5q31 and 6q12, and 1 patient had four-way translocation: t(5;9;17;15)(q31;q13.1;q21;q22). Interestingly, 3 patients showed translocation at the same locus (5q31). 41% of patients had additional chromosomal abnormalities. 9%, 6% and 3% of patients had ider(17)(q10)t(15;17), trisomy 8 and der(7)t(7;8)(q31.1;q21.1), respectively. For variant of inv(16)/t(16;16), 1 patient (4%) had t(16;16;17)(p13;q22;q11.2) and another patient had der(16)inv(16)(p13q22)t(X;16)(p11.2;q22). Trisomy 22 was the most common additional abnormality in inv(16)/t(16;16) and was found in 6 patients (17%). Partners of MLL translocation were 9p22(19), 19p13.1(11), 19p13.3(2), 1p32(2), 3p21(1), 6p27(1), 10p12(1), 10q22(1), 16p13.3(1), 17q11.2(1) and 17q25(1). Trisomy 8 was the most frequent additional chromosomal abnormality with MLL translocation and was found in 7 cases (17%).

Summary and Conclusions: Variants of t(8;21), t(15;17), and inv(16)/t(16;16) were not rare. Therefore, application of FISH and PCR tests was essential for the detection of variant translocations. To the best of our knowledge, all variants, except 2 cases, were novel translocations. Considering 3 cases with 5q31 involvement in t(15;17), 5q31 might possibly be a nonrandom breakpoint in acute promyelocytic leukemia, and the gene involved in 5q31 should be investigated.

P962

MUTATIONAL STUDY OF DNMT3A IN ACUTE MYELOID LEUKEMIA
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Background: The *DNMT3A* gene mutation was identified as a recurrent somatic mutation in acute myeloid leukemia (AML) and suggested to be a poor prognostic marker. As DNMT3A is the key enzyme in DNA methylation, its mutation would have a role in epigenetic and biological alterations in leukemia.
Aims: The present study was performed to investigate this gene mutation's biological impact on gene expression.
Methods: Sequence analysis of exons 9 and 23 encoding the proline-tryptophan-tryptophan-proline (PWWP) domain and catalytic domain of DNMT3A, respectively, was conducted in 29 cytogenetically normal (CN) AML samples. Constructs of wild-type *DNMT3A* and three recurrent mutants (R882H, R882C, and A368D) by site-directed mutagenesis were transfected into Hek293 cells. Western blotting analysis and direct immunofluorescence microscopy of the transfected cells were performed to determine the recombinant protein expression and localization. Gene expression profiles were determined by microarray analysis of transfected cells.
Results: *DNMT3A* mutations were found in 6 of 27 acute leukemia samples. R882C (c.2644C>T) and R882H (c.2645G>A) mutations in exon 23 encoding the catalytic domain of DNMT3 were detected. Recombinant wild-type, R882H, and R882C DNMT3A proteins were identified at higher levels in nuclear extracts than in the cytoplasm and found to show nuclear localization. However, the DNMT3A A368D mutant protein was detected only in cytoplasmic extracts by immunoblotting and was also localized to the cytoplasm as determined by fluorescence microscopy. Microarray analysis indicated that genes under regulation by CEBPA were downregulated and those under regulation by MEISA1A and HOXA9 were upregulated compared to the wild type and the R882H mutant, and *TP53TG3* was highly downregulated in both the R882H and R882C mutants (Figure 1).

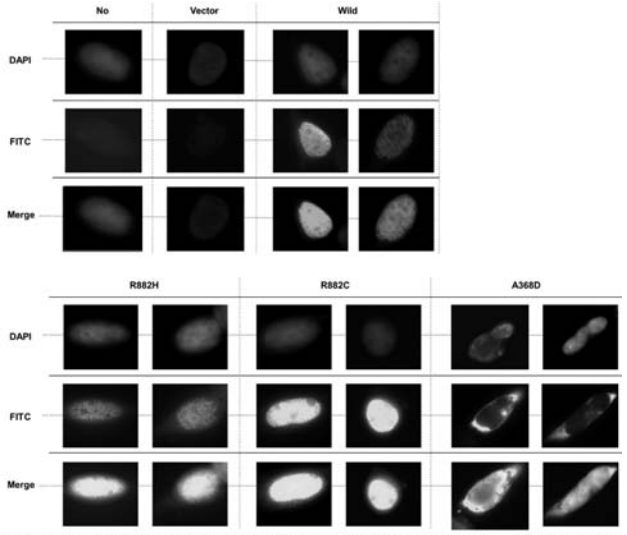

Figure 1. Localization of DNMT3A mutants in Hek293 cells.

Summary and Conclusions: The recurrent *DNMT3A* mutation at the R882 site previously implicated as a prognostic marker in CN-AML was also found in Korean leukemic samples. In the PWWP domain A368D mutant, loss of localization may result in modification of the gene expression profile. The effects of R882H and R882C mutations in the catalytic domain of DNTM3A on CEBPA transcriptional activation and the *TP53TG3*-associated pathways should be examined in future studies.

P963

RAPID AND RELIABLE DETECTION OF BCR-ABL MAJOR AND MINOR TRANSCRIPTS BY THE ONE-STEP, ISOTHERMAL RT-LOOP MEDIATED AMPLIFICATION REACTION MONITORABLE IN REAL TIME ON THE LIAISON IAM INSTRUMENT

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Background: The molecular identification of the BCR-ABL transcripts in patients affected by chronic myeloid leukemia or acute lymphoblastic leukemia is routinely based on conventional RT-PCR, a multistep procedure that takes few hours to identify the translocation and define the breakpoints
Aims: to allow a rapid and simultaneous molecular detection of major and minor BCR-ABL transcripts by using the one-step RT-LAMP and the semi-automated Liaison IAM instrument (DiaSorin)
Methods: RT-LAMP is an isothermal, non-PCR method which amplifies targets directly form RNA, thanks to the employment of a DNA polymerase with reverse transcription activity. This multiplex, close format, fluorescent assay, allows to simultaneously detect and distinguish the Minor (p190) and Major (p210) BCR-ABL transcripts and the internal control Gusb. Fluorescence variation derived from targets amplification are collected through different channels in real time by the Liaison IAM instrument (DiaSorin) that automatically analyses data releasing final result
Results: the triplex BCR-ABL RT-LAMP has been initially tested on RNA samples extracted from cell lines. Serial dilutions of RNA derived from TOM-1 or K-562 cell lines (p190 and p210 positive, respectively) into wild type RNA from HL-60 cell line have been tested for sensitivity assessment. The p190 and p210 transcripts have been detected and distinguished down to 10^{-4} and 10^{-5}respectively within 50 minutes. To establish the specificity of the assay, 70 replicates of wild type RNA from 7 cell lines have been tested and all resulted BCR-ABL negative in the presence of the correct amplification of the GUSb internal control (100% specificity). The BCR-ABL RT-LAMP assay was validated on 65 clinical samples previously proved positive by conventional RT-PCR (35 for p210, 30 for p190 transcript) and fully concordant results have been obtained. Furthermore, thanks to the real time monitoring on the Liaison IAM instruments, the amplification of positive samples has been already detectable after 15-20 minutes of reaction. Specificity was finally confirmed on 60 negative RNA samples (30 healthy donors and 30 Ph negative patients) all resulted BCR-ABL negative as expected
Summary and Conclusions: the triplex p190-p210-GUSb RT-LAMP performed on the Liaison IAM instrument represents an efficient system for simultaneous detection of both major and minor BCR-ABL transcripts starting directly from patients RNA. This assay simplifies the procedure and the close format reduces the contamination risks of conventional multi step RT-PCR. Furthermore, the high sensitivity, specificity and rapidity significantly enhance the patient management and improve the diagnostic laboratory routine.

P964

SCREENING METHOD TO DETECT MOST PREVALENT MUTATIONS IN GAUCHER DISEASE

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Background: Sequencing approaches are commonly used to identify mutations in rare inherited disorders, *e.g.* Gaucher disease. Over 300 different mutations are described so far, four of which - N370S, L444P, 84GG, IVS2+1G>A- are the most common and represent up to 90% of the mutant alleles. To screen populations not yet characterized for incidence of Gaucher disease PCR methods to identify 4 most prelevant mutationt could be more effective.
Aims: To elaborate approach suitable for high throughout detection of common Gaucher mutations.
Methods: We designed allele-specific (AS) primers to discriminate normal alleles from mutant and functional gene from pseudogene for multiplex real-time polymerase chain reaction (Taqman). AS primers were chosen to anneal directly to the proposed mutation site, regardless to the homology with preudogene. Second nucleotide from the 3 '- end of each AS primer was intentionally changed to mismatched (G to C, A to T and vice versa) to increase the specificity of the reaction. To avoid pseudogene amplification reverse (opposite to AS) primers were chosen from the regions showing dissimilarity between gene and pseudogene. These dissimilarities (one nucleotide or more) are mismatched with the 3'-end of chosen reverse primers.Thus, the sequences of all primers, taken together, provide an opportunity to analyze the mutations located only in the functional gene but not in the pseudogene.
Results: We have tested DNA of 100 patients with Gaucher disease type I and 80 DNA of control group (non-Gaucher patients) for the presence of N370S, L444P, 84GG and IVS2+1G>A mutations. Mutation N370S was detected in 93% DNA of Gausher patients, L444P was found in 22%, IVS2+1–in 2%, 84GG–in 1% of patients. None of these mutations were found in DNAs from the control group. Direct sequencing of beta-glucocerebrosidase gene in 20 Gausher patients was used to verify PCR results. No mismatches between PCR and sequencing data were found.
Summary and Conclusions: We designed a set of primers for one-step multiplex real-time PCR detection of most prevalent Gaucher mutations. Elaborated protocol is adequate to detect mutations located only in the functional gene but not in the pseudogene. Proposed approach could be useful to design primers for detecting other genetic markers which require pseudogene discrimination.

Drug resistance and pharmacology

P965

EX VIVO SENSITIVITY AND RESISTANCE MECHANISMS TO CLOFARABINE IN CHILDHOOD ACUTE LEUKEMIA

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Background: Nucleoside analogues are drugs widely used in the treatment of childhood acute leukemia. Clofarabine, a nucleoside analogue of second generation looks promising in these diseases. Nonetheless, in adult leukemia, resistance mechanisms may compromise the anti-tumoral effect of nucleoside analogues and might impair the prognosis of these diseases. While second generation of nucleoside analogues has successfully entered phase I/II clinical trials in children, these mechanisms have not been fully explored in this population.
Aims: Our study aims to compare the effect of nucleoside analogues of first generation to the effect of clofarabine on primary samples of childhood acute leukemia, to test the impact of this effect on the outcome of patients and to explore mechanisms of resistance to nucleoside analogues in childhood.
Methods: 42 primary samples at diagnosis or at relapse from childhood and adolescents/young adults' acute leukemia (25 ALL and 17 AML) were studied. In accordance with the Declaration of Helsinki, informed consent was obtained from patients or their legal representatives. All samples but one were bone-marrow with 90% of median infiltration by blast-cells. Samples were tested for apoptosis after exposure to, as a reference, first generation of nucleoside analogues (aracytine and fludarabine) in parallel to clofarabine at doses range from 100µM to 1µM for aracytine and from 33µM to 0.1µM for fludarabine and clofarabine. Apoptosis was assessed by Propidium iodide/AnnexinV double-staining for the whole CD45+ blastic population. Resistance mechanisms were explored by quantification of gene transcripts (qPCR) involved in trans-membrane transport of nucleoside analogues (human Equilibrative Nucleoside Transporter 1), in their intracellular metabolism (deoxycytidine kinase, deoxyguanosine kinase and 5'-nucleotidases cN-II and cN-III) or in their intracytoplasmic target (Ribonucleotide reductase 1/2).
Results: Median age for the whole population was 10yrs [1-21yrs] and M/F sex-ratio=2.2. Median inhibitory concentration (IC) 50 was higher in ALL than in AML, 350µM *vs.* 89µM, 111µM *vs.* 69µM and 65µM *vs.* 16µM, for aracytine, fludarabine and clofarabine, respectively, but the difference was significant for aracytine only (P=0.001). Based on IC50, ALLs as well as AMLs were more sensitive to clofarabine than to aracytine (P<0.001 and=0.04, respectively) and in a lesser extent to fludarabine (non-significant). Median IC50 of each drug was used as the cutoff to define sensitivity/resistance. Among the different gene transcripts quantified only the level of deoxycytidine kinase was unexpectedly found significantly lower in ALL and AML samples sensitive to clofarabine compared to resistant ALL/AML samples. At this point, the deep meaning of this data remains elusive. Outcome of patients was analyzed with respect to the level of sensitivity to each drug. At a median follow-up of 24 months, disease-free survival was 88% and 38% (P=0.04) for AML patients sensitive or resistant to aracytine, respectively and 100% and 43% (P=0.05) for AML patients sensitive or resistant to clofarabine, respectively. Resistance to nucleoside analogues tested didn't impact the outcome of ALL patients.
Summary and Conclusions: Childhood ALLs and AMLs are more sensitive to clofarabine than to first generation analogue nucleosides. This better effect might be at least in part explained by the overcoming of known nucleoside analogue mechanisms of resistance. Low sensitivity to clofarabine unfavorably impacts the outcome in AML.

P966

KNOCKDOWN-INDUCED CELL CYCLE ARREST SENSITIZES TUMOR CELLS FROM PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA TOWARDS TRAIL-INDUCED APOPTOSIS IN VITRO AND *IN VIVO*

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Background: Resting tumor cells represent a huge challenge during anti-cancer therapy due to their increased treatment resistance. TNF-related apoptosis-inducing ligand (TRAIL) is a putative future anti-cancer drug currently in phase I and II clinical studies. We recently showed that TRAIL is able to target leukemia stem cell surrogates.
Aims: Here we tested the ability of TRAIL to target cell cycle arrested tumor cells.
Methods: Primary tumor cells from patients with acute lymphoblastic leukemia were xenografted into mice, amplified and re-isolated for experiments. Xenograft cells were lentivirally transduced for expression of luciferase allowing the use of *in vivo* imaging for follow up of the preclinical treatment trial using TRAIL. Cell cycle arrest was induced in tumor cell lines and xenografted tumor cells in G0, G1 or G2 using cytotoxic drugs, phase-specific inhibitors or RNA interference against cyclinB and E. Knockdown efficiency was tested by Western Blot.
Results: Biochemical or molecular arrest at any point of the cell cycle increased TRAIL-induced apoptosis. Accordingly, when cell cycle arrest was disabled by addition of caffeine, the anti-tumor activity of TRAIL was reduced. Most important for clinical translation, tumor cells from three children with B-precursor or T-cell acute lymphoblastic leukemia showed increased TRAIL-induced apoptosis upon knockdown of either cyclinB or cyclinE arresting the cell cycle in G2 or G1, respectively. *In vivo*, systemic treatment with TRAIL inhibited and even erradicated tumor cells from patients with acute lymphoblastic leukemia in immuno-compromised mice.
Summary and Conclusions: Taken together and in contrast to most conventional cytotoxic drugs, TRAIL exerts enhanced anti-tumor activity against cell cycle arrested tumor cells. Therefore, TRAIL might represent an interesting drug to treat static tumor disease, *e.g.*, during minimal residual disease.

P967

TRANSCRIPT LEVELS AND COPY NUMBER VARIATION OF THE ABCC3 EFFLUX TRANSPORTER CAN PREDICT CLINICAL OUTCOME IN IMATINIB-TREATED CHRONIC MYELOID LEUKAEMIA PATIENTS

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Background: Imatinib has dramatically improved survival in chronic myeloid leukaemia (CML) but disease progression may still occur in some patients. Imatinib is a substrate for the ABC efflux transporters ABCB1 (MDR1) and ABCG2 (BCRP), whereas its uptake into the cells is highly dependent on the expression and/or the activity of the influx transporter hOCT1 (SLC22A1). Additional transporters from the ABC family could also affect the balance of imatinib influx/efflux within the CML cell, and may thus contribute to resistance mechanisms. In this study, we focus on ABCC3, an efflux transporter.
Aims: To determine whether imatinib is a substrate for ABCC3, define the importance of copy number variations in the gene and correlate its expression and activity with clinical outcome.
Methods: Peripheral blood mononuclear cells (PBMC) were collected from 4 normal subjects and 90 newly-diagnosed untreated chronic phase CML patients. Following 12 months imatinib treatment, cases were classified as: a) optimal b) sub-optimal c) failure and d) blast crisis (BC), according to ELN 2009 criteria. ABC and hOCT1 transporter mRNA expression and ABCC3 gene copy number variation (CNV) were assessed by qPCR using commercially available TaqMan® assays; GAPDH and RNaseP were used as normalising controls respectively. Data were interpreted using the Comparative Ct method (ΔΔCt) and the ΔCt method using CopyCaller™ software respectively. Unidirectional efflux (transwell assay) of imatinib was investigated using confluent monolayers of the Marin-Darby canine kidney (MDCKII) cell line stably transfected to over-express ABCC3. Imatinib uptake studies, with and without probenecid, were also undertaken on mononuclear cells of 10 CML patients (5 optimal and 5 failures) at diagnosis. Mann-Whitney test was used to correlate transporter mRNA expression with clinical outcome. ABCC3 CNV was assessed by Kaplan-Meier survival plots using the Mantel-Cox Log-rank statistical test. Unpaired 2-tail t test was used on the efflux assays. All statistical analyses were performed using the GraphPad Prism 5 statistical software.
Results: ABCC3 mRNA expression was significantly higher in patients failing treatment (P=0.0002) when compared with those classified as optimal responders. When comparing the ratio of hOCT1 (influx) to ABCB1, ABCC1 and ABCC3 (efflux) transporters, patients with a lower ratio of hOCT1/ABCC3 were more likely to fail imatinib treatment (P=0.0004). CNV analysis also revealed that patients with 3 or more copies of the ABCC3 gene were destined to fail imatinib (P=0.001). In MDCKII cells with polar ABCC3 expression, imatinib was transported unidirectionally in a manner consistent with ABCC3 efflux. This was blocked by the ABCC3 inhibitor probenecid (Net efflux on MDCII-ABCC3 monolayers: 3.61 without *vs.* 1.26 with probenecid). Imatinib uptake studies in primary patient cells also demonstrated that optimal responders had higher imatinib uptake than patients destined to fail (P=0.019); this was also abolished by probenecid.
Summary and Conclusions: Our data clearly illustrate that imatinib is a substrate for the ABCC3 efflux transporter. CML patients with high ABCC3 expression were less likely to have a favourable response to imatinib treatment. Given that imatinib is transported by a number of transporters, it is likely that a complex interplay in the expression and activity of a number of influx and efflux transporters will determine intracellular concentrations, and the likelihood of imatinib resistance and/or treatment failure.

P968

TARGETING THE DBC1-SIRT1-P53 AXIS IS A PROMISING THERAPEUTIC STRATEGY IN FLT3-ITD-POSITIVE AML

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Background: Acute myeloid leukemia (AML) is a heterogeneous disease of the hematopoietic progenitor cell. Several genetic alterations have been detected so far, of these FLT3-internal-tandem duplications (FLT3-ITD) can be found in about 25% of AML patients. FLT3-ITDs are associated with a high risk of relapse and poor overall survival. To improve patient outcome several tyrosine kinase inhibitors (TKI) have been developed, but often duration of response is short and resistance common. The NAD+ dependent protein SIRT1 has been shown to act as an important negative regulator of p53 activity and recent reports have demonstrated increased expression of SIRT1 in leukemic cells. Of note, SIRT1 seems to be specifically essential to maintain stem cell activity in CML leukemic stem cells (Li, Bhatia *et al.*, Cancer Cell 2012). The impact of SIRT1 in the context of other oncogenic tyrosine kinases is poorly understood.
Aims: To determine the role of SIRT1 in AML driven by FLT3-ITD mutations and other oncogenic tyrosine kinases.
Methods: Expression levels of SIRT1 and DBC1 in AML cell lines, BaF/3 cells expressing oncogenic tyrosine kinases (FLT3-ITD, cKIT D816V, JAK2 V617F, TEL/PDGFRβ) and primary AML samples were investigated by immunoblotting and RQ-PCR analysis. To examine FLT3-ITD mediated SIRT1 regulation, cells were treated with midostaurin or transduced with shRNA directed against FLT3. Further, we performed co-immunoprecipitation analyses using SIRT1 and DBC1 antibodies +/- midostaurin treatment. To explore the functional role of SIRT1, leukemic cells were treated with the specific SIRT1-inhibitors Tenovin 6 and EX527 or transduced with SIRT1-shRNA and apoptotic cell death and p53-acetylation were determined. Finally, we analyzed the role of Sirt1 in an FLT3-ITD$^+$ AML mouse model.
Results: Immunoblotting experiments of FLT3-ITD$^+$ leukemic cell lines and primary patient samples showed increased expression of SIRT1, whereas DBC1 levels were unaffected compared to FLT3-ITD$^-$ leukemic controls. Interestingly, increased SIRT1 expression was also observed in cells expressing other oncogenic tyrosine kinases. No elevated mRNA levels were observed suggesting tyrosine kinase mediated post-translational modifications. Treatment of FLT3-ITD positive cells with midostaurin or FLT3-knockdown was followed by reduced expression of SIRT1, p53 acetylation and enhanced DBC1-phosphorylation. As DBC1 acts as a major inhibitor of SIRT1 function, we performed co-immunoprecipitation experiments. Treatment with midostaurin caused strong association of DBC1 with SIRT1 in MOLM14 and MV4;11 cells. To explore whether targeting SIRT1 can enhance the effects of FLT3-TKIs several cell lines and primary murine FLT3-ITD$^+$/MLL-AF9 cells were treated with Tenovin 6 and EX527 +/- midostaurin. Whereas continuous treatment with SIRT1-inhibitors caused inhibition of proliferation, the combination with midostaurin induced significant apoptotic cell death. Similar effects were observed upon shRNA-mediated SIRT1 knockdown.
Summary and Conclusions: Our date indicate that SIRT1 expression is regulated by FLT3-ITD and other oncogenic tyrosine kinases, likely due to post-translational stabilization mediated by DBC1. Further, targeting SIRT1 in the context of oncogenic tyrosine kinases might represent a promising approach to increase sensitivity of TKIs and prevent the development of resistance. To investigate SIRT1 inhibition *in vivo*, experiments are ongoing.

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TRANSCRIPTIONAL PROGRAMMES ASSOCIATED WITH EVI1 MEDIATED CHEMOTHERAPY RESISTANCE OF HUMAN MYELOID CELLS

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Background: Overexpression of the oncogenic transcription factor *EVI1* is associated with resistance to chemotherapy and a dismal prognosis in acute myeloid leukemia (AML). Nevertheless, the molecular mechanisms through which *EVI1* decreases drug sensitivity of myeloid cells have only recently begun to be explored.
Aims: In this study, we aimed to establish an *in vitro* model system that would recapitulate the chemotherapy resistance conferred by *EVI1* overexpression in human disease, and to use this model to explore transcriptional targets that may play a role in EVI1 mediated drug refractoriness.
Methods: The human myeloid cell lines U937 and HL60 were infected with retroviral vectors mediating overexpression of *EVI1*, as well as the corresponding control vectors. Differentiation was assessed by fluoresence activated cell sorting for the cell surface marker CD11b and by morphological analysis. *In vivo* growth of U937_EVI1 and control U937_vec cells was monitored after subcutaneous injection into CB17 scid/scid mice. Metabolic activity and apoptosis after treatment with cytotoxic drugs were measured using standard assays. Genome wide gene expression analyses of U937_EVI1 and U937_vec cells cultured for 48 h in the absence or presence of 400 nM etoposide were conducted using the Human Gene ST1.1 array.
Results: Constitutive overexpression of *EVI1* in human myeloid U937 cells reduced differentiation *in vitro*, and strongly enhanced the growth of xenograft tumors in SCID mice. Most importantly with respect to the properties of *EVI1* overexpressing AML, U937_EVI1 cells displayed increased resistance to the double strand break inducing drugs etoposide and daunorubicin, both of which are used in the therapy of AML, compared to control U937_vec cells. Similar results were obtained with HL60_EVI1 cells. Gene expression profiling of U937_EVI1 and U937_vec cells revealed 1486 unique genes that were regulated at least 1.5-fold by etoposide in the absence of *EVI1* (875 up, 611 down), and 362 genes that were regulated at least 1.5-fold by *EVI1* in the absence of etoposide (126 up, 236 down). Notably, 119 genes were regulated both by *EVI1* and etoposide (51 induced and 30 repressed by both conditions, and 38 induced by etoposide but repressed by *EVI1*). This enrichment was highly significant ($P<10^{-14}$; Fisher's exact test). Of the genes regulated by etoposide, *EVI1*, and both stimuli, 80, 21, and 11 were related to the gene ontology (GO) term apoptosis, respectively. We further searched for genes whose regulation by etoposide was counteracted by *EVI1*. Indeed, *EVI1* diminished the induction by etoposide of 54 unique genes, but the reverse pattern - an alleviation by *EVI1* of repression by the cytotoxic drug - was not observed. Three of the 54 genes whose induction by etoposide was reduced by *EVI1* were related to the GO term apoptosis.
Summary and Conclusions: We have established an *in vitro* model system that recapitulated the chemotherapy resistance associated with *EVI1* overexpression in human AML patients. Gene expression profiling suggested that *EVI1* mediated resistance to cytotoxic drugs through a number of target genes and at least two different mechanisms: firstly, it may "pre-adapt" cells to cytotoxic drug exposure by activating gene expression patterns that are also elicited by chemotherapeutic agents, and may at least in part represent cellular defense mechanisms against their action. Secondly, *EVI1* counteracted the induction of genes that may take part in mediating the cytotoxic action of chemotherapeutic agents.

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KNOCKDOWN OF NUCLEOPHOSMIN BY RNA INTERFERENCE REVERSES MULTIDRUG RESISTANCE IN RESISTANT LEUKEMIC HL-60 CELLS

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Background: Nucleophosmin, a multifunctional nucleolar phosphoprotein, is involved in many cellular activities. Abnormal expression of *NPM* gene, such as over-expression, mutation, rearrangement or deletion, could lead to abnormal cellular proliferation, which is an important mechanism of malignant transformation in tumor cells. A number of previous studies have indicated that the expression of *NPM* was more abundant in the proliferative cells than the resting cells, especially in many kinds of solid tumors cells. Furthermore, it is showed that over-expression of NPM in solid tumor cells was probably one of the poor prognostic factors, and related to the tumor progression and drug-resistance development. Other than the solid tumors, it has been confirmed that *NPM* is mutated in around 30-50% of adult AML with a normal karyotype. These mutations are proven to have prognostic significance. Our previous study has revealed that NPM was obviously over-expressed in many leukemia cells, especially in the resistant leukemia cell lines and in relapsed/refractory patients. But, relatively little is known about the role of NPM in resistance of leukemia.
Aims: In this study, in order to explore the potential role of NPM in resistance of leukemia, the recombinant eukaryotic expression vector of *NPM*-shRNA was constructed.
Methods: In this study,adriamycin sentitive and resistant HL-60 cell lines (HL-60 and HL-60/ADR) were used as target cell for lentiviral-mediated RNAi. We designed and selected one shRNA targeting on *NPM* gene for transfection into HL-60 and HL-60/ADR cell lines. Cell proliferation and differentiation were measured by growth curve, clone formation assay, NBT reduction assay and CD11b analysis. qRT-PCR and western blotting were used to access the expression of related genes and proteins. Cell cytotoxicity assay was used for evaluation of cell sensitivity to chemotherapeutic agents.
Results: The results showed obvious down-regulation of NPM mRNA and protein levels after *NPM* RNAi. The *NPM*-targeted RNAi resulted in many cellular changes, such as, suppressing cell proliferation and inducing cell differentiation. Down-regulation of *NPM* gene could arrest the cell cycle progression, an increase in the proportion of G0/G1 phase (38.51±1.33% *vs.*52.84±2.08% of control and knockdown groups on HL-60 cells, 41.90±1.35% *vs.* 60.89±2.71% of control and knockdown groups in HL-60/ADR cells, respectively). *NPM* gene silencing could also induce pro-apoptotic genes and proteins expression, and inhibit anti-apoptotic genes/proteins expression.Furthermore, IC50 of two chemotherapeutic agents (ADR, DNR) to HL-60 and HL-60/ADR cells decreased, especially more remarkable on HL-60/ADR cells. IC50 of ADR on HL-60/ADR cells was reduced from 12.544±0.851μmol/L (before *NPM* RNAi) to 6.331±0.522μmol/L (after *NPM* RNAi), IC50 of DNR was reduced from

2.152±0.143μmol/L (before *NPM* RNAi) to 1.116±0.093 μmol/L (after *NPM* RNAi). The relative reversal rate of HL-60/ADR cells on ADR was 50.2%, and on DNR was 48.9%.
Summary and Conclusions: In conclusion, our results demonstrated that shRNA expression vectors could effectively reduce *NPM* expression and restore the drug sensitivity of resistant leukemic cells to conventional chemotherapeutic agents.

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SYNTHETIC LETHALITY IN P53 MUTATED B-CELL MALIGNANCIES: CHK-1 INHIBITION AND DNA-PK INHIBITION EFFECT IN CELL LINES AND PRIMARY CLL CELLS
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Background: Mutations in the *TP53* gene are usually associated with very poor prognosis in hematological malignancies, including strong resistance to therapy. The concept of synthetic lethality based on inhibition of Chk1 kinase represents a promising approach for elimination of highly aggressive cancer cells. After this inhibition, the blockade of all three major cell cycle checkpoints is presumed, ie, the G1/S (owing to *TP53* mutation), and S together with G2/M (both through the Chk1 inhibition). This should result in uncontrolled cell proliferation followed by mitotic catastrophe and cell death. DNA-PK is a kinase responsible for DNA damage signaling within the nonhomologous end-joining DNA repair. It has been reported that especially high risk cancer cells harboring *TP53* mutations can be protected from chemotherapy through enhanced activation of this pathway. The sensitization to chemotherapeutic drugs using DNA-PK inhibition has been reported in CLL.
Aims: To analyze potential sensitization of B-cell leukemia and lymphoma cells with *TP53* mutations to nucleoside analog fludarabine using: (i) Chk1 inhibition in growing cell lines, (ii) Chk1 inhibition in non-dividing CLL cells, (iii) DNA-PK inhibition in growing cell lines, (iiii) DNA-PK inhibition in non-dividing CLL cells.
Methods: Following cultures were used: 15 B-cell leukemia or B-cell lymphoma permanent cell lines with 10 of them having *TP53* mutation and 24 primary CLL cultures with 12 of them having *TP53* mutation (PBMNC containing >85% of CLL lymphocytes). The inhibitors were following: SCH900776 (racemic isoform, 200 nmol) for Chk1 and NU7026 (10 μmol) for DNA-PK. After 2h pre-incubation with inhibitors, fludarabine was administered at four different concentrations (assessed individually for cell lines - see Results, and 25; 6.25; 1.6 and 0.4 μg/mL for CLL cultures). Cell viability was measured by the metabolic WST-1 assay (Roche). The sensitization effect was evaluated if appearent in at least two bordering concentrations using two-way analysis of variance (ANOVA).
Results: The detailed *TP53* status (deletion and/or mutation presence) was assessed for all used cell lines and primary cultures by I-FISH and by the yeast functional analysis with subsequent sequencing. Concentrations of fludarabine ranged from 20 to 0.125 μg/mL in the tested cell lines, with those having *TP53* defects being obviously more resistant. The Chk1 inhibitor on its own exhibited none or only negligible effect on cell viability (≥90% in comparison with untreated control). The significant sensitization effect (P<0.01) was observed in 5/10 (50%) mut-*TP53* cell lines and 1 out of 5 (20%) wt-*TP53* cell lines. By contrast, no sensitization effect was observed in tested primary CLL cultures. The DNA-PK inhibitor on its own reduced a viability in most of the cultures (average 73%). The sensitization effect (P<0.01) was apparent in 2/10 (20%) mut-*TP53* cell lines and none out of 5 wt-*TP53* cell lines. Similarly to cell lines, also some primary CLL cultures responded to DNA-PK sensitization: positive effect was observed in 4/12 (33%) *TP53*-defected samples and none out of 12 wt cultures. Using western blot for phosphorylated histone H2AX (γH2AX), we verified that both Chk1 and DNA-PK sensitization was mediated through enhanced accumulation of DNA damage (dsDNA breaks).
Summary and Conclusions: Our work shows that both Chk1 kinase inhibition and DNA-PK inhibition may represent feasible approach how to eliminate some aggressive leukemia and lymphoma cells. Some other cultures remain, however, inert to the sensitization. Concerning primary non-dividing CLL cells, we confirm that the DNA-PK approach might be promising. Our preliminary data suggests that Chk-1 inhibition should be tested in CLL cells after these are stimulated to proliferation. The work was supported by the project FNUSA-ICRC (CZ.1.05/1.1.00/02.0123), MUNI/A/0784/2012, and NT13519-3.

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POLYMORPHISMS OF MULTIDRUG RESISTANCE TRANSPORTER (ABCG2 AND ABCB1) GENES IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA
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Background: ATP-binding cassette (ABC) transporters such as P-glycoprotein (encoded by the *ABCB1* gene) and breast cancer resistance protein (*ABCG2*), appear to be expressed in hematopoietic stem cells, and presumed to play a protective role against a broad spectrum of toxic substrates. ABC-transporters may also be responsible for the efflux of small lipophylic substrates, such as steroids, and their expression may play a regulatory role in inducing growth, differentiation or apoptosis. Alteration in the putative physiological function of ABC-transporters may result in hematopoietic stem cell disorders. In addition, both ABC-transporters have been linked to drug resistance mediating the efflux of several cytotoxic substances.
Aims: To investigate common single nucleotide polymorphisms with functional consequences in ABCB1 and ABCG2 genes, whether they influence the predisposition to haematopoietic stem cell disorders and contribute to the development of chemotherapy resistance in patients with acute myeloid leukemia (AML).
Methods: 395 AML patients (185 males/210 females; median age: 50; range: 16-93 years) diagnosed and followed between 2001-2009 were enrolled in the study. Remission and relapse rates and survival were analyzed for 328 patients younger than 60 years and treated with curative intention. Single nucleotide polymorphisms of *ABCG2* (c.34G>A and c.421C>A) and *ABCB1* (c.3435C>T) were analysed from genomic DNA samples of 390 AML patients and 204 healthy controls by LightCycler allele discrimination technique.
Results: Allele frequencies (AF) were not different in AML patients as a whole cohort compared to controls (c.34G>A AF±95%CI: 4.2±1.4% in AML *vs.* 3.9±1.9% in controls; c.421C>A: 8.1±2.0% in AML *vs.* 10.0±3.0% in controls; c.3435C>T AF±95%CI: 49.6±3.6% in AML *vs.* 49.8±5.0% in controls). On the other hand, AML subgroup analysis revealed that AML1-ETO [t(8;21)(q22;q22) translocation] positive patients (n=18) showed higher c.34G>A AF (16.7±12.4%) compared to control individuals (P=0.02). Among AML patients younger than 60 years receiving daunorubicin based induction chemotherapy (n=256), ABCG2 c.421C>A carriers (CA+AA genotypes) displayed longer overall (48-month OS: 42.5±8.1% *vs.* 28.4±3.1%; P=0.072) and disease free survival (48-month DFS: 38.3±8.3% *vs.* 26.0±3.1%; P=0.048) compared to individuals carrying the major allele (CC genotype). Early death due to toxicity, resistant disease, remission and relapse rates were not different between ABCG2 c.421C>A carriers and major allele carriers. ABCB1 polymorphism tested in this study did not alter disease predisposition or treatment outcome.
Summary and Conclusions: Our results suggest that common ABCG2 polymorphisms might influence AML predisposition and treatment outcome differentially depending on AML genetic background or chemotherapy applied.

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INHIBITION OF PI3K/MTOR OVERCOMES NILOTINIB RESISTANCE IN BCR-ABL1 POSITIVE LEUKEMIA CELLS
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Background: Chronic myeloid leukemia (CML) is a cytogenetic disorder resulting from expression of the Philadelphia chromosome (Ph), that is, the t(9;22) chromosomal translocation and the formation of the BCR-ABL1 fusion protein. Tyrosine kinase inhibitors (TKI), such as imatinib and nilotinib, have emerged as leading compounds to treat CML. Translocation t(9;22) does not only occur in CML, 20-30% of acute lymphoblastic leukemia (ALL) are also found with expression of Ph. However, TKI are not comparably effective for the treatment of Ph+ ALL. BEZ235, a dual inhibitor of PI3K/mTOR, shows broad preclinical anti-leukemia activity *in vitro* and *in vivo* and is currently undergoing clinical evaluation.
Aims: The aim of this study was to investigate TKI resistance mechanisms and sensitization of Ph+ tumor cells to TKI treatment.
Methods: Cell apoptosis was investigated by flow cytometry with annexin V/propidium iodide (PI) staining. Quantitative RT-PCR was used to check mRNA level and protein level was analyzed by Western blot. Cleavage of caspase 3 and PARP was also assessed by Western blot. Cell cycle arrest assay was carried out using flow cytometry with PI staining.
Results: The Ph+ cell lines JURL-MK2 and SUP-B15 were used in this study. The annexin V/PI assay revealed that nilotinib induced apoptosis in JURL-MK2 cells, but not in SUP-B15 cells. There was no mutation in the tyrosine kinase domain of BCR-ABL1 in cell line SUP-B15. Consequently, the cells were not generally irresponsive to TKI, evidenced by dephosphorylation of the BCR-ABL1 downstream target STAT5. The resistance to apoptosis after nilotinib treatment was accompanied by the constitutive and nilotinib irresponsive activation of the PI3K pathway. Treatment of SUP-B15 with the dual PI3K/mTOR inhibitor BEZ235 alone induced apoptosis in a low percentage of cells. Combination of nilotinib and BEZ235 led to a synergic apoptotic effect in SUP-B15 cells. Caspase3 and PARP cleavage confirmed that apoptosis was induced after combined treatment. The main effect of PI3K/mTOR inhibition and reason for apoptosis in the nilotinib resistant cells

was the block of the translational machinery, leading to the rapid down-regulation of anti-apoptotic protein MDM2.
Summary and Conclusions: In conclusion, the results demonstrated that BEZ235 overcomes nilotinib resistance to apoptosis in SUP-B15 cells through translational down-regulation of MDM2. The current findings suggest that MDM2 may be a therapeutic target to sensitize TKI-resistant BCR-ABL1 positive leukemia cells.

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ACTIVITY OF ABL1 KINASE INHIBITOR, IMATINIB AND THE JAK KINASE INHIBITOR TG101348: THE TARGETED THERAPY FOR RESIDUAL BCR-ABL POSITIVE LEUKEMIA CELLS

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Background: ABL kinase inhibitor, imatinib is highly effective therapy against chronic myeloid leukemia (CML) patients. However, imatinib is not curative for most CML patients. Residual CML cells are present in bone marrow microenvironment. Bone marrow microenvironment is a source of soluble factors such as cytokine and regulates the proliferation of leukemia cells. The hematopoietic cytokine receptor signaling is mediated by tyrosine kinases termed Janus kinases (Jaks) and downstream transcription factors, signal transducers and activators of transcription (STATs). Jak-STAT signaling is also activated in CML cells. One of the Jak kinase inhibitor, TG101348 (SAR302503) is an orally available inhibitor of Jak2 and developed for the treatment of patients with myeloproliferative diseases.
Aims: Combination therapy using a BCR-ABL tyrosine kinase inhibitors and a Jak inhibitor, TG101348may help prevent stroma-associated drug resistance-and these approaches may be expected to improve the outcomes of CML patients.
Methods: In this study, we investigated the ABL tyrosine kinase inhibitor, imatinib and TG101348 efficacy by using the BCR-ABL positive cell lines, K562 and primary CML samples when leukemic cells were protected by the feeder cell lines (HS-5 and S9).
Results: 72 hours treatment of imatinib exhibits cell growth inhibition and induced apoptosis against K562 cells in a dose dependent manner. However, the treatment of imatinib exhibits cell growth inhibition partially against K562 cells in the presence of HS-5 conditioned media. Treatment of TG101348 did not exhibit cell growth inhibition against K562 cells directly. However, the combination treatment with imatinib and TG101348 abrogated the protective effects of HS-5 conditioned media in K562 cells. We next investigated the intracellular signaling of imatinib and TG101348. Phosphorylation of BCR-ABL, Crk-L was not reduced after TG101348 treatment. However, phosphorylation of BCR-ABL, Crk-L was significantly reduced and increased apoptosis after combination treatment with imatinib and TG101348. We next investigated the efficacy between imatinib and TG101348 by using CD34 positive primary CML samples. The treatment of imatinib exhibits cell growth inhibition partially against CD34 positive CML samples in the presence of feeder cells. Combined treatment of CD34 positive primary samples with imatinib and TG101348 caused significantly more cytotoxicity and induced apoptosis. We also found that mitogen-activated protein kinase (MAPK) was also inhibited by imatinib and TG101348 treatment. We next investigated the intracellular signaling of imatinib and TG101348 by using the CD34 positive primary samples. Phosphorylation of BCR-ABL, Crk-L was significantly reduced and increased apoptosis after treatment with imatinib and TG101348. Moreover, combination of imatinib and TG101348 inhibited the colony growth of Ph-positive primary samples. We also investigated the TG101348 activity against feeder cell. Phosphorylation of STAT5 was reduced by TG101348 in a dose dependent manner. The cytokine production was analyzed by using cytokine array systems. The cytokine production such as granulocyte macrophage colony-stimulating factor (GM-CSF) from HS-5 was also reduced by TG101348 treatment.
Summary and Conclusions: Data from this study suggested that administration of the imatinib and Jak inhibitor, TG101348 may be a powerful strategy against stroma-associated drug resistance of Ph-positive leukemia cells and enhance cytotoxic effects of imatinib in those residual CML cells.

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TP53 MUTATIONS BUT NOT ATM MUTATIONS ASSOCIATE WITH HIGHER IN VITRO RESISTANCE OF CLL CELLS TO RITUXIMAB

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Background: *TP53* and *ATM* defects have been associated with inferior outcome in chronic lymphocytic leukemia (CLL). The response of corresponding patients to conventional chemotherapy is usually poor. Monoclonal antibodies targeted to leukemic cells are supposed to act primarily independently on apoptosis induction and should, therefore, bypass a block imposed by the *TP53* or *ATM* inactivity. Despite that, treatment outcome using rituximab on its own or in combination with chemotherapy is usually unsatisfactory among *TP53*-affected patients. This can be accounted either to a higher primary resistance to rituximab or to a faster and more aggressive relapse.
Aims: To compare *in vitro* primary response of CLL cells with well defined *TP53* and *ATM* defects to rituximab.
Methods: Deletions 17p and 11q were detected by I-FISH; *TP53* mutations were identified by yeast functional analysis and direct sequencing; *ATM* mutations were captured through resequencing microarray. All selected aberrant samples harbored complete p53 or ATM inactivation as evidenced by defective response to IR and doxorubicin; by contrast, all selected wild-type samples were functioning in these tests. The analysis of CLL cells sensitivity to rituximab was performed on 70 characterized samples using a metabolic WST-1 assay; 20% active human serum was added to allow cell lysis by complement; non-specific immunoglobulin was used as a negative control in all samples. The ability of rituximab to induce apoptosis under our experimental conditions was verified by flow-cytometry using propidium iodide *vs.* annexin V measurement.
Results: The following main observations have been made: (1) Concentration of 10 μg/mL was sufficient for rituximab *in vitro* testing; higher concentrations (20 and 30 μg/mL) showed the same effect in all cultures; (2) Final viability of tested cultures ranged from 45% to 100% in comparison with untreated control; all cultures were inert to non-specific immunoglobulin. There were clear differences in sensitivity to rituximab between the tested genetic groups; the *TP53*-mutated group (n=27 samples) was substantially more resistant than both wild-type samples (n=31) (P=0.04) and especially than *ATM*-mutated samples (n=12) (P=0,017; Wilcoxon test) (3) rituximab used at concentration of 10 μg/mL (and without active serum) was able to induce apoptosis (annexin V positivity) after 24 h treatment in tested cultures.
Summary and Conclusions: Our results showing significantly different response to rituximab for CLL cells harboring *TP53* mutation *vs.* those harboring *ATM* mutation were quite unexpected, especially because active serum was assumed to allow the lysis by complement. This observation indicates that apoptosis might play important role during primary CLL cells response to rituximab.
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EVIDENCE THAT THE PREGNANE X AND RETINOID RECEPTORS PXR, RAR AND RXR MAY REGULATE TRANSCRIPTION OF THE IMATINIB TRANSPORTER HOCT1 EXPRESSION IN CHRONIC MYELOID LEUKAEMIA CELLS

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Background: The expression and activity of the imatinib uptake transporter human organic cation transporter 1 (hOCT1; SLC22A1) is an independent predictor of response to imatinib treatment in chronic myeloid leukaemia (CML) patients. It has been recently shown by our group that peroxisome proliferator-activated receptors (PPAR) activation can increase the killing effect of imatinib in CML cells, due to upregulated *hOCT1* gene expression and imatinib uptake.
Aims: To investigate the role, if any, of activation of nuclear receptors other than PPAR in the transcriptional regulation of hOCT1 in CML cells.
Methods: The CML cell line KCL22 (chosen because of its low basal hOCT1 expression) was cultured and treated for 16 hours with agonists for adopted orphan receptors and endocrine receptors including Pregnane X receptor (PXR), Constitutive androstane receptor (CAR), Farnesoid X receptor alpha (FXRalpha), Liver X receptor alpha (LXRalpha), Retinoid acid receptor (RAR), Retinoid X receptor (RXR), Glucocorticoid receptor (GR), Oestrogen receptor (ER), Androgen receptor (AR), Mineralocorticoid receptor (MR), Vitamin D receptor (VDR), Progesterone receptor (PR) and Thyroid hormone receptor (TR). 3-6 replicate experiments were performed for each receptor. Fresh peripheral blood mononuclear cells from 9 cases of newly diagnosed untreated chronic phase CML patients were treated with agonists for nuclear receptors. A standard MTT assay was used to define the optimal treatment doses of each receptor that had no effect on cell viability. hOCT1 gene expression levels were measured using real-time quantitative RT-PCR on a LightCycler.
Results: In KCL22 cells, it was found that the PXR agonists Rifampin, T0901317 and SR12813 increased hOCT1 expression by an average of 29.4, 3.28 and 2.99 fold above untreated control levels respectively (P<0.05 for Rifampin and T0901317, P=0.07 for SR12813, Wilcoxon rank test). hOCT1 expression was increased 2.9 fold compared with untreated control after KCL22 cells were treated with the RAR and RXR agonist 9-cis retinoic acid, although this effect had borderline significance (P=0.07). Treatment with agonists for the rest of the nuclear receptors did not significantly alter the hOCT1 expression in KCL22 cells. In peripheral blood mononuclear cells from newly diagnosed untreated chronic phase CML patients, the PXR agonist SR12813 significantly increased hOCT1 expression by 1.6 fold compared with untreated control (P=0.04, Wilcoxon rank test, n=5). However, unlike the findings in KCL22 cells, there was no change after treatment with the alternative PXR agonist Rifampin (P>0.05, n=9). hOCT1 expression was also significantly

upregulated by the RAR agonist All-trans-retinoic acid (ATRA) and the RAR and RXR agonist 9-cis retinoic acid by 1.7 and 2.5 fold above untreated control (P=0.04 for both; n=5; Wilcoxon rank test). Agonists for FXRalpha, LXRalpha, GR, ER, AR, MR and PR were also tested and showed no significant effect on hOCT1 expression in these primary CML cells, in concordance with the findings in KCL22 cells.
Summary and Conclusions: It is shown here that PXR, RXR and RAR may play important roles in the transcriptional regulation of the hOCT1 gene in CML cells. Further study is needed to explore the potential effects of agonists of PXR, RXR and RAR on imatinib uptake and cell killing in combination with imatinib in CML cells.

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THE ROLE OF CYP2J2 IN CYCLOPHOSPHAMIDE METABOLISM
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Background: Cyclophosphamide (CP) is an alkylating agent that is used in high doses in the treatment of hematological malignancies and solid tumors and in the conditioning regimen before stem cell transplantation. It is also a potent immuno-suppressive agent that is used in low doses for the treatment of rheumatoid arthritis, systemic lupus erythematosus and other autoimmune diseases. CP alkylates DNA at N7 (guanine) of the imidazole ring and trigger apoptosis by the formation of Guanine- Adenine intrastrand crosslinks. CP is a prodrug that is converted through cytochrome P450 (CYP) in the liver to 4-hydroxycyclophosphamide (4-OH-CP) which is the main active metabolite (90% of the total CP). CYP2B6 was reported to metabolize about 90% of the total CP. However, other enzymes such as CYP2A6, CYP2C8, CYP2C9, CYP2C19 and CYP3A4 were also reported to be involved in the bioactivation of CP.
CYP2J2 in human is involved in metabolic transformation of xenobiotics. It is encoded by the *CYP2J2* gene that has been mapped on the short arm of chromosome 1. It is mainly expressed in intestine and cardiovascular system with low expression level in the liver. CYP2J2 has been reported to metabolize several drugs despite that its role in drug metabolism is not yet fully understood. CYP2J2 can contribute to drug metabolism in the extrahepatic tissues, particularly the intestine, and may be the main enzyme responsible for first-pass metabolism for certain drugs.
Aims: To determine the role of CYP2J2 in cyclophosphamide metabolism.
Methods: Eleven patients went through HSCT and were conditioned with CP and total body irradiation. Blood samples were collected and analyzed before conditioning start and at 0.5,1,2,4,6, 8 hr after CP infusion then one sample before the start of CP second dose and another sample 6hr after the infusion. Gene array and genotyping were carried out using Nimblegen gene expression arrays while GeneSpirng GX 12.0 software was used for analysis. CP and 4-OH-CP concentrations were determined using high performance liquid chromatography (HPLC) with UV detector for CP and fluorescence detector for 4-OH-CP. Pharmacokinetics was analyzed using WinNonLin software. The clinical outcome including engraftment, rejection, chimerism, transplantation-related morbidity and mortality and relapse have been evaluated.
To confirm the findings, microsomes containing cDNA-expressed human CYP2J2 were incubated with different concentrations of CP (0-50 mM). Microsomes were incubated for one hour and reaction was terminated by adding equal volume of acetonitrile (ACN). 4-OH-CP concentrations were measured as previously mentioned using HPLC with fluorescence detector.
Results: All patients showed high expression of CYP2J2 mRNA (over 2-folds higher than their level before the start of conditioning). However, high inter-individual variation was observed for gene expression which most probably can be explained by the different polymorphic forms of CYP2J2 gene. The gene expression variability was in proportion to the ratio of 4-OH-CP/CP areas under curves (AUCs) indicating that CYP2J2 may play a major role in CP metabolism. No correlation between diagnosis and/or other clinical data and the results of CYP 2J2 was observed.
In-vitro results have confirmed that CP was metabolized through CYP2J2 microsomes. V_{max}/K_m was 8.98 mL/min/ pmol P450 after incubation in quadruplicates. These results are in agreement with our previous findings for CYP 2B6.
Summary and Conclusions: This investigation showed that *CYP2J2* gene is uP-regulated during the treatment with cyclophosphamide which indicated that CYP2J2 is induced by CP and might be involved in CP metabolism. The results obtained from *in-vitro* incubations have confirmed the *in-vivo* results. The present results may help to better understanding of cyclophosphamide metabolism especially in the extrahepatic tissues and hence lead to new strategies for personalized dosing with increased safety and decreased drug-drug interactions.

P978

ABCB1 HAPLOTYPE IS ASSOCIATED WITH ALTERATIONS IN FUNCTIONAL ACTIVITY OF P-GP AND AFFECTS MAJOR MOLECULAR RESPONSE IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH STANDARD-DOSE OF IMATINIB
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Background: Despite the excellent results of Imatinib Mesylate (IM) treatment in CML patients, some individuals develop resistance due to impaired bioavailability. IM interacts with membrane transporters such as ABCB1 (P-gp) and SLC22A1. It was demonstrated that haplotype for *ABCB1* c.1236C>T/c.3435C>T/c.2677T>A polymorphisms strongly affect the secondary structure of ABCB1 mRNA and its activity. These modifications could affect the efflux transporter activity and response to treatment with IM.
Aims: The aim of this study was to investigate the P-gp functional activity in peripheral blood mononuclear cells from CML patients with different haplotypes for *ABCB1* c.1236C>T, c.3435C>T and c.2677TG>T polymorphisms.
Methods: Twenty eight patients in chronic phase of CML were selected according to haplotypes for *ABCB1* c.1236C>T, c.3435C>T and c.2677G>T polymorphisms. Ten patients with *ABCB1* 1236CC/3435CC/2677GG haplotype comprised the wild-type group and 18 patients carriers of haplotypes with at least one mutated allele in each genotype for three *ABCB1* polymorphisms (10 patients with 1236CT/3435CT/2677GT and 8 with 1236TT/3435TT/2677TT) comprised the mutated group. All patients were in chronic phase of CML, treated with a standard dose of IM (400 mg/day) for a mean time of 63.5±12.6 months and with complete cytogenetic response. Major molecular response (MMR) was defined as a reduction of *BCR-ABL1* transcripts levels to ≤0.1% in the peripheral blood standardized on the International scale. Complete molecular response (CMR) was defined as a reduction to ≤0.0032% *BCR-ABL1* transcripts levels. Real-Time PCR was performed to evaluate the *ABCB1* and *SLC22A1* mRNA expression using GAPDH gene as control. P-gp functional activity was determinated by rhodamine123 efflux assay. Analysis of P-gp expression and functional activity were performed by flow cytometry.
Results: P-gp activity in wild-type group was higher than in mutated group (59.1 *vs* 38.3%; P=0.001).The groups of haplotypes had no difference in *SLC22A1*, *ABCB1* mRNA and P-gp expression. Patients who did not achieve MMR showed a higher rate of efflux mediated by P-gp compared with individuals who did not achieve this response (64.7% *vs.* 45.7%, P=0.001). All patients who did not achieve MMR presented efflux above 60%.Unlike MMR, no association was found for CMR. Higher levels of *SLC22A1* mRNA were found in patients who achieved MMR (P=0.042). The *ABCB1* mRNA expression showed a positive and strong correlation with P-gp expression (r=0.747; P=0.001).P-gp activity had positive and moderate correlation (r=0.570; P=0.001), whereas SLC22A1 mRNA expression showed negative and moderate correlation with *BCR-ABL1* transcripts (r=-0.407; P=0.032).
Summary and Conclusions: *ABCB1* mRNA and P-gp expression were similar in haplotypes groups for c.1236C>T, c.3435C>T and c.2677G>T polymorphisms, however, patients with the 1236CT/3435CT/2677GT and 1236TT/3435TT/2677TT haplotypes exhibit less efflux mediated by P-gp presenting highest frequency of MMR, probably due to higher intracelullar concentrations of IM.

P979

DIAGNOSTIC AND THERAPEUTIC POTENTIALS OF EXPRESSION LEVELS OF BIOACTIVE SPHINGOLIPID GENES IN NEWLY DIAGNOSED AND DRUG-RESISTANT CHRONIC MYELOID LEUKEMIA PATIENTS
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Background: Ceramide is an important apoptotic sphingolipid that affects vital cell signaling pathways such as growth, proliferation, apoptosis, senescence, migration, and cell cycle arrest. *de novo* synthesis of ceramide is carried out by Ceramide synthase gene family (CerS1-6). Glucosylceramide synthase (GCS) and sphingosine kinase-1 (SK-1) are powerful anti-apoptotic molecules triggering cell proliferation and division, and also repressing apoptosis.
Aims: In this study, we aimed to investigate expression levels of bioactive sphingolipid genes in bone marrow samples of newly diagnosed CML patients; imatinib-, nilotinib-, or dasatinib-resistant CML patients; and CML patients showing minimum hematological response to imatinib, nilotinib or dasatinib, and

then examine the correlation between the changes in expression levels and progression of CML.
Methods: Mononuclear cells from bone marrow are seperated in these groups of CML patients by Ficoll Paque method. Total RNAs of mononuclear cells were isolated, and then converted to cDNA by reverse transcription. Expression levels of BCR/ABL, CerS1-6 genes, GCS and SK-1 genes were analyzed by real-time PCR. Thirtyfive patients were involved in this study (7 newly diagnosed, 5 imatinib resistant, 4 dasatinib resistant, 2 nilotinib resistant, 12-, 3-, and 2 showing minimum hematological response to imatinib, dasatinib, and nilotinib respectively.
Results: Expression levels of CerS1, CerS2, Cers4, Cers5, and CerS6 genes were increased significantly in CML patients showing minimum hematological response to nilotinib treatment and in imatinib-treated CML patients than newly-diagnosed and drug-resistant CML patients. On the other hand, expression levels of GCS and SK-1 genes were significantly higher in drug-resistant patients than that of the newly-diagnosed CML patients and drug-treated patients showing minimum hematological response.
Summary and Conclusions: We previously demonstrated that enforced expression of CerS1 gene or application of ceramide mimetics increased apoptotic effects of tyrosine kinase inhibitors with a strong synergism in CML cells while inhibition of GCS and SK-1 synergistically increased TKI-induced cell death. We also previously determined that SK-1 regulates expression and protein stability of BCR/ABL. In conclusion, the results of this study confirmed and showed for the first time a significant correlation between the expression levels of bioactive sphingolipid genes and the sensitivity or resistance of CML patients to TKIs. A correlation between expression levels of SK-1 and BCR/ABL was also determined. Taking together, all these data showed that expression levels bioactive sphingolipids might be novel markers in determination of the resistancy in CML patients. More importantly, bioactive sphingolipids could be novel targets for the effective treatment of resistant CML patients.
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Novel therapeutics, targeted therapies and gene therapy

P980

SMO INHIBITOR SPECIFICALLY TARGETS THE HEDGEHOG PATHWAY AND REVERTS THE DRUG-RESISTANCE OF LEUKEMIC STEM CELLS

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Background: Hedgehog (Hh) pathway activation contributes to leukemia development and growth and its inhibition is likely to offer an efficient therapeutic opportunity. SMO inhibitor (SMOi), Hh pathway inhibitor, is a new selective inhibitor of leukemia self-renewal and is currently being evaluated in clinical trials.
Aims: In this study we explored the '*in vitro'* and '*ex vivo'* activity of SMOi, as single-agent or in combination with conventional chemioterapy, and we investigated potential biomarkers of functional inhibition.
Methods: We studied CD34+ Leukemia Stem cells (LSCs) collected before and after 28 days treatment in a phase I dose escalation protocol enrolling selected hematological malignancies (MF, MDS, CML-BP, *CMML* and AML). We were able to collect and separate highly purified (98%) bone marrow CD34+ cells from 5 AML,1 MF and 2 CML patients (pts) by immunomagnetic separation, and analysed them for Gene Expression Profile (GEP) (Affimetrix HG-U133 Plus 2.0) with Partek Genomics Suite and software GeneGo.
Results: The GEP analysis on CML pts showed that 1197 genes were differentially expressed between CD34+ cells collected before and after 28 daysof SMOi dose finding oral therapy and among these mostly were related to Hh signalling (P-value 0.03), providing further evidences that SMOi really therapeutically targets the Hh pathway. Regarding genes involved in Hh signaling pathway, *Gas1* and *Kif27* were strongly upregulated (P-value 0.01 and 0.02 respectively), suggesting that they may work as biomarkers of activity. Moreover, analysis performed on AML patients, with Partek Genomic Suite and GeneGo, showed that 589 genes were differentially expressed between CD34+ cells collected before and after 28 daysof SMOi dose finding oral therapy and among these mostly were related to Hh signalling (P-value 0.0002), providing further evidences that SMOi really therapeutically targets the Hh pathway. Regarding genes involved in Hh signaling pathway, Casein Kinase I, Gli3 and β-catenin were strongly down-regulated (P-value 0.012, 0.012 and 0.043 respectively. Other genes were differentially expressed after *'ex-vivo'* treatment with SMOi as compared to the baseline: we observed a down-regulation of *Bcl2* (fold change -1.03004), *ABCA2* (fold change -1.08966), *Gli1* (fold change -1.0775), *Smo* (fold change -1.07702), and an upregulation of *Gli2* (fold change 1.08191). The GEP results for Gli1, Gli2 and Smo were confirmed by RT-PCR. This analysis included also Gli3, Abcb1 and Abcg2 genes. To investigate the *'in vitro'* efficacy of SMOi we treated MOLM-13, HL-60, KG1α, BV-173, SUPB-15 and K562 cell lines with increasing concentration of SMOi (10 nM - 100 μM) for up to 72 hours. SMOi had no efficacy on cell viability. Consistent with the WST-1 results, AnnexinV/PI staining analysis did not show any apoptosis process at the same time points. SMOi had no efficacy on cell viability on primary cells from healthy donors. To support the '*ex vivo*' data we have confirmed '*in vitro*' that SMOi specifically targets and down-regulates the Hh Pathway by Real Time PCR and Western Blot, as soon as 48 hrs of incubation with SMOi (10 μM). We treated K562 cell line (Tirosin Kinase (TK)-resistant) with SMOi (10 μM) in combination with Nilotinib, Imatinib, and Bosutinib (1 μM). We observed a synergistic and additive effect by WST-1 assay and AnnexinV/PI staining analysis, suggesting that SMOi reverts the chemio resistance mechanism of K562 to TKs.
Summary and Conclusions: SMOi specifically targets the Hh Pathway in CD34+ cells, suggesting that Hh inhibition may impair LSC maintenance. New potential biomarkers (Gas1 and Kif27) were identified. '*In vitro'* treatment with SMOi did not show efficacy neither apoptosis and in the viability. Therefore, the combination of SMOi with TK inhibitors or conventional chemotherapy could represent a valid new therapeutic approach in these haematological malignancies. Work supported by European LeukemiaNet, FIRB 2008, AIRC, AIL, COFIN, University of Bologna and BolognAIL.

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A PHASE3, RANDOMIZED, DOUBLE BLIND, PLACEBO CONTROLLED, MULTI CENTER STUDY (ENGAGE) TO INVESTIGATE THE EFFICACY AND SAFETY OF ELIGLUSTAT IN PATIENTS WITH GAUCHER DISEASE TYPE 1: 9 MONTH RESULTS

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Background: In Gaucher disease type 1 (GD1), acid-beta-glucosidase deficiency leads to accumulation of glucosylceramide in tissue macrophages (Gaucher cells). Clinical manifestations include hepatosplenomegaly, anemia, thrombocytopenia and skeletal disease. Eliglustat, a novel oral substrate reduction therapy, is in late-stage development for the treatment of GD1. ENGAGE (NCT00891202), a Phase 3 registration trial, is a randomized, double-blind, placebo-controlled, multinational trial to investigate the efficacy and safety of eliglustat in untreated GD1 patients.
Aims: We present the results of the primary analysis period of the ENGAGE trial.
Methods: A total of 40 patients (mean age: 31.8 years; 20 males, 20 females) meeting the main entry criteria for splenomegaly and either thrombocytopenia or anemia or both were enrolled. Patients were stratified by spleen volume and randomized 1:1 to receive eliglustat (50 or 100 mg twice daily depending on plasma levels) or placebo. Efficacy assessments include changes from baseline in spleen and liver volumes, hemoglobin, platelets, skeletal manifestations, disease-related biomarkers, Gaucher assessments (*e.g.*, therapeutic goals), and quality of life. Safety monitoring includes adverse-event reporting (severity, seriousness, treatment-relatedness), physical examination and scheduled laboratory and electrocardiographic evaluations.
Results: After 9 months of treatment, eliglustat demonstrated superior efficacy compared to placebo in the primary endpoint (change in spleen volume in multiples of normal) with an absolute difference of 30% (28% decrease in eliglustat-treated patients *versus* 2% increase in placebo patients; $P<0.0001$). All secondary endpoints were met and showed statistically significant improvements compared to placebo; hemoglobin levels increased from baseline by an absolute difference of 1.2 g/dL compared with placebo ($P<0.0006$); liver volume decreased from baseline by an absolute difference of 7% compared with placebo ($P<0.0072$); and platelet levels increased from baseline by an absolute difference of 41% compared with placebo ($P<0.0001$). Among tertiary endpoints, a statistically significant improvement in total bone marrow burden score was observed among patients in the eliglustat arm compared to placebo, and all other markers of bone disease showed trends towards improvement. No serious adverse events were reported in either treatment group. All adverse events reported were mild or moderate, with the most common being headache, arthralgia and diarrhea. One patient withdrew from the trial, for a reason not treatment-related and the remaining 39 patients completed the blinded period and transitioned to the open-label extension period.
Summary and Conclusions: Eliglustat continues to show promising efficacy and safety in its clinical development program.

P983

CHARACTERIZATION OF THE PHARMACOKINETIC AND PHARMACODYNAMIC PROPERTIES OF PACRITINIB (SB1518), A NOVEL ORAL JAK2/FLT3 INHIBITOR, IN PATIENTS WITH MYELOFIBROSIS, AML AND LYMPHOMA

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Background: Pacritinib is a novel selective JAK2-FLT3 inhibitor with demonstrated antitumor activity in two mouse models of FLT3- (MV4-11) and JAK2-dependent (BaF3-JAK2V617F) leukemia. To date, a total of 4 clinical trials of over 3 years in duration are either ongoing or have been completed in patients with advanced malignancies (n=191) along with 2 single dose pharmacokinetic studies in healthy volunteers (n=42).
Aims: To evaluate whether the PK/ PD profile of pacritinib supports further clinical development of pacritinib in myelofibrosis.
Methods: Two PK studies were conducted in healthy volunteers to assess the effect of food on the PK of pacritinib and the inter- and intra-individual PK variability of pacritinib. Moreover, population PK of pacritinib was characterized following multiple dose administration of pacritinib in patients with advanced myeloid malignancies. To provide proof of concept in support of the starting dosage selection for pacritinib in myeloid malignancies, the effect of pacritinib on phos-STAT3 was assessed in myeloid cell lines Karpas 1106P and 32D. The effects of pacritinib on phos-STAT5 were also examined on *ex vivo* expanded erythroid progenitors (EPs) from patients with PV and on the EPs of healthy volunteers.
Results: Pooled analyses of PK assessments from the completed clinical trials in patients at pacritinib dose levels up to 600 mg QD showed slow absorption (Tmax~6 hrs) and dose-related increases in exposure. In addition, the results demonstrated long elimination half-life (mean Day 1 $t_{1/2}$=47 hrs) supporting QD administration of pacritinib. Comparison of drug concentrations on Days 1 and 15 showed a 1.5- to 2-fold increase in exposure at steady-state. However, no additional accumulation of drug was observed after repeated administration over several 28-day cycles. While between-subject variability was relatively high (28-45%), the within-subject variability was low (13-15%), highlighting the consistent systemic exposure for pacritinib in individual subjects. Pacritinib is not a PgP substrate and no significant formation (*i.e.*, <10% of parent exposure) of metabolites of pacritinib was observed in metabolism studies indicating limited liability of pacritinib to metabolic and PgP-related drug-drug interactions. There is no significant effect of food on pacritinib PK; hence pacritinib can be orally administered without regard to timing of meals. At a pacritinib 100 mg QD regimen, mean steady-state plasma levels of pacritinib exceeded the *in vitro* IC50 values for inhibition of targeted kinases (JAK2/FLT3) and inhibition of whole cell proliferation (BaF3-JAK2 and MV4-11 cells), lending support to dosage selection in Phase1/2 clinical trials for pacritinib. Pacritinib potently inhibited the proliferation of only a few tumor cell lines at submicromolar concentrations, consistent with its target selectivity. The most sensitive cell lines were either JAK2-dependent or mutant FLT3-dependent, including murine 32D (IC50=160 nM), human Karpas 1106P (IC50=240 nM), and mutant FLT3-dependent MV4-11 cells (IC50=32 nM). In a study using *ex vivo* expanded erythroid progenitors (EPs) treated with pacritinib, phos-STAT5 levels were inhibited in a dose-dependent manner (IC50 <200 nM) and reduced the viability of expanded EPs from both normal volunteers with JAK2wt (IC50=260 nM) and PV patients with JAK2V617F (IC50=230 nM), with no significant differences observed between groups. Moreover, pacritinib treatment had no effect on the JAK2V617F allele frequency in EPs from PV patients, indicating similar drug sensitivity for EPs from the same patient, regardless of the presence of JAK2 mutation. A study to assess the effects of pacritinib on intracellular JAK2 signaling showed that phos-STAT3 was reduced in a dose-dependent manner in both Karpas 1106P and 32D cells (Figure 1).

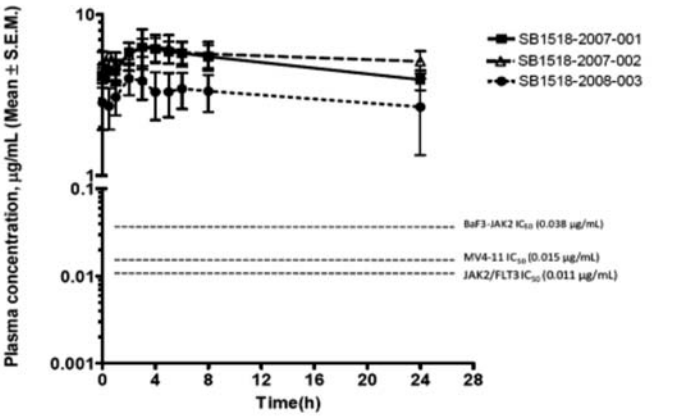

Figure 1. Mean Pacritinib (SB1518) plasma levels at day 15 assessments for patients receiving 100 mg QD. (Error bars indicate the S.E.M.).

Summary and Conclusions: Overall, the favorable PK/PD profile of pacritinib along with an acceptable safety, tolerability and efficacy profile of pacritinib support further clinical development of pacritinib in myelofibrosis.

P984

PRECLINICAL PK/EFFICACY RELATIONSHIPS FOR MLN9708, AN INVESTIGATIONAL SMALL MOLECULE PROTEASOME INHIBITOR, IN MM.1S HUMAN MULTIPLE MYELOMA XENOGRAFTS

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Background: MLN9708 (ixazomib citrate) is an investigational oral proteasome inhibitor currently in Phase III trials in relapsed and/or refractory (RR) multiple myeloma (MM) and in relapsed or refractory light chain amyloidosis (RRAL). In Phase I/II studies, MLN9708 has demonstrated preliminary activity and a generally well-tolerated safety profile as a single agent in RRMM and RRAL and in combination with lenalidomide and dexamethasone in newly diagnosed MM. In plasma, MLN9708 completely hydrolyzes to the pharmacologically active compound MLN2238. In preclinical studies performed in immunocompromised mice, twice weekly IV or PO dosing of MLN2238 at or near its maximum tolerated dose (MTD) has demonstrated antitumor activity against the MM.1S human plasmacytoma xenograft model. However the pharmacokinetic/efficacy (PK/E) relationship for MLN2238 in MM.1S xenograft-bearing mice has not yet been determined.
Aims: To develop a PK/E model for MLN2238 in MM.1S xenograft-bearing

mice at multiple dose levels and to compare the findings to human plasma PK data from Phase I clinical trials to place the clinically achieved exposures in the context of the preclinical findings.
Methods: Single dose studies were conducted to model the plasma PK of MLN2238 using SCID mice dosed IV at2, 3.5, 7 and 14 mg/kg. Studies to determine antitumor activity of MLN2238 in SCID mice bearing MM.1S xenografts were performed with IV twice weekly (BIW) dosing at 0.5,1, 1.5,2,3, and 7 mg/kg. Exponential regressions were performed on the tumor volume data to determine the tumor growth rate in individual mice. Individual tumor growth rates were used to calculate the end of treatment (day 14) tumor volumes in each mouse. Percentage tumor growth inhibition (%TGI) was calculated as (avg day 14–avg day 1 tumor volume in treated mice)/(avg day 14 - avg day 1 volumes from the control mice).PK/E models were built by fitting the average growth rate and%TGI values to 14-day AUC estimated from the PK model.
Results: An IV three compartmental model was found to best capture the drug kinetics in SCID mice. The PK model exhibited a linear relationship between dose and exposure (AUC) for the range tested, which is consistent with the population PK model from clinical Phase I trials. Pronounced antitumor activity, including complete regression, was observed in MM.1S xenografts.We found a linear relationship between average growth rate and AUC. The dose resulting in tumor stasis was estimated to be 2.4 mg/kg IV BIW. The drug exposure to%TGI relationship was captured with a sigmoidal Emax model, with steepness of 2.4 and with an IC_{50} value of 1.1 mg/kg. Next, we sought to place the derived PK/efficacy relationships in the context of the clinically achieved human exposures by accounting for the differences in MLN2238 plasma protein binding in mouse and human. At the average MTD exposure determined in Phase I clinical trials [single dose IV $AUC_{0\text{-}72}$ of 429 ng.hr/mL (28% CV)], the MM.1S xenograft model exhibited%TGI of 30-70%.
Summary and Conclusions: We have developed a dose-response relationship for MLN2238 in MM.1S xenograft model. To the best of our knowledge, this is the first time a PK/E relationship has been constructed for a proteasome inhibitor *in vivo*. Overlaying the free-fraction corrected clinical exposure data from Phase 1 trials of MLN9708 on the preclinical PK/E relationship, we find that the MM.1S model exhibits antitumor activity at exposures well-tolerated in humans.Additional preclinical studies are in progress to develop an oral PK/E model for MLN2238 in MM.1S xenografts.

P985

THERAPEUTIC POTENTIAL OF DUAL PI3K DELTA/GAMMA INHIBITION IN T-CELL LYMPHOMAS

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Background: Activation of the PI3K pathway triggers multiple events including cell growth, cell cycle entry, cell survival and motility. While α and β isoforms are ubiquitous in their distribution, expression of δ and γ is restricted to cells of the hematopoietic system. Because these isoforms contribute to the development, maintenance, transformation, and proliferation of immune cells, dual targeting of PI3Kδ and γ represents a promising approach in the treatment of lymphomas.
Aims: The objective of the experiments was to explore the therapeutic potential of RP6530, a novel, small molecule PI3Kδ/γ inhibitor, in T-cell lymphomas.
Methods: Activity and selectivity of RP6530 for PI3Kδ and γ isoforms and subsequent downstream activity was determined in several enzyme and cell-based assays. Additionally, RP6530 was tested for potency in viability, apoptosis, and Akt phosphorylation assays using immortalized T-cell lymphoma cell lines. ADME and pharmacokinetic properties of the molecule were also determined.
Results: RP6530 had high potency against PI3Kδ (IC_{50}=24.5 nM) and γ (IC_{50}=33.2 nM) enzymes with selectivity over α (>10,000-fold) and β (>100-fold) isoforms. Cellular potency was confirmed in target-specific assays, namely anti-FcεR1-(EC_{50}=30.5 nM) or fMLP (EC50=36.9 nM) induced CD63 expression in human whole blood basophils, LPS induced CD19+ cell proliferation in human whole blood (EC50=250 nM), and LPS induced CD45R+ cell proliferation in mouse whole blood (EC50=101 nM). RP6530 caused a dose-dependent inhibition (>50% @ 2-7 μM) in growth of immortalized (Jurkat, MOLT-4, CCRF-CEM, HuT-78, and Hut-102) T-cell lymphoma cell lines. Reduction in viability was accompanied by a reduction in pAKT (>50% @ 1 μM) along with induction of apoptosis (>50% @ 0.5-3.0 μM in the aforementioned cell lines. Pharmacokinetic studies across species indicated good oral absorption (>70% bioavailability for rat, and dog) with favourable plasma concentrations (>10 μM @ 10 mg/kg for rat, and dog) relevant for efficacy.
Summary and Conclusions: Results demonstrate that RP6530 is a potent and selective dual PI3Kδ/γ inhibitor with therapeutic potential in T-cell lymphomas. Further pre-clinical testing in murine xenografts and patient derived primary leukemic cells as well as toxicological evaluation of the molecule is in progress.

P986

EVALUATION OF GROWTH-INHIBITORY AND HISTAMINE RELEASE-TARGETING EFFECTS OF PKC412 AND ITS METABOLITES ON NEOPLASTIC MAST CELLS BY CHEMICAL PROTEOMICS PROFILING AND TARGET VALIDATION

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Background: The multikinase inhibitor midostaurin (PKC412) is a promising agent and currently investigated in clinical trials in advanced systemic mastocytosis (SM). However, although mediator-related symptoms and organ damage often improve, no long-lasting hematologic remissions are seen. *In vivo* two major metabolites of midostaurin are detectable, CGP52421 and CGP62221.
Aims: We examined the effects of PKC412 and its metabolites on proliferation of the human mast cell leukemia cell line HMC-1 and primary neoplastic mast cells (MC), as well as IgE-dependent histamine release in human basophils.
Results: All three compounds suppressed IgE-dependent histamine secretion in basophils, with IC_{50} values <1 μM. PKC412 and CGP62221 produced growth inhibition in HMC-1.1 cells (KIT D816V-) and HMC-1.2 cells (KIT D816V+), with IC_{50} values ranging between 50 and 250 nM, whereas CGP52421 showed no substantial growth-inhibitory effects below 1 μM. Corresponding results were obtained with primary neoplastic MC, although in some patients, CGP52421 showed growth-inhibitory effects. As assessed by light microscopy, active caspase 3 staining and TUNEL assay, midostaurin and CGP62221 induced apoptosis in HMC-1.1 cells and HMC-1.2 cells, whereas CGP52421 did not produce apoptosis in HMC-1 cells. Midostaurin and CGP62221 were found to inhibit phosphorylation of KIT D816V in HMC-1.2 cells, whereas CGP52421 showed no substantial effects. Chemical proteomics profiling and drug-competition experiments revealed that PKC412 interacts with several tyrosine kinases such as KIT, FES, AAK1, BIKE, and SYK. Interestingly, the key downstream-regulator of KIT D816V, FES, showed affinity for PKC412 and CGP62221, but not for CGP52421 in HMC-1.2 lysates. Finally, we were able to show that both metabolites exert synergistic growth inhibitory effects with cladribine (2CdA) in HMC-1.2 cells.
Summary and Conclusions: Together, our data show that the PKC412 metabolite CGP52421 inhibits IgE-mediated histamine release in basophils, but is a weak inhibitor of MC proliferation, presumably because of altered target-binding capacity. These observations may have clinical implications and may explain why mediator-related symptoms improve in PKC412-treated patients with SM, even if no hematologic remission is obtained.

P987

PULMONARY EFFECTS OF PERFLUOROCARBON EMULSION THERAPY ON INFECTED SICKLE CELL MICE

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Background: Pulmonary crises in patients with sickle cell disease (HbSS) are linked to occlusion of pulmonary blood vessels and bacterial infection, most commonly *Streptococcus pneumoniae.* Patients experiencing a pulmonary crisis are often hypoxemic which induces red cell 'sickling' and further vaso-occlusion. Patients with HbSS are profoundly sick. Treatment is supportive with antibiotics, fluids and occasionally exchange blood transfusion. Perfluorocarbon emulsions (PFCEs) are a potential alternative therapy to blood transfusion in severe sickle cell lung disease. However, there are few data on the effects of PFCEs in sickle cell disease. In this study, we investigated the effects of intravenous therapy with PFCE on transgenic sickle cell and control mice infected with *Streptococcus pneumoniae*.
Aims: Although blood transfusion can be life saving in patients with sickle cell anemia experiencing life-threatening crises, it is not without risk. Perfluorocarbon emulsions (PFCE) are blood substitutes that may have therapeutic value in patients with HbSS. This study set out to determine whether intravenous PFCE therapy alters the clinical course of transgenic HbSS mice infected with *Streptococcus pneumoniae*.
Methods: HbSS and C57 (control) mice infected with *S. pneumoniae* and treated with IV PFCE or PBS (3mL/kg) were managed in air with some HbSS mice also transiently exposed to oxygen (50%). Mice were culled and lungs were harvested at 72 hours or on showing signs of 2+ lethargy. A second group of mice were injected with PFCE daily for 1 week. Histological analysis of lungs was performed using H&E sections and light microscopy. In addition, white blood cell analysis was performed using flow cytometer and cytokine quantifications were performed using lung homogenate and sandwich ELISA assay.

Results: All mice infected with *S. pneumoniae* developed outward signs of infection within 72 hours. HbSS mice infected with *S. pneumonia* treated with PBS died significantly faster than C57; almost 50% of C57 mice were still alive (not culled) at 72 hours. In contrast, all HbSS mice treated with PBS were culled by 72 hours. There was no difference in survival comparing HbSS mice treated with PFCE-Air and PBS-Air following *S. pneumoniae* infection. However, HbSS mice treated with PFCE-O_2 showed a greater resilience to *S. pneumoniae* than those treated with PBS-O_2 or PFCE-Air. Treatment with PFCE did not improve resilience of C57 mice to infection with *S. pneumoniae*.
Pulmonary cytokine levels were significantly higher in lungs of *S. pneumoniae*-infected HbSS mice in comparison to *S. pneumoniae*-infected C57 mice. There were no statistical differences in levels cytokines comparing HbSS mice treated with PFCE or PBS. However, HbSS mice treated with PFCE-O_2 following *S. pneumoniae*-infection had significantly lower levels of cytokines than *S. pneumoniae*-infected HbSS mice treated with PFCE-Air
Non-infected HBSS mice were found to have significant airway and interstitial fibrosis. Following repeated exposure to PFCE daily for a week, the lungs of HbSS and C57 mice showed no significant histological difference. However, PFCE treatment for a week was found to significantly reduce the percentage of lung covered by fibrotic tissue from approximately 80% to near 40%. In addition, the spleens of HbSS mice exposed to PFCE daily for a week were significantly smaller than spleens of PBS-treated HbSS mice.
Summary and Conclusions: HbSS mice treated with PFCE and oxygen survived significantly longer than HbSS mice managed with PFCE and air. The improved outcomes were associated with lower pulmonary inflammation. PFCE therapy had no effect on survival of *S. pneumonia*-infected C57 mice or on *S. pneumoniae* growth. Repeated exposure to PFCE was associated with reduced lung fibrosis in HbSS mice and decreased spleen size. The findings suggest that PFCE may have a role in treating patients with sickle cell anemia.

P988

THE BTK-SPECIFIC INHIBITOR ONO-4059 SYNERGIZES WITH THE BCL2 INHIBITOR ABT-263.

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Background: Bruton's tyrosine kinase (BTK) mediates signaling from a number of receptors including the B-cell receptor (BCR). BTK is not a genetic "driver" of B-cell malignancy, but the maintained dependence of certain B-cell malignancies on BCR signaling has allowed successful therapeutic introduction of the BTK inhibitor (BTKi) Ibrutinib; CLL and mantle cell lymphoma are most sensitive. However, the Ibrutinib kinome is broad and more specific inhibitors might be clinically advantageous.
Aims: ONO-4059 is a specific BTKi currently in Phase I development in NHL/CLL. We explored the activity of ONO-4059 as single agent and in combination using a panel of derived cell lines.
Methods: We used *in vitro* assays of cell death and proliferation in a panel of 60 hemopoietic cell lines including 16 BCP-ALL, 14 DLBCL, 7 BL, 4 MCL and 3 myeloma cell lines. ONO-4059 was used alone and in combination with wide variety of clinically relevant compounds, including the BCL2 inhibitor ABT-263 (Navitoclax).

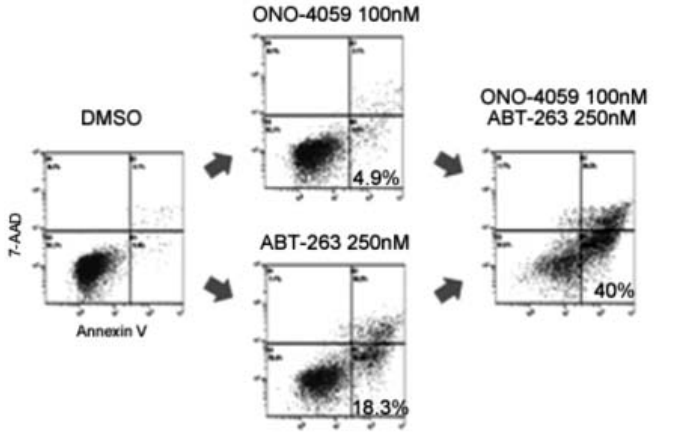

Figure 1.

Results: 2 ABC-DLBCL (TMD-8, OCLy10) and 1 MCL cell line (REC-1) were the most sensitive with EC50s of 5.5nM, 15.8nM and 19.6nM respectively. Genome-wide exomic DNA sequence analysis of these three cell lines showed no common mutational spectrum. ONO-4059 induced rapid decreases of P-BTK and P-ERK in these cell lines. However, cell death with morphological features of apoptosis and caspase activation did not occur until between 48 and 72 hours after exposure to ONO-4059. Interestingly, two ABC and one GCB DLBCL cell lines showed partial sensitivity to ONO-4059, with only a fraction of cells undergoing cell death. To assess possible pharmacological interactions, the cell lines were assessed for synergy with ABT-263. There was synergy with ONO-4059 in the BTKi sensitive cell lines as shown in the FACS profiles below obtained with the U2932 ABC-DLBCL cell line. This synergy may reflect increased BIM protein expression following pERK inhibition, causing increased sensitivity to BCL2 inhibitors (Figure 1).
Summary and Conclusions: These data indicate mechanism-based synergy between BCL2 and ONO-4059 and may have therapeutic implications.

P989

EFFICIENCY OF CD19 TARGETING BY MONOCLONAL ANTIBODIES IN *EX VIVO* AND MOUSE MODELS OF CHILDREN ACUTE B-LYMPHOBLASTIC LEUKEMIAS

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Background: Treatment of B-acute lymphoblastic leukemia (B-ALL), first cancer of children, is based on conventional chemotherapy, mostly permitting to obtain recovery. Nevertheless, the high cure rate is still associated with acute toxicities, long-term sequelae and refractory forms. Monoclonal antibodies (Mabs), as shown in rituximab model, can have direct effects in inducing apoptosis or arresting proliferation of tumor cells and indirect effects, including antibody dependant cytotoxicity (ADCC) and complement dependant cytotoxicity (CDC). Widely used in clinical oncology, no one is currently recommended in B-ALL treatment. The CD19 glycoprotein has already successfully been targeted in B-cell lymphoma and chronic lymphoid leukemia pre-clinical studies; because of its high expression on the surface of nearly all the B-ALL cells, it could represent an attractive target in children B-ALL.
Aims: We would demonstrate an antitumor efficiency of anti-CD19 Mabs in B-ALL mouse models, and we will explore their main cytotoxicity mechanisms in *ex vivo* pediatric B-ALL samples.
Methods: IDD001 or IDD002 humanized anti-CD19 IgG were generated by iDD biotech with a similar epitope site and different Fc domains. ADCC and CDC activities were tested using a calcein fluorescence-based assay, *in vitro* on B-ALL cell lines (REH and RS (4;11)) and *ex vivo* on pediatric B-ALL samples; effector lied in modified NK-cells (highly expressing CD56) for ADCC assay and in rabbit serum (20%) for CDC assay. Pediatric B-ALL were reproduced in severe combined immunodeficiency (SCID) mouse model by injecting REH or RS (4;11) cell line with a 6 to 12 weeks long tumor development; efficiency of Mabs was then tested in a 5 groups experiment (5 mouses per group in REH model, 8 in RS (4;11) model); dexamethason was administrated to a group as a reference treatment (1 mg/kg per day 5 days a week), rituximab as a negative control (30 mg/kg twice a week); IDD001 and IDD002 were tested by intraperitoneal injection at 30 mg/kg twice a week in REH model and 10 mg/kg twice a week in RS (4;11) model) (Figure 1).

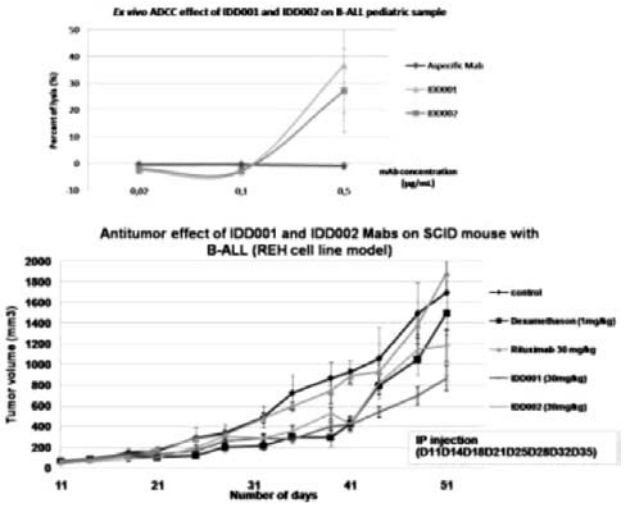

Figure 1.

Results: From its proprietary MAbs library, iDD biotech has generated next-generation, humanized, Fc optimized and afucosylated MAbs anti-CD19 (patent WO/2012/010561/01056), to enhance binding of FcγRIIIa and ADCC against a broad range of B-lymphoma and leukaemia cell lines with or without CDC (MAb anti-CD19 IDD001 *versus* MAb anti-CD19 IDD002). Importantly, iDD

biotech MAbs anti-CD19 mediated significantly higher level of apoptosis compared with other anti-CD19 MAbs under development. Moreover, generic wild type CHO cell lines can be used as host cells to produce IDD001 or IDD002 anti-CD19 MAbs. *In vitro*, IDD001 or IDD002 Mabs enhanced strongly and consistently ADCC, on B-ALL cell lines as well as on B-ALL samples (fig 1); this was found to be specifically due to CD19 targeting. In REH mouse model, IDD001 or IDD002 MAbs significantly inhibited B-ALL growth during the 8 weeks long treatment (fig 2); the same effect was observed in RS (4;11) mouse model with Mabs administrated for 3 injections. For these two experiments, growth inhibition induced by Mabs was similar than inhibition induced by dexamethason during the treatment period, and rebound effect at the end of treatment wasn't significantly different between Mabs and dexamethason.

Summary and Conclusions: These pre-clinical findings provide strong support for further experiments and for clinical development of humanized IDD001 or iDD002 anti-CD19 Mabs, either as a monotherapy in B-ALL refractory forms or in combination with conventional chemotherapy for severe prognosis forms. Based on rituximab experience, we could expect a good tolerance in human therapeutics with limited toxicities, mainly drug infusion reactions or prolonged lymphopenia.

P990

INFLUENCE OF HISTONE DEACETYLASE INHIBITORS (HDACI) ON CD20 EXPRESSION AND EFFICACY OF ANTI-CD20 MONOCLONAL ANTIBODIES

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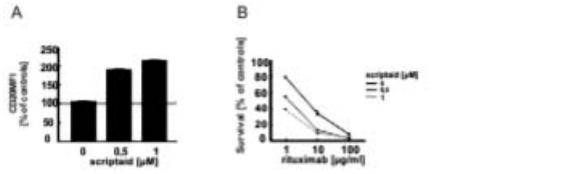
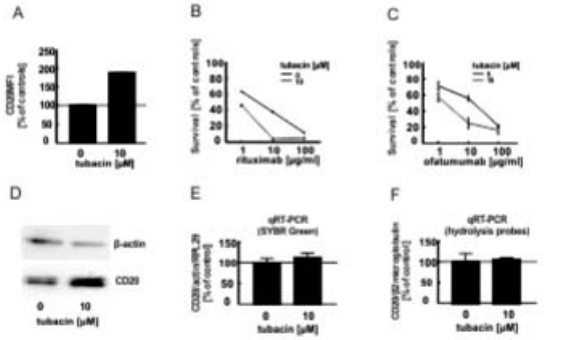
Background: CD20 is an integrate membrane protein widely expressed on the surface of normal and malignant B-cells. It is an excellent molecular target for monoclonal antibodies (mAbs) that are widely used in the therapy of non-Hodgkin's lymphomas and chronic lymphocytic leukemia (CLL). Anti-CD20 mAbs trigger effector mechanisms of immune response and such a therapy is characterized by high efficacy, low toxicity and minor side-effects. Accumulating evidence indicates that CD20 can be modulated at several levels, both transcriptional and posttranscriptional and its uP-regulation would result in increased efficacy of anti-CD20 mAbs. CD20 antigen has been reported to be regulated epigenetically *e.g.* by blocking the activity of histone deacetylases (HDACs). Such observations has been made in B-lymphoma cells with very low basal CD20 level. The use of non-selective pan-inhibitors of HDACs (HDACi) gives promising results both *in vitro* and *in vivo* in several tumor models, including hematologic malignancies. The results of our preliminary experiments show that use of HDACi leads to uP-regulation of CD20 protein in B-cell lymphoma independently of basal CD20 levels and subsequent increase of the efficacy of therapy with anti-CD20 mAbs (Figure 1).



Influence of non-selective inhibition of HDACs on CD20 level and efficacy of rituximab.
Raji cells pretreated for 48h with scriptaid were incubated with saturating amount of FITC-conjugated anti-CD20 mAb for 30 min in room temperature in the dark. The binding of mAb was determined with flow cytometry (A).
Raji cells pretreated for 48 h with scriptaid were washed and incubated for 60 min with increasing concentrations of rituximab and 10% human AB serum. Cell viability was measured with PI staining using flow cytometry (B).



Influence of selective inhibition of HDAC6 on CD20 level and efficacy of rituximab.
Raji cells pretreated for 48h with tubacin were incubated with saturating amount of FITC-conjugated anti-CD20 mAb for 30 min in room temperature in the dark. The binding of mAb was determined with flow cytometry (A).
Raji cells pretreated for 48 h with tubacin were washed and incubated for 60 min with increasing concentrations of rituximab (B) or ofatumumab (C) and 10% human AB serum. Cell viability was measured with PI staining using flow cytometry.
Lysates from Raji cells preincubated for 48 h with tubacin were separated in a polyacrylamide gel. CD20 and actin were detected with corresponding antibodies (D).
RNA from Raji cells preincubated for 48 h with tubacin was isolated with TRIzol and used for first strand cDNA synthesis. cDNA was used for quantitative real time PCR (*qRT-PCR*) amplification of CD20 and actin/RPL29 products (with SYBR Green I) (E) or quantitative real time PCR amplification of CD20 and β2-microglobulin products with the corresponding hydrolysis probes labeled with Fam and Dabcyl (F).

Figure 1.

Aims: The aim of this study was to understand which HDAC isoforms are responsible for the observed effect of CD20 uP-regulation. Determination of a specific isoform influencing CD20 expression could help us decipher the molecular mechanism in which HDAC inhibition increases CD20 expression in human B-cell tumors.

Methods: This study required use of B-cell lymphoma cell lines as well as lymphocytes infected with EBV. Several HDAC pan-inhibitors as well as class/isoform selective inhibitors were tested. To assess the membrane level of CD20 antigen, FITC-conjugated anti-CD20 antibody staining was performed followed by cytometric analysis. The influence of HDACi on total level of CD20 protein was assessed in Western blotting using specific antibodies. The complement-dependent cytotoxicity (CDC) assay was performed using rituximab and ofatumumab as well as human serum as a source of complement. Cell cytotoxicity was assessed by propidium iodide staining followed by cytometric analysis. The influence of HDAC inhibition on the transcription of CD20 was examined by qRT-PCR using both SyBR Green as well as hydrolysis probes.

Results: The results of our study strongly suggest that inhibition of HDAC6 with its specific inhibitor tubacin is sufficient of increase in CD20 level. Inhibition of HDAC6 isoform results in the increase if the efficacy of anti-CD20 mAbs. Tubacin augments total level of CD20 protein but it does not influence CD20 on transcriptional level. These results suggest that blocking HDAC6 activity might influence CD20 at posttranscriptional level.

Summary and Conclusions: The results of our study strongly suggest that combining HDACi with anti-CD20 antibodies can be an effective therapeutic modality for patients suffering from B-cell malignancies. Our experiments indicate that selective inhibition of HDAC6 is sufficient for uP-regulation of CD20 level and may have potential clinical application in hematological malignancies. This observed regulation does not seem to involve transcriptional mechanism. However, the molecular mechanisms of the observed phenomenon need to be elucidated. Extensive experiments aiming at determining what factors are engaged in the regulation of CD20 by HDAC6 will be performed.

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BOTANICAL ALKYL HYDROQUINONE HQ17(3) EXHIBITS CYTOTOXIC EFFECT ON SUP-B15 ALL CELLS HARBORING T(9;22) PHILADELPHIA CHROMOSOME THROUGH INDUCING IRON-DEPENDENT LYSOSOMAL EVENTS AND OXIDATIVE STRESS

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Background: Acute lymphoblastic leukemia with Philadelphia chromosome (Ph^+-ALL)(t(9;22)*BCR-ABL*) is a very high risk (VHR) hematological neoplasm constitutes 20-30% adult ALL and 2-3% of childhood ALL. Multiple cooperative genetic lesions together with constitutive BCR-ABL onco-protein contribute to a very aggressive clinical cause, hence tyrosine kinase inhibitors (TKIs) is not sufficient to convey long-term control of the disease. Thus, searching for agents with higher specificity to leukemias and investigating the molecule mechanisms involved in the selective cytotoxic effects on leukemic cells will be beneficial for finding new anti-leukemic therapeutics for the Ph^+-ALL. HQ17(3) [10'(Z),13'(E),15'(E)-heptadecatrienyl-hydroquinone], is a natural product isolated from the sap of *Rhus succedanea*. HQ17(3) has been reported to have cytotoxic activity on tumor cell lines and HL-60 AML cells. We found HQ17(3) exhibited very effective cytotoxic effect on the TKI-resistant Ph^+-ALL cell line SUP-B15 (IC_{50}: 1.9 μM), but spared normal peripheral blood mononuclear cells.

Aims: To investigate the characters of, and the molecular pathways involved in the HQ17(3)-induced cytotoxic effects in Ph^+-ALL (VHR ALL harboring t(9;22)) cells.

Methods: HQ17(3)-treated and control SUP-B15 cells were subjected to the following tests: 1) membrane lipid disturbance was analyzed by Annexin V/PI stain, 2) DNA fragmentation was defined as sub-G1 fraction of cellular DNA content after the PI staining, 3) mitochondrial membrane potential loss were stained by DiOC6(3). The stained cells were subjected to flow cytometric analysis. Pan-caspase inhibitor (zVAD-fmk), receptor interacting protein 1 (RIP1) inhibitor (necrostatin-1, Nec-1), or iron-chelator (deferroxamine, DFO) were used in combination with HQ17(3) in some experiment. 4) Acridine orange stain and confocal microscopy are used to visualize the changes of lysosomes in the presence of HQ17(3).

Results: Introduction of HQ17(3) induced extensive cell death in 24 hours. Loss of plasma membrane integrity (PI^+) occur concomitant with phosphatidylserine exposure (Annexin V^+), which was not prevented by zVAD-fmk, Nec-1 or both, indicating a caspase-independent necrotic death program. HQ17(3)-induced cell death displays mitochondrial membrane potential loss and profound nuclear DNA fragmentation. ROS scavengers (GSH or vitamin C) attenuated HQ17(3)-induced cell death and the associated damages, indicating ROS production/oxidative stress account for important part of the cell demise. Of notice, DFO abolished the HQ17(3)-induced cell death, suggesting iron-dependent event(s) (such as hydroxyl radical production by Fenton reaction took place in lysosomes) is critical for the HQ17(3)-induced cell destruc-

tion. We also found both the number and size of acidic vesicles (lysosomes) are significantly increased after 4-hour treatment of HQ17(3) and diminished after 10 hours (when cell death is evident).
Summary and Conclusions: Naturally-derived HQ17(3) displayed selective, and significant anti-leukemic activity in Ph+ ALL (SUP-B15) cells by a caspase-independent, necrotic death program. Iron-dependent events and ROS production contribute to this potent cell destruction process. Lysosomal enlargement and membrane permeability are evident in HQ-induced cell death. These results suggest that agents selectively induce or sustain ROS in leukemic cells may induce lysosomal events, and would potentially augment the treatment for VHR-ALL with t(9;22) translocation.

P992

REACTIVATION OF SELECTED P53 MUTANTS IN B-CELL TUMOR CELL LINES USING TARGETED SMALL MOLECULES

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Background: The tumour suppressor protein p53 is a transcription factor that has an essential role in guarding the cell from genotoxic stress. By contrast, mutated p53 can be extremely dangerous as it may gain new oncogenic properties and thus enhance tumorigenesis. Mutated p53 frequently accumulates in cancer cells and it has been shown that subtle molecular manipulation can lead in some cases (in some mutations) to p53 reactivation to its wild-type conformation. Although p53 mutations are frequent in various types of tumors, prognosis of affected patients is especially poor in hematological malignancies.
Aims: To assess a feasibility of mutated p53 reactivation in B-cell lines using comercially available small molecules.
Methods: For p53 reactivation, the following cell lines were used: RAMOS (Burkitt lymphoma; mutation I245D and 17P-); RAJI (Burkitt lymphoma; mutations R213 and Y234H); and SU-DHL-4 (DLBCL; mutation R273C and 17P-). All cell lines expressed high level of mutated p53 protein. Small molecules PRIMA-1 (Cayman Chemical Company) and ellipticine (Calbiochem) were used as reactivation agents. Cell lines were cultured under standard conditions (37 °C, 5% CO_2); Since RAJI cell line harbors temperature-sensitive p53 mutation (Y234H), these cells were also tested for reactivation at 30 °C. The reactivation was tested at the p53 protein level by western blot (WB) and at p53 downstream pathway by quantitative real-time PCR.
Results: In all three cell lines, both PRIMA-1 and ellipticine afftected cellular viability in a concentration-dependent manner (50; 25; 12.5 and 6.25 μM and 10; 5; 2.5 and 1.25 μM, respectively). Time-dependent effect (24, 48, and 72 h) was substantially more apparent in the case of ellipticine. The same concentration range was subsequently applied for testing of p53 reactivation by WB. In RAMOS cells, PRIMA-1 reduced mutated p53 level at two highest concentrations and already after 24 h. In RAJI cells the reduction of mutated p53 protein level was obvious only at 30 °C; under these conditions the two highest concentrations again led to disappearance of mutated p53, prominently after 72 h. Situation with SU-DHL-4 cells was similar, as the two highest concentrations led once again to disappearance of mutated p53. For ellipticine, WB was performed after6,12, and 24 h. In RAMOS cells, the most pronounced effect was observed at highest concentration after 6 and 12 h cultivation; the cells cultured 24 h exhibited already massive apoptosis. In RAJI cells, the reduction of mutated p53 was only very subtle at both 37 °C and 30 °C. Analysis of p53 downstream target genes induction was performed after 24, 48, and 72 h. Concerning PRIMA-1, RAMOS cell line was inert to any reactivation. In RAJI cell line, we observed a subtle induction (up to 350% compared to untreated control set at 100%) of *p21* and *PUMA* genes. In SU-DHL-4 there was a clear induction (620%) of *p21* gene, but only at a single time interval. Regarding ellipticine, we observed subtle induction of *BAX* in RAJI cells and no induction of the studied genes in RAMOS cells.
Summary and Conclusions: Our results show that the small targeted molecules can manipulate with mutated p53 protein in cancer B-cells. These molecules can lead to mutated p53 reduction (clinically highly desirable due to predicted mutated p53 oncogenic gain-of-function) and to induction of cell cycle regulatory and proapoptotic genes. However, further experiments are necessary to more deeply understand exact mechanisms standing behind these effects. Supported by grant NT/13519-4/2012 by IGA MH CR and by project MUNI/A/0723/2012.

P993

NF-KB IS A POTENTIAL MOLECULAR TARGET OF EBV-POSITIVE T- OR NK-CELL LYMPHOPROLIFERATIVE DISORDERS

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Background: Epstein–Barr virus (EBV) rarely infects T- or NK cells, in addition to B cells, and causes EBV-positive T-/NK-cell lymphoproliferative disorders (EBV-T/NK-LPDs) such as extranodal NK/T-cell lymphoma (ENKL), aggressive NK-cell leukemia, and chronic active EBV infection (CAEBV). Because the mechanisms responsible for development of these EBV-induced malignancies have not been elucidated yet,optimal chemotherapy, especially for CAEBV, has not been established and the EBV-T/NK-LPDprognosis remains very poor.NF-κB is a transcription factor that mediates anti-apoptotic molecular signaling and promotes cancer cell proliferation. It is constitutively activated in some malignancies, and the proteasome inhibitor bortezomib can suppress its activity and is clinically used as an anti-cancer reagent. NF-κB is also activated by EBV infection in B-cells and contributes to infected cell survival.
Aims: To clarify the molecular mechanism underlying the development of EBV-T/NK-LPD by investigating the role of NF-κB
Methods: Four EBV-positive T- and NK-cell lines, SNT8, SNK6, SNT15, and SNT16, were examined. The EBV-negative T-cell lines Jurkat and Molt4 and the EBV-negative NK-cell line KHYG1 were used as the negative controls. Clinical samples were obtained from CAEBV patients diagnosed according to the criteria of Okano M. *et al.* (Am J Hematol 80:64-9, 2005). To detect and isolate EBV-infected cells, T- and NK-cells were separated using magnetic beads from peripheral bloodmononuclear cells (PBMCs). For *in vitro* EBV infection, EBV was prepared from the culture medium of B95-8 cells and added to MOLT4 cells (PNAS 100:7836-40, 2003). NF-κBactivation was examined by western blotting, electrophoretic mobility shift assay (EMSA), and reporter assay. The proteasome inhibitor bortezomib was used for NF-κB inhibition. The effects were examined in not only *in vitro* but also in a xenograft model of EBV-T/NK-LPD generated by transplantation of PBMCs from the CAEBV patients to NOD/Shi-scid, IL-2R γKO mice.
Results: Constitutive NF-κB activation represented by nuclear localization and DNA binding of p50, p52, and RelA, was detected in the cell lines and EBV-infected T- or NK-cells derived from the CAEBV patients (infected cell types: CD4 in 3; CD8 in 3; γ δ in 1; and CD56 in 3). Supershift EMSA revealed that NF-κB–DNA binding complexes in these cells involved p50, p52, and RelA. EBV infection of MOLT4 cells induced constitutive NF-κB activation and inhibited cell death induced by serum depletion or VP16 treatment in the infected cells. Luciferase assay demonstrated that LMP1, in particular, and LMP2A, to a lesser extent, upregulated NF-κB-dependent reporter gene expression in MOLT4 and KHYG1 cells, whereas LMP2B and EBNA1 did not, suggesting that LMP1 and LMP2A mediated NF-κB activation in T- and NK cells. Bortezomib inhibited NF-κB activation, suppressed proliferation, and induced apoptosis in the EBV-T/NK cell lines and primary cells of CAEBV. Furthermore, intraperitoneal bortezomib administration reduced the EBV-DNA titer in peripheral blood of mice transplanted with EBV-T/NK-LPD cells.
Summary and Conclusions: EBV induces NF-κB-mediated anti-apoptotic signals in T- and NK cells and can contribute to tumor development. Suppression of NF-κB by bortezomib induces anti-tumor effects onEBV-T/NK-LPD not only *in vitro* but also *in vivo*.

P994

THE PI3K INHIBITOR NVP-BKM120 INDUCES APOPTOSIS AND AUTOPHAGY IN T-ALL AND BURKITT LYMPHOMA CELL LINES

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Background: Constitutively active PI3K/Akt/mTOR signaling has been well described in T-ALL. Recently, PI3K activity was reported to cooperate with the development of Burkitt lymphoma. Thus, the role of PI3K/Akt/mTOR in cell growth and survival, two important features of leukemogenesis, has rendered it as a potential pharmacological target in different types of hematological malignancies.
Aims: Herein, we studied the therapeutic potential of a highly selective pan-class I PI3K inhibitor, NVP-BKM120, a2,6-dimorpholino pyrimidine derivative developed by Novartis, on both T-ALL and Burkitt lymphoma cell lines.
Methods: The T-ALL cells lines, Jurkat and Molt-4, and the Burkitt lymphoma cell lines, Namalwa and Daudi, were obtained from ATCC. NVP-BKM120 was prepared as a 10mM stock solution in DMSO and different concentrations were used (0.5 μM, 1μM, 2 μM, 10μM and 50μM). Cell viability was measured using the MTT assay. Clonogenicity was determined by a colony-formation assay. Apoptosis was assessed by Annexin-V/PI staining and by caspase cleavage. Western blot analysis was performed by standard methods. Vital staining and flow cytometry analysis with acridine orange was performed for the detection and quantification of acidic vesicular organelles (AVOs). The comparisons between the groups were performed by the Mann–Whitney or *t* test. *P* value <0.05 was considered statistically significant.
Results: Rates of survival after NVP-BKM120 treatment were determined by MTT assays. All cell lines tested displayed an IC_{50} of around 10μM. NVP-BKM120 at 1μM was able to interfer with the long-term proliferative potential

of the cell lines, as observed by a significant decrease in colony formation. After 6 hours of NVP-BKM120 treatment, the percentage of annexin-V cells increased, as did the cleavage of procaspase-8, procaspase-9 and procaspase-3. Western blot analysis showed a dose-dependent decrease in Ser473 P-Akt, followed by a decrease in the antiapoptotic BCL2 and increase of proapoptotic BAX protein expressions, along with P70S6K dephosphorylation in all cell lines studied. Total Akt and P70S6K levels were unaffected by the drug. To identify the development of AVOs in Molt-4 and Namalwa cells, we used acridine orange, which fluoresces bright red in acidic compartments. NVP-BKM120 (2mM) was found to increase the strength of the bright red fluorescence signal in Molt-4 (from 6.8% to 50.8%) and in Namalwa cells (from 10.2% to 21.4%).
Summary and Conclusions: NVP-BKM120 treatment resulted in the apoptosis of T-ALL and Burkitt lymphoma cell lines, with the cleavage of procaspase-8 and procaspase-9, suggesting that both the intrinsic and extrinsic pathways of apoptosis are activated upon drug treatment. NVP-BKM120 treatment induced dephosphorylation of P70S6K, a mTORC1 downstream target, and also decreased Ser473 P-Akt. These results suggest that this molecule overcomes the signaling feedback loops between mTORC1, PI3K and Akt, commonly described after treatment with mTOR inhibitors. The decrease in BCL2 levels together with increased AVOs indicates the activation of autophagy, supporting the evidence of inhibition of mTOR signaling, the major negative regulator of autophagy in human cells. In conclusion, our findings suggest that the NVP-BKM120 compound represents a promising candidate for the therapy of T-ALL and Burkitt lymphoma patients with aberrant activation of the PI3K/Akt/mTOR pathway.

Cellular immunotherapy and vaccination

P995

THE DONOR DEPENDENT IMMUNE MODULATORY POTENTIAL OF MESENCHYMAL STROML CELL PREPARATIONS CAN BE PREDICTED BY GALECTIN-9

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Background: Therapeutic approaches using multipotent mesenchymal stromal cells (MSCs) are advancing in regenerative medicine, transplantation and autoimmune diseases. Until now the responsible factors for MSC-mediated immune suppression are still controversially discussed. Most probably a multifactorial set of factors determine the immune suppressive potential of MSCs and might explain the huge variations observed between single MSC preparations. It has been described that immunosuppression by MSCs is enhanced via stimulation with interferon-γ (IFN-γ). Recently galectins, a β-galactoside binding lectin family, have been added to the group of immune modulatory molecules that are responsible for MSC mediated immune suppression.
Aims: We aimed to find possible marker, which help to predict the immune modulatory potential between MSC donor preparations.
Methods: We randomly chose MSC donors and demonstrated that galectin-9 (Gal-9) expression is strong uP-regulated upon activation with interferon-γ (IFN-γ). We compared donors be mRNA and protein level and determined their immune modulatory potential on T-and B-cells with a proliferation assay. The *in vivo* effect of Gal-9 and MSCs was investigated in mice immunized with human coagulation factor VIII (FVIII) in the presence of human MSCs, anti-murine Gal-9, or murine Gal-9. FVIII was used as positive control.
Results: We demonstrated that Gal-9 is a major mediator of the anti-proliferative effect of MSCs on both, T-and B-cells. Activation of MSCs with IFN-γ resulted in a major decrease of proliferation of both T-cells and B-cells. In addition, Gal-9 and activated MSCs contribute to the suppression of triggered immunoglobulin release. Activation of MSCs with IFN-γ decreased the IgG release, whereas blocking Gal-9 neglected the effect almost completely. Additionally, we provide results that Gal-9 expression levels (mRNA and protein) can distinguish between different MSC donor preparations. In this context we confirmed the correlation between high Gal-9 levels and immune modulatory potential. Because compared to immune suppression by recombinant Gal-9, only relatively low Gal-9 levels from MSCs had a strong immune suppressive effect, the question emerged whether their suppressive nature results from cell-cell contacts or by secreted factors. Initial experiments give evidence that the effect is cell-cell contact related, since we observed no changes in immune cell proliferation, when we separated MSCs and immune cells via transwells. Further, only minimal amounts of Gal-9 could be found in supernatants after activation. In addition we demonstrated that MSCs, which were transfected with a his-tagged Gal-9 distributed this recombinant protein to immune cells in close proximity to the labelled Gal-9 expressing cells. I*n vivo* Both, MSCs and murine Gal-9 suppressed immunoglobulin induction. Murine Gal-9 suppresses $CD4^+$subsets, while only MSCs were able to reestablish the equilibrium of B-and T-cells in spleens. Additional experiments with isolated cells demonstrated that, in contrast to human immune cells, murine-derived T- and B-cells do not respond to human recombinant Gal-9, but human IFN-γ activated MSCs. These discrepancies may be based on only 60% homology of murine and human Gal-9. Other immune modulatory factors may then play a stronger role in this context.
Summary and Conclusions: In conclusion, we identified Gal-9 as a new player involved in MSC mediated immune modulation, which interfers with multiple cell types including T-and B-cells. Also, Gal-9 may serve as a predictive indicator for MSC preparations and clinical therapy. We are the first who demonstrate that Gal-9 influences B-cell functions and proliferation in a concentration dependent manner. In addition, Gal-9 is a major effector of MSC mediated immune modulation.

P996

INCREASING SAFETY AND EFFICACY OF TCR TRANSFER BY EMPLOYING A MINIMALLY MURINIZED HUMAN TUMOR-REACTIVE SINGLE-CHAIN TCR

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Background: Adoptive transfer of T cells modified by tumor antigen-specific T-cell receptor (TCR) gene transfer is a valuable strategy to induce tumor regression in cancer patients. However, transfer of α/β-double-chain (dc)TCR into human T cells harbors the risk of forming hybrid dimers with naturally expressed TCR chains.
Aims: In this study, we used a human single-chain (sc)TCR specific for the

melanoma-antigen gp100(280-288) constructed by linking the variable (V)α-chain to the TCR β-chain to prevent TCR mispairing.
Results: Retroviral cotransduction of the human scTCR together with the constant(C-) domain of the TCRα-chain (Cα) into human T cells did not result in sufficient TCR expression on the cell surface. Expression was restored when human C-domains were replaced by mouse C-domain counterparts. As mouse-derived proteins could be immunogenic in patients, we replaced only selected amino acids in the human constant domains of the scTCR/Cα by their corresponding mouse amino acids. However, this strategy resulted only in a moderate enhancement in scTCR-expression levels. To further improve stability, additional disulfide bonds between the scTCR and the Cα were introduced. This yielded in a substantial increase of expression, cytokine production and lysis of target cells by T cells bearing the minimally murinized (mm) scTCR/Cα. To study if the mm scTCR mispairs with TCRα-chains of other specificities, TCR-deficient Jurkat cells were transduced with different combinations of the scTCR and full length TCRα-chains. Cell surface expression of the mm scTCR by pairing with different human and murine TCRα-chains was demonstrated by anti-Vβ14-staining. However, low Vβ14-MFI in Jurkat cells transduced with the scTCR/TCRα in comparison to scTCR/Cα correlates with a low density of mispaired scTCR/TCRα-dimers on the cell surface. After co-incubation with gp100(280-288) peptide-loaded T2-cells, Jurkat cells harbouring mispaired scTCR/TCRα produced no IFN-γ, demonstrating that the scTCR is not able to interact with the gp100(280-288)-antigen when it mispairs with the irrelevant TCRα-chain. Similar experiments in human T cells showed a marginal expression of the scTCR in combination with an irrelevant TCRα-chain, probably due to favourable pairing of the full length TCRα-chain with the naturally expressed TCR.
Summary and Conclusions: These optimized human scTCR/Cα molecules represent promising candidates for adoptive T cell transfer by providing effective antitumor responses. Mispairing of scTCR with endogenous TCR chains cannot be excluded, but clinical relevance might be limited due to low expression levels of hybrid TCRs.

P997

LONG-TERM FOLLOW-UP OF ADOPTIVE IMMUNOTHERAPY WITH HAPLOIDENTICAL KIR-L MISMATCHED NATURAL KILLER CELLS AS POST-REMISSION CONSOLIDATION STRATEGY IN ELDERLY HIGH RISK ACUTE MYELOID LEUKEMIA PATIENTS

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Background: The most common cause of treatment failure in acute myeloid leukaemia (AML) patients is early relapse. Then, new consolidation strategies represent now one of the most interesting issues. Data from literature suggest that KIR mismatched NK cells may be transferred and expanded *in vivo* and may significantly impact on tumor cell killing, particularly in AML patients.
Aims: Aim of this work is to establish the feasibility and efficacy of adoptive immunotherapy with haploidentical KIR-L mismatched NK cells as consolidation therapy in elderly high-risk AML patients, who achieved CR.
Methods: Fourteen patients with high-risk AML (2 in molecular relapse and 12 in morphological complete remission (CR); with a median age of 62 years (range 53-73) received highly purified $CD56^+CD3^-$ NK cells from haploidentical KIR-ligand mismatched donors after fludarabine/cyclophosphamide –containing-immunosuppressive chemotherapy, followed by interleukin-2 subcutaneous administration (10×10^6 IU/day, 3 times weekly for 2 weeks -6 doses total) after NK cell infusion.
Results: The median number of infused NK cells was 2.74×10^6/Kg. No NK cell-related toxicity, including graft-*versus*-host disease (GVHD), was observed. Hematological recovery was comparable to standard chemotherapy: median time to neutrophil count recovery (ANC $>0.5 \times 10^9$/l) was 18 days (range 12-45), median time to platelet recovery (PLTs $>20 \times 10^9$/l) was 20 days (range 13-45). Both patients in molecular relapse achieved molecular CR, which lasted 9 months for both patients. Among 12 patients in morphological CR, 7 patients are disease-free after 28, 25, 63, 59, 49, 10 and 9 months (median 28 months; range 9-63), whereas 4 relapsed after3,5, 24 and 3 months; 3 relapsed patients ultimately died due to disease progression, one is receiving re-induction chemotherapy. One patient died during the neutropenic phase due to overwhelming bacteria pneumonia. Seven AML patients, who had achieved CR after the same induction/consolidation therapy and with similar prognostic features as the study patients, were excluded from NK therapy because they did not have KIR-ligand mismatched donors. Interestingly, analysis of clinical outcomes showed that 6/7 patients relapsed at a median of 4 months and died of disease progression, whereas one patient is alive and disease-free after 24 months post autologous stem cell transplant. After infusion, donor NK cells were found in the peripheral blood (PB) of all evaluable patients (peak value on day 10). They were also detected in the bone marrow (BM) in some cases (peak value on day 5). An association between serum IL-15 concentration and donor chimerism after NK cell infusion was observed. Particularly, the rise in IL-15 serum level was followed by increase in donor chimerism, thus supporting the conclusion that homeostatic IL-15 drives *in vivo* expansion and survival of adoptively transferred NK cells. Donor-*versus*-recipient alloreactive NK cells were demonstrated *in vivo* by the detection of donor-derived NK clones that killed recipient targets, including leukemic blasts.
Summary and Conclusions: Infusion of purified NK cells is feasible in elderly patients with high risk AML. Adoptively transferred NK cells were alloreactive against recipient cells and might have induced anti-leukemic activity.

P998

STAINING OF ANTIGEN-SPECIFIC CD4+ T CELLS USING MHC CLASS II TETRAMERS GENERATED ACCORDING TO A NOVEL METHOD OF PEPTIDE-MHC CLASS II MONOMER PRODUCTION AND PURIFICATION

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Background: CD4+ T cells recognize linear peptides bound in the peptide-binding cleft of MHC class II molecules displayed on the surface of antigen-presenting cells. CD4+ T cells are key players in adaptive immunity orchestrating both CD8+ T cells (*e.g.* activation, differentiation, proliferation, maintenance) and B cells (*e.g.* antibody class switching, high affinity antibody production). The use of MHC class I tetramers to stain antigen-specific CD8+ T cells emerged in 1996 and has since transformed the field of cellular immunology, effectively becoming the golden standard for direct enumeration, analysis and manipulation of CD8+ T cells. On the other hand generation of functional MHC class II complexes and MHC class II tetramers has proven challenging.
Aims: To develop an alternative method of MHC class II tetramers/multimer generation for the staining of antigen-specific CD4+ T cells.
Methods: Recombinant MHC class II alpha and beta chains were refolded *in vitro* in the presence of peptides that had been extended by a hexa-histidine sequence. The resulting peptide-MHC class II complexes could readily be purified and concentrated by immobilized metal affinity chromatography, and subsequently tetramerized using fluorochrome-labeled streptavidin.
Results: We have developed a novel method of MHC class II tetramer synthesis. With common HLA-DRB-alleles and various viral epitopes stemming from human cytomegalovirus and Influenza A virus we have subsequently demonstrated that these MHC class II tetramers can stain and purify antigen-specific, MHC class II-restricted CD4+ T lymphocytes.
Summary and Conclusions: The use of MHC class II tetramers could provide important information about the reconstitution of virus-specific CD4+ T cells and possibly the development of minor histocompatibility antigen-specific CD4+ T cells following allogeneic hematopoietic cell transplantation.

P999

PMHC ARRAYS DEMONSTRATE THAT "UNTOUCHED" T CELL POPULATIONS FROM PRESENTATION ACUTE MYELOID LEUKAEMIA PATIENTS PREDOMINANTLY RECOGNIZE THE CANCER-TESTIS ANTIGEN, PASD1

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Background: Peptide major histocompatibility complex (pMHC) arrays, also known as tetramer arrays, were developed to identify "untouched" populations of $CD8^+$ T cells in the peripheral blood of cancer patients. The technique is able to analyse multiple T cell populations using small numbers of $CD8^+$ T cells (~1.2×10^6 cells/array) and pMHC (1ng per spot, 1/1,000th of that used in flow cytometry) spotted onto polyacrylamide gels. The pMHC array can simultaneously analyse a large number of T cell populations without haplotype restriction.
Aims: To determine the tumour antigens and epitopes therein recognized by leukaemia patients at disease presentation.
Methods: Negatively isolated $CD8^+$ T cells were obtained, following informed consent and local ethical approval, from the peripheral blood of leukaemia

patients. T cells were lipophillically dyed with DiD fluorescent tracer and incubated with arrays printed with pMHCs from more than 50 tumour-associated antigen and viral epitopes (including HLA-A2/CMV and Flu controls). Positive scoring of T cell populations were only made when T cells were consistently bound to 3 of 6 of the same pMHC spots in two distinct regions on the array.
Results: We have analysed 33 leukaemia patients (25 AML, 3 ALL, 5 CML) and 18 normal donors. Although normal donor T cells recognized HLA-A2/Flu M1 and/or CMV pp65 and/or IE1 epitopes, no binding to tumour antigen epitopes were found. However we found that 11 of the 12 patients who had specific-T cell populations recognising epitopes within tumour antigens were AML patients and 8 of these recognized PASD1. The other four patients (3 AML and 1 ALL) had T cells which recognized epitopes within MUC1, Tyrosinase, CEAM5 and MelanA.
Summary and Conclusions: We have developed a robust method for the simultaneous analysis of T cell populations in leukaemia patients, the use of which can indicate a short-list of T cell populations for further investigation of T cell function, minimising reagent and sample use.

P1000

CYTOKINE INDUCED KILLER CELLS ARE ACTIVE AGAINST NON-SMALL CELL LUNG CANCER AND OVERCOME THE ACQUIRED RESISTANCE TO TARGET THERAPIES

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Background: Non-small cell lung cancer (NSCLC) patients initially responding to target therapies invariably develop resistance to these drugs. This poses the question of further *therapeutic approach*. In our study, we assessed a combined treatment of target therapy and immunotherapy with CIK (Cytokine Induced Killer) cells. CIK cells are able to kill tumor in MHC-unrestricted manner. This antitumor activity is mediated by the interaction of CIK's membrane receptor NKG2D with MIC A/B and ULBP 1-3 ligands expressed on the tumor cells.
Aims: In this preclinical study, we investigated the efficacy of immunotherapy with CIK cells in NSCLC and, concomitantly, the possibility of overcoming the acquired resistance to target therapies: EGFR tyrosine kinase inhibitors (TKI) and MET tyrosine kinase inhibitors.
Methods: CIK cells were expanded from healthy donors, starting from PBMC, with the timed addition of IFN-gamma, Ab anti-CD3 and IL2. EGFR TKI resistant NSCLC cells were generated by treating EGFR-addicted cells (PC-9, H 4006, H 3255, HCC 827) with increasing concentrations of EGFR TKI:erlotinib, gefitinib, dacomitinib, afatinib and AZD8931. MET TKI resistant NSCLC cells are generated by treating EBC 1 and H 1993 cells with increasing concentrations of crizotinib and JNJ38877605.
Results: Both wild type NSCLC cell lines and correspondent resistant sublines expressed MIC A/B and ULBP ligands which render them good potential targets for immunotherapy with CIK cells. Mean expression values of MIC A/B were 34% in EGFR TKI sensitive cells; values of 34%. 36%, 36%, 35% and 43% were observed on corresponding sublines respectively resistant to erlotinib, gefitinib, dacotinib, afatinib and AZD8931. ULBP 2 was expressed in EGFR TKI sensitive cells with mean of 94%; values observed on corresponding resistant sublines for all EGFR TKI drugs were comparable. MET TKI sensitive and resistant lines had equal expression of 100% of ULBP 2. CIK cells efficiently and comparably killed both, sensitive and drug resistant NSCLC cell sublines *in vitro* up to 91% of specific killing (n=21). Preliminary *in vivo* experiments showed that weekly intravenous infusions of CIK cells into NOD/SCID mice (n=6), implanted with NSCLC cells (EBC1), significantly delayed tumor growth compared to untreated controls (n=5) (V1=2764±376 mm^3, *vs* V2=477±379 mm^3, average±sem, P=0,02). Similar trend was observed treating animals implanted with EBC1-originated tumor made resistant to MET TKI *in vivo*.To rule out potential impairment by targeted therapies, CIK cells were exposed for 1 week with EGFR TKI (erlotinib 400nM, gefitinib 100nM, dacomitinib 5nM, afatinib 5Nm, AZD8931 5nM) and MET TKI (crizotinib 150nM) without observing any significant change in phenotype or functionality.
Summary and Conclusions: CIK cells demonstrated intense activity *in vitro* and *in vivo* against NSCLC targets. This beneficial effect was retained also against tumor cells that developed resistance to molecular targeted therapy with either EGFR TKI or MET TKI. Our data support and encourage further investigation in this direction with promising clinical benefit in NSCLC patients and the perspective potential to overcome resistance to molecular targeted approaches.

P1002

NATURAL KILLER CELL EXPANSION UNDER GOOD MANUFACTURING PRACTICE CONDITIONS FOR CLINICAL USE IN ADULT PHILADELPHIA+ ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Background: The management of adult Philadelphia (Ph)+ acute lymphoblastic leukemia (ALL) has profoundly changed with the utilization of tyrosine kinase inhibitors (TKI). Two GIMEMA studies have shown that virtually all patients, irrespective of age, obtain a complete remission (CR) with TKI plus steroids without systemic chemotherapy. Most patients tend over time to relapse and the optimal post-CR treatment is unclear. The possibility of controlling/eradicating minimal residual disease (MRD) through an immunotherapy-based strategy represents a very attractive therapeutic option. Previous studies have shown that cytotoxic natural killer (NK) cells with killing activity against autologous blasts may be expanded and activated from ALL patients in CR, suggesting the use of NK-based immunotherapeutic protocols for the management of these patients.
Aims: Aim of this study was to design a method of NK cell expansion under good manufacturing practice (GMP) conditions to be used in clinical protocols based on the *in vivo* infusion of *ex vivo* expanded autologous NK cells for adult patients affected by Ph+ ALL in CR.
Methods: PBMCs were collected from 20 healthy donors and 3 adult Ph+ ALL patients in CR. For NK cell enrichment, a two-step immunomagnetic procedure was used, consisting of an initial $CD3^+$ T-cell depletion followed by a $CD56^+$ positive selection. Isolated NK cells (1×10^5/mL) were cultured for 14 days in SCGM serum-free medium supplemented with 5% autologous plasma,500 U/mL IL-2 and 50 ng/mL IL-15 in the presence of irradiated autologous feeder cells (2.5×10^5/mL). Only GMP and clinical grade materials were used. The phenotype of freshly-isolated and expanded NK cells was assessed using mAbs against CD56, CD16, CD3, DNAM-1 and NKG2D receptors, being the last two proteins involved in NK-cell recognition and killing of primary blasts. The cytolytic properties of expanded NK cells against the K562 cell line and against primary adult Ph+ ALL blasts were determined in a standard ^{51}Cr release assay.
Results: NK cells from healthy donors and Ph+ ALL patients could be expanded respectively up to a 31.8±15.5 and 39.1±19.3 fold increase. Expanded NK cells contained a homogenous cell population displaying a high expression of CD56 and CD16 in the absence of CD3. DNAM-1 and NKG2D activatory receptors presented a significantly increased expression after expansion both from healthy donors (DNAM-1 P=.0007; NKG2D P=.0004) and from Ph+ ALL patients (DNAM-1 P=.0012; NKG2D P=.045). Fresh and cryopreserved expanded NK cells from healthy donors and Ph+ ALLs induced an efficient lysis of K562 cells (mean cytotoxicity at a 50:1 E:T ratio 75.6%±9.7% and 62.9%±2.3%, respectively). When NK cells expanded from healthy donors were used as effectors against adult Ph+ ALL blasts, a marked cytotoxicity was observed (n=5; mean cytotoxicity at a 50:1 E:T ratio 21.4%±7.2%), indicating the susceptibility of Ph+ ALL blast cells to NK cell cytotoxicity. In addition, preliminary data show that NK cells expanded from Ph+ ALL patients in CR exert a cytotoxic activity also against autologous blasts cryopreserved at diagnosis, thus confirming the above data.
Summary and Conclusions: These results open the way to a possible new immunotherapeutic strategy for adult Ph+ ALL patients with evidence of MRD, based on the *in vivo* infusion of autologous NK cells expanded *ex vivo* according to GMP-suitable procedures. The ultimate goal is a non-chemo/non-transplant management of Ph+ ALL - particularly the elderly - with the use of TKI (and steroids) plus an immune-mediated control of the disease.

P1003

EFFICIENT GENERATION OF HLA-A2 AND A24-RESTRICTED WT1-SPECIFIC CYTOTOXIC T LYMPHOCYTES USING GENE-ENGINEERED ARTIFICIAL ANTIGEN-PRESENTING CELLS

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Background: Adoptive transfer of antitumor T cells to patients with malignant melanoma has been shown to have a significant clinical impact. However, widespread use of this novel modality has been hampered by the difficulties in consistently generating antitumor lymphocytes in a timely manner for every patient. To overcome this issue, we previously reported the generation of K562-based artificial antigen-presenting cells (aAPCs). Using this aAPC-based culture system, we are able to reproducibly generate HLA-restricted antigen-specific $CD8^+$ cytotoxic T lymphocytes (CTL). Recent clinical trials have demonstrated that melanoma-specific CTL, expanded using this aAPC system, could survive for prolonged periods in advanced-stage melanoma patients without lymphodepletion or cytokine treatment. Moreover, these CTLs trafficked to the tumor, mediated biological and clinical responses, and established antitumor immunologic memory.
Aims: Wilms' tumor 1 suppressor (WT1) is one of the ideal tumor-associated antigens for cancer immunotherapy. Using our aAPC-based T cell culture system, we expanded and characterized WT1-specifc CTL restricted by frequent HLA-A alleles, A2 and A24.
Methods: HLA-A*02:01 (A2) and A*24:02 (A24)-positive peripheral blood mononuclear cells were obtained from leukemia and cancer patients (n=5). To establish antigen-specific T cells, $CD8^+$ T cells were purified by positive selection using a magnetic beads method (Miltenyi Biotec). aAPCs expressing either HLA-A2 or A24 as a single HLA allele were pulsed with HLA-A2 or HLA-A24 restricted, wild type or modified 9-mer WT1 immunodominant peptides (RMF-

PNAPLY for HLA-A2 and CYTWNQMNL for HLA-A24). aAPCs were then irradiated with 200 Gy and added to purified CD8+ T cells at a ratio of 1:10 in 96-well plates in PRMI1640 supplemented with 10% human AB serum. Between stimulations, IL-2 (10 U/mL) and IL-15 (10 ng/mL) (both from Peprotec) were added to the cultures.
Results: Flow cytometry (FACS) analysis confirmed that aAPCs stably expressed HLA class I, CD80 and CD83. Following 3 rounds of weekly stimulation with peptide-pulsed aAPCs, WT1-peptide-specific CTLs were evaluated by a tetramer staining and an enzyme-linked immunosorbent spot (ELISPOT) assay. The percentage of HLA-A2 tetramer-positive cells was 0.051±0.023% before stimulation. It increased to 0.258±0.165% (5.1 fold increase) following three stimulations. In contrast, the percentage of HLA-A24 tetramer-positive cells before and after stimulation was 0.070±0.008% and 0.787±0.873% (11.2 fold increase), respectively, indicating greater magnitude of increase compared with HLA-A2 tetramer positive cells. Neither HLA-A2 nor A24-restricted WT1-specific CD8+ T cells were stained by irrelevant tetramers, confirming their strict HLA restriction and antigen specificity. Interestingly, IFN-gamma ELISPOT assays revealed that the magnitude of increase in WT1-specific CTLs generated from HLA-A2 patients following stimulation was higher than those generated from A24 patients (10.8 times and 6.7 times, respectively). FACS analysis showed that majority of WT1-specific CTLs expanded using WT1 peptide-pulsed aAPCs expressed an effector memory phenotype. These results suggest that there could be difference in functional maturation between HLA-A2 and A24-restricted WT1-specific CTL generated from leukemia and cancer patients.
Summary and Conclusions: These results demonstrated that HLA-A2 and A24-restrited WT1-specific CTLs with an effector memory phenotype can be generated *in vitro* using peptide-pulsed gene-engineered aAPCs within a short period of time.

P1004

OPTIMIZATION OF ALLOGENEIC NK CELLS FOR ADOPTIVE TRANSFER THERAPY

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Background: Natural killer (NK) cells have long been considered as potential agents for adoptive cell therapy for solid cancer patients. Triggering of effector NK function depends on the balance of inhibitory (mostly Killer-Ig-like Receptors; KIR) and stimulatory (NK Lysis Receptors; NKLR) signals. Although NK cells are able to eliminate malignant cells without prior antigenic stimulation, most clinical studies utilizing autologous NK cells yielded disappointing results.
Aims: To develop the most efficient strategy employing allogeneic NK cells for adoptive cell transfer in solid tumors.
Methods: We analyzed various modular approaches, including donor-recipient KIR-ligand mismatching, *ex vivo* expansion and activation of NK cells, induction of NKLR, and co-administration of antibodies to elicit antibody-dependent cell cytotoxicity (ADCC) in a melanoma model.
Results: In agreement with previous models, we could show that melanoma cells were more efficiently killed by allogeneic NK cells derived from KIR-ligand mismatched donors. Specific anti-tumor activity of NK cells could further be enhanced by the addition of ADCC-inducing antibodies. The ADCC effect was independent from the KIR-ligand mismatched setting and was only observed in NK cells over-night pre-activated with Interleukin-2 (IL-2), but not in fresh NK cells. To investigate the efficacy of ex-vivo activated and expanded NK cells, we developed a clinically-compliable expansion protocol. After CD3 T cell depletion, cells were subjected to various growth media, IL-2 concentrations,+/- anti-CD3 antibody and+/- irradiated feeder cells. The addition of irradiated feeder cells together with anti-CD3 antibody (to stimulate feeder cells) in AIM-V or X-Vivo 10 culture media resulted in the highest fold expansion of CD3-depleted cells (average of 112-fold after 2 weeks and 420-fold after 3 weeks). *Ex vivo* expanded NK cells displayed an enhanced killing activity when compared to over-night incubation with IL-2, as well as in the mismatched setting, as compared to the matched setting. The improved killing activity of *ex vivo* expanded NK cells could be explained by the uP-regulation of NK lysis receptors, mainly NKG2D and NKp30. Addition of ADCC-inducing antibody modestly increased the killing activity of already expanded NK cells.
Summary and Conclusions: NK cell *ex vivo* expansion and activation, and optimization of NKLR expression seem to be the most potent strategy for the generation of anti-tumor reactive NK cells, followed by ADCC induction and lastly by KIR-ligand mismatching. This study rationalizes a clinical trial that combines adoptive transfer of highly potent *ex vivo* expanded allogeneic NK cells from selected donors (high NKLR expression) and antibody therapy in patients with malignancies.

P1005

LENALIDOMIDE MODULATES AN INHIBITORY EFFECT OF HUMAN REGULATORY T CELLS ON THE PROLIFERATION OF B CELL LYMPHOMA

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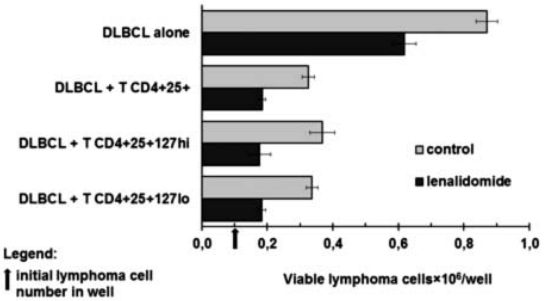
Background: High numbers of intra-tumoral regulatory T-cells (Tregs) have been reported to correlate with improved survival in germinal center B cell-like diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), and classical Hodgkin lymphoma. Thus Tregs, which are capable of directly suppressing activation of normal B cells, can be similarly involved in the regulation of lymphoma B cell proliferation. Studies on B-cell lymphoma models have shown that lenalidomide modulates the immune system. Inhibition of the proliferation and function of naturally occurring Tregs was reported as one of multiple immunomodulatory effects of lenalidomide. This raises the question of whether lenalidomide interferes with the regulation of lymphoma cell proliferation by Tregs.
Aims: To investigate interactions between Tregs and lymphoma B cells and to evaluate effects of lenalidomide on the regulation of lymphoma B-cell proliferation by Tregs.
Methods: Established cell lines of FL, DLBCL, Burkitt lymphoma, plasma cell myeloma, mantle cell lymphoma, and Hodgkin lymphoma were co-cultured with freshly isolated or *ex vivo* expanded Tregs, with or without 1microM or 2microM lenalidomide (Celgene Corp.), *i.e.* at concentrations compatible with clinical concentrations. The equivalent volume of DMSO was added to control cultures without lenalidomide. To evaluate cell proliferation by flow cytometry, lymphoma cells were stained with carboxyfluorescein succinimidyl ester (CFSE), and Tregs with the cell proliferation dye eFluor670. CD4+CD25+Tregs were obtained from peripheral blood of healthy donors by magnetic separation. To expand Tregs, CD4+CD25+T cells were cultured either in the presence of irradiated allogeneic monocyte-derived dendritic cells (allo-DC) or anti-CD3/28/2 Ab-coated beads, with IL-2 (10 or 50 U/mL, respectively), with or without rapamycin (100 ng/mL).
Results: Effects of freshly isolated Tregs on the proliferation of different lymphoma cell lines ranged from help to suppression. Lenalidomide neither induced nor enhanced suppression of lymphoma cell proliferation by freshly isolated Tregs, unless anti-CD3/28/2 Ab-coated beads or "third party" allo-DC were added to the mixed cultures to activate Tregs. Tregs expanded in the presence of rapamycin for 2 to 5 weeks following polyclonal activation or activation by "third party" allo-DC, suppressed proliferation of 10/12 lymphoma cell lines tested, and proliferation of polyclonally activated autologous and allogeneic conventional Tcells. Tregs expanded without rapamycin proliferated for a shorter period of time, and did not develop equal regulatory function. Expanded Tregs constantly expressed FOXP3 and maintained demethylation of *FOXP3* intronic Treg-specific demethylation region (TSDR), secreted IL-10 and IFN-gamma, expressed CTLA-4, CD39, CD73, GARP, TIGIT, GITR, and granzyme A, but not granzyme B or perforin. Proliferation of lymphoma B cell lines was moderately reduced or not affected by lenalidomide alone. In mixed leukocyte cultures, lenalidomide potentiated suppression of lymphoma proliferation exerted by Tregs pre-expanded *ex vivo*. The inhibitory effects of lenalidomide or Tregs on lymphoma proliferation were additive, even when lenalidomide moderately inhibited Treg proliferation. Tregs expanded from sorted CD127(lo) and CD127(hi) subsets of CD4+CD25+T cells similarly suppressed lymphoma proliferation, and lenalidomide similarly potentiated suppression exerted by these Treg populations (Figure 1).



Effect of 2 μM lenalidomide on the regulation of diffused large B cell lymphoma (DLBCL) cell proliferation by Treg expanded either from CD4+CD25+ T cells or CD4+CD25+CD127(lo) T cells or CD4+CD25+CD127(hi) T cells. Lymphoma cells were plated at a ratio 4:1 to T cells. The bars represent mean values +/- S.D. of viable lymphoma cell count in triplicate 5-day cultures.
Treg were activated polyclonally and expanded for 21 days with rapamycin (100 ng/ml) prior to plating with lymphoma cells in mixed leukocyte cultures.

Figure 1.

Summary and Conclusions: Tregs could be considered for adoptive therapy post-autologous hematopoietic transplantation (HCT) to eradicate residual lymphoma and to counteract both graft-*versus*-host disease and lymphoma regrowth after allogeneic HCT. Tregs expanded *ex vivo* with rapamycin could

be particularly useful. Lenalidomide potentiates the regulatory function of Tregs directed against B lymphoma under the condition that Tregs are activated. Indirect activation of Tregs in the absence of lymphoma B cells induces regulatory function of Tregs against B lymphoma cells.
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P1007

CENTRALIZED PRODUCTION AND DISTRIBUTION OF CMV-SPECIFIC CYTOTOXIC T CELL LYMPHOCYTES

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Background: Investigator-led Phase I/II studies have shown that adoptive transfer of donor virus-specific cytotoxic T cell lymphocytes (CTL) can reconstitute immunity on a long-lasting basis in patients at risk for viral reactivation following allogeneic hematopoietic stem cell transplantation (allo HSCT).
Aims: In order to support routine clinical use for virus-specific CTL, it is critical to establish a reliable and convenient approach for supply of the cell product to transplant centers across Europe.
Methods: Requests for Cytomegalovirus-specific (CMV) CTL are accepted for specific patients from transplant physicians under a cell therapy agreement with Cell Medica, employing standard request forms. Eligibility is confirmed, including HLA-type and presence of CMV-specific cells, in donor samples. A proprietary technology is utilized for direct selection of CTL based on MHC-multimer technology (STAGE Cell Therapeutics GmbH). After final testing the CMV-specific CTL are released as Cytovir™ CMV and shipped to the hospital in a temperature controlled manner for direct infusion according to local SOPs for donor lymphocyte infusions. In addition, Cell Medica is sponsoring two clinical studies investigating the use of Cytovir™ CMV for high risk patients (CMV D+/R+) prophylactically (CMV~IMPACT study) as well as therapeutically post-CMV reactivation (CMV~ACE/ASPECT trial).
Results: The centralized production reliably results in a product that after microbiological clearance can be delivered in 10-14 days from receipt of starting material. Based on the provision of over 50 cell products to support clinical trials to 15 centers, no logistics failures have occurred. The Table 1 shows data from the last 27 products manufactured after process optimization.

Table 1. Manufacturing data.

(n=27)	Median	Range
% CMV+ cells in donor starting material (of CD8+)	0.85 %	0.11 – 13.3 %
% CMV+ cells in final product (of CD3+)	93.57 %	8.72 – 99.56 %
% CD3+ cells in final product	65.66 %	10.20 – 98.51 %
% viability in final product	95.62 %	76.86 – 98.78 %
T cell dose /kg infused	$2.7x10^4$	$7.61x10^2 – 5x10^4$
Total number of T cells infused	$1.92x10^6$	$5.63x10^4 – 4.2x10^6$

Summary and Conclusions: Cell Medica have developed a model for centralized production and delivery of virus-specific CTL for transplant centers across Europe. Detailed manufacturing data demonstrate a high quality cell product. According to the scientific recommendation from the European Medicines Agency (EMA) Cytovir™ CMV is not classified as an advanced therapy medicinal product (ATMP) (date of adoption of recommendation by the EMA 26/01/2010). Routine use of CMV-specific CTL is feasible across Europe. Regulatory approvals pursuant to national legislation will be sought in the next 8 months with all necessary requirements already fulfilled to allow UK supply.

P1008

LAK CELLS AND ANTI-CD20 MONOCLONAL ANTIBODIES AS INMUNOTHERAPY ON FOLLICULAR LYMPHOMA: ENCHANCED ANTIBODY-DEPENDENT CELL CITOTOXICITY OF LAK CELLS IN ASSOCIATION WITH GA101 RATHER THAN RITUXIMAB

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Background: Administration of "*in vitro*" expanded autologous cytokine-activated killer cells (LAK cells) has been proposed to obtain an immune control of follicular lymphoma (FL). Culture of peripheral blood lymphocytes with IL-2 generate LAK cells with cytotoxic capacity. Combination of these LAK cells with mAb could achieve a synergistic effect by enhancing the ADCC activity with potential clinical interest. GA101 is a new anti-CD20 mAB that binds with higher affinity to the CD20 increasing ADCC effect comparing to rituximab.
Aims: To study the cytotoxic capacity of LAK cells generated from peripheral blood of patients with FL. In addition we assess whether the combination of rituximab and LAK could improve functional activity of these cells. Finally, we conducted a comparative study of two anti-CD20 antibodies, rituximab and GA101, and we compare subgroups of patients with different polymorphisms in Fc and complement proteins.
Methods: LAK cells were expanded "*in vitro*" from peripheral blood samples of patients with FL. Cytotoxic capacity of these cells was compared to cells without culture. Cytotoxicity studies were performed using chromium release assays using K562 cells, Daudi and CRL-1596 as target cells. The effect of rituximab and GA101 was evaluated on the capacity of the effector cells to lyse CRL-1596 cell line. Cetuximab was used as a control. To perform these studies target cells were incubated with effector cells and antibody at 10 mg/mL concentration. SNPs genotype was performed with TaqMan Applied Biosystems technology using the detection systemABI Prism 7900HT
Results: Mononuclear cells were isolated and basal cytotoxicity was measured in 35 peripheral blood samples from patients with FL. Basal cytotoxicity against K562, Daudi and CRL-1596 cells was 17.2%, 9.46% and 1.21% respectively. After culture with IL-2, the cytotoxicity activity against the same cell lines was studied. In all cases a statistically significant increase was observed: K562 (38.43%), Daudi (38.78%) and CRL-1596 (20.5%). Furthermore we studied the rituximab effect on the cytotoxic capacity against CRL-1596 of culture cells. The observed cytotoxicity of LAK cells with rituximab was 33.90% *vs* 17.2% for LAK cells alone (P<0,001). The cytotoxicity of LAK cells with GA101 was 44.72% *vs* 17.2% for LAK cells alone (P<0,001). No differences were observed against CRL1596 cell line with the irrelevant antibody cetuximab (20.5% *vs* 17.2). Finally when comparing the ability to enhance the ADCC of both anti-CD20 antibodies we found significant differences in favor to GA101 (GA101: 44.72% *vs* rituximab: 33.90%). Results of Fc receptor and complement activation proteins polymorphisms and correlation of these results with ADCC will be provided in the meeting.
Summary and Conclusions: LAK cells generated from peripheral blood lymphocytes by culture with IL-2 in patients with FL show a higher cytotoxic activity than non culture lymphocytes. The observed cytotoxic capacity of LAK cells against a CD20 positive cell line is enhanced by means of anti-CD20 monoclonal antibodies addition. GA101 is more effective than rituximab in enhancing the cytotoxic capacity of generated LAK cells.

P1009

ROLE OF CIRCULATING AB-DOUBLE NEGATIVE T CELLS (DNT) IN LYMPHOMA PATIENTS: PRELIMINARY RESULTS OF A PROSPECTIVE STUDY

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Background: Numerous aspects of lymphoma pathophysiology indicate mutual interactions between the host immune system and lymphoma cells. These interactions may either promote or control lymphomagenesis. An unconventional subset of $CD4^-CD8^-$ double-negative T cells (DNTs) has been recently described to specifically contribute to anti-tumor immunity.Indeed, DNTs are involved in immune regulation and tolerance as well as in host defence and inflammation, acting as both regulatory T cells and/or cytotoxic T cells.DNTs are T lymphocytes expressing either αβ or γδ T-cell receptor (TCR) and lacking of CD4,CD8 and CD56.In healthy human donors and murine models, they constitute about the 1-5% of lymphocytes in the peripheral blood and in lymphoid organs. No data are available on the role of DNT cells in human anti-lymphoma immunity. Translating information from murine models expanded DNT cells would not impair host immunity against lymphoma and perhaps stimulate it.On the other hand,DNT cells also demonstrated to have a direct *in vitro* anti-tumor activity against lymphoma. Few data are available on the prognostic significance of DNTs in lymphomas,on their interaction with other immune cells and on their functional attitude.
Aims: The aim of this study is to assess the frequency and the functional attitude of circulating DNTs in Lymphoma patients and healthy donors as controls, in order to evaluate the role of DNTs on clinical outcome
Methods: For phenotypic and functional characterization of DNTs peripheral blood samples of 30 Lymphoma patients and 16 healthy donors wereprospectively collected.The staining of circulating DNT subset was performed with the following conjugated monoclonal antibodies (MoAbs) for surface and intracellular markers: CD3, CD4, CD8, CD56, CD45, TCRαβ, CD45Ra, CD45Ro, CCR7, CD27, CD28, CD30, CD69, GITR, CD95, CD178, CD152, IFN-γ, TNF-α, granzymeB, perforin. Isotype-matched MoAbs will be used as staining controls.

For functional studies, DNTs were purified from PBMCs of pts through a negative selection by using specific MACS microbeads and then cultured for 2 week in complete medium supplemented with anti-CD3 (OKT3), rhIL-2 and rhIL-4. Data was acquired using a 8-colour flow cytometer and analyzed using Kaluza software. Data were compared among the groups using the Mann-Whitney non parametric test or Kruskal–Wallis one-way analysis of variance.The study was approved by the local Ethics Committeeand all patients provided their informed consent in accordance with the Declaration of Helsinki.

Results: We observed a significant decrease (P=0.006) of αβ-DNTs in the PB of patients with untreated lymphoma (20.5±4.8 SE,) (Mean±SE) as compared with healthy controls (31.3±3.4),and their number correlated with disease relapse/progression (Figure 1 A and B).In Hodgkin's Lymphoma patients the αβ-DNTs frequencies were significantly increased as compared with other histotypes (Figure 1 D). Interestingly, after *ex vivo* expansion, DNTs, acquired a immunomodulatory cytokine profile, characterized by the secretion of IFN-γ and granzyme B which are known as central component of anti-tumor immune responses (Figure 1 C).

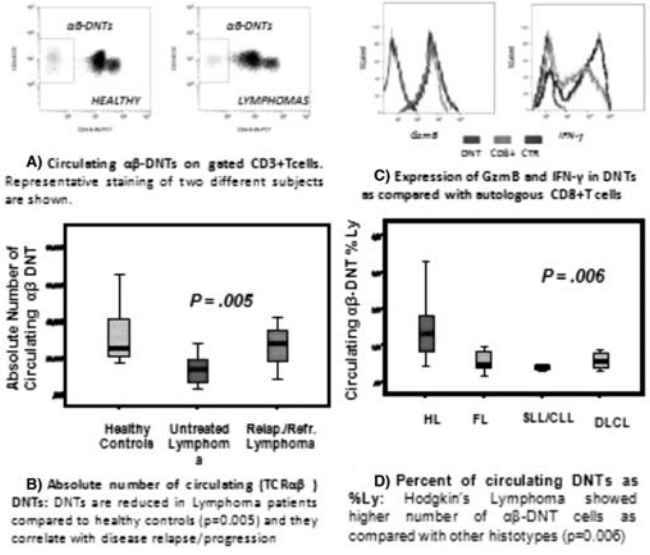

Figure 1.

Summary and Conclusions: To date,no data has been reported on DNTs phenotypic and functional characterization in Lymphoma pts. Our study has demonstrated for the first time that αβ-DNTs may play an important role in both the development and the progression of lymphomas. In addition, based on our preliminary results,it is likely that ex-vivo expanded DNTs exert an anti-tumor activity,thus suggesting their possible use as new strategy for adoptive immune-therapy.

Red blood cells and iron; physiology and disease (anemia) - Biology

P1010

EFFECT OF HISTONE METHYLTRANSFERASE EZH2 INHIBITOR 3-DEAZANEPLANOCIN A (DZNEP) ON ERYTHROPOIESIS

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Background: EZH2, a core component of Polycomb repressive complex 2 (PRC2), plays a role in transcriptional repression through mediating H3K27 trimethylation, and is involved in various biological processes, including hematopoiesis. Studies have indicated that 3-deazaneplanocin A (DZNep), an inhibitor of EZH2,preferentially induces apoptosis in various hematological malignancies, implying that EZH2 may be a potential new target for epigenetic treatment.On the other hand, we have demonstrated that DZNep also has a potential to promote erythroid differentiation of K562 cells (ASH 2012). In the present study, we have extended our study to assess the effect of DZNep on erythropoiesis.

Aims: We conducted iTRAQ (isobaric tags for relative and absolute quantitation)-based proteomic analysis in DZNeP-treated K562 cells, and also assessed the effect of DZNep on primary erythroblasts.

Methods: K562 cells were treated with DZNep at doses of 0.2 and 1 uM for 72 h.siRNA-mediated knockdown of EZH2 was conducted with nucleofector (Amaxa Inc.). For transcription profiling, SurePrint G3 Human GE 8 × 60K (Agilent) and Human Oligo chip 25K (Toray) were used for DZNeP-treated and EZH2 knockdown K562 cells, respectively. iTRAQ analysis was conducted with Triple TOF 5600 (AB Sciex). To obtain human primary erythroblasts, CD34-positive cells isolated from cord blood were induced in liquid suspension culture, and DZNep was treated at doses of 0.01 and 0.1 uM for 96 h.

Results: DZNep treatment decreased EZH2 protein expression without significantly affecting EZH2 mRNA levels. We also confirmed that DZNep treatment significantly inhibited cell growth, accompanied with accumulation of p27. Interestingly, the treatment significantly induced erythroid differentiation of K562 cells, as determined by benzidine staining. Transcriptional profiling with untreated and DZNeP-treated K562 cells (1 uM) revealed that 789 and 698 genes were upregulated and downregulated (> 2-fold), respectively.The DZNeP-induced gene ensemble included prototypical GATA-1 targets, such as *SLC4A1*, *EPB42*, *ALAS2*, *HBA*, *HBG*, and *HBB*. Concomitantly, DZNep treatment at both 0.2 and 1 uM upregulated GATA-1 protein level, whereas the effect on its mRNA levels was weak (1.02- and 1.43-fold induction with 0.2 and 1 uM DZNep treatment, *P*= 0.73 and 0.026, respectively). Next, we conducted iTRAQ-based proteomics analysis to further obtain an insight into the molecular mechanisms of DZNeP-mediated erythroid differentiation.The analysis identified a total of 120 unique proteins (P<0.05), including significant enrichment of hemoglobins (HBZ and HBE), whereas EZH2 and GATA-1 failed to be detected.

To examine whether the observed results of DZNep treatment were due to the direct inhibition of EZH2 or hitherto unrecognized effects, we conducted siRNA-mediated knockdown of EZH2 in K562 cells. Quantitative RT-PCR analysis demonstrated that EZH2 knockdown had no significant effect on the expression of erythroid-lineage related genes. Furthermore, transcription profiles of the genes in the quantitative range of the array were quite similar between control and EZH2 siRNA-treated K562 cells (r=0.977), implying that DZNeP-mediated erythroid differentiation might not be directly related to the EZH2 inhibition.

Finally, we assessed the effect of DZNep on primary erythroblasts, demonstrating that the treatment (0.1 uM)significantly induces *HBB* and *HBG* expression in CD34-positive cell-derived primary erythroblasts.

Summary and Conclusions: DZNep promotes the expression of erythroid genes in primary erythroblasts. Our microarray and proteomic analyses may provide a better understanding of the mechanism of action of DZNep.

P1011

HS3 LOCUS CONTROL REGION SEQUENCES ARE NOT REQUIRED FOR HIGH BETA GLOBIN EXPRESSION

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Background: The Locus Control Region (LCR) is a genetic element located several Kb upstream the β globin cluster. This element is composed by 5 regulatory sites (HS1 to HS5), in which the LCR functions seem to reside. The LCR restricts globin expression to the erythroid cell lineage, enhances gene expression and protects these genes from the negative effects of surrounding chro-

matin. In fact, deletions affecting the entire regulatory region abolish the β cluster expression, resulting in εγδβ thalassemia.
Furthermore, LCR was the first described regulator that could exert its effects over long distances. As a consequence, it has been extensively studied in different biological models, from KO mice to cell lines, in order to understand the human functionality of this element. However, several problems arise from these studies, such as the lack of reproducibility when using the same biological model or the contradictory results in the study of different models. The challenge of understanding how the LCR works *in vivo* derives in part by the fact that there are a very limited number of known LCR human naturally occurring deletions.
Aims: Here we described 3 cases of the same family, who are carriers of a novel deletion limited to the HS3 element. This could be helpful in order to understand the HS3 and LCR functionality in humans.
Methods: Propositus and her family (mother and brother) were submitted for screening of thalassemia based on mild microcytosis. An HS3 deletion, inside the LCR, was detected in all patients by MLPA. In addition, propositus and her mother showed an extra α globin gene copy (genotype: ααα/αα), whereas the brother showed no alterations in the α cluster. Patient's red blood cell indices are detailed in Figure 1. Finally, we have performed a gapPCR to precisely define the deletion breakpoints.
Results: We have found an HS3 deletion removing 1992bp. 5' breakpoint is located at position 11836 in the β cluster (NG_000007.3), whereas 3' breakpoint lies inside an Alu sequence, at position 13827. This novel alteration has been named as Toledo deletion.

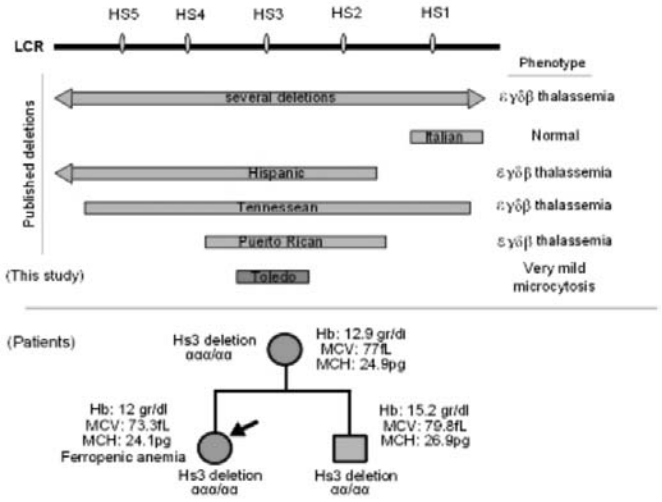

Figure 1.

Summary and Conclusions: Figure 1 shows a schematic view of previously known human LCR deletions. Alterations affecting both HS2 and HS3 lead to εγδβ thalassemia. Thus, those elements were supposed to be necessary for high levels of β globin transcription. However, there were no natural deletions removing just one of those key elements.
Toledo HS3 deletion is relevant because it only affects HS3, keeping HS2 intact. Surprisingly, this deletion is associated to a virtually normal phenotype, slightly aggravated when there is coinheritance of extra α gene copies. Thus, we conclude that HS3 is not required for high β globin transcription.
This deletion gives a human *in vivo* confirmation of the results obtained by the group of Peterson working on transgenic mice containing a human β globin cluster. They described HS3 as an element with no observable effect over β gene. Nevertheless, their results clearly point the existence of HS3 enhancer specificity for ε and γ genes. This kind of Hs site specificity for globin gene activation has not been observed studying the native murine β cluster in KO mice models, where a deletion of any of the individual Hs results in a 20% reduction of adult globin expression in the β cluster.
The cases exposed here also argue in favour of the limited value of mouse gene regulation studies for understanding human disease.
In conclusion, we have found a new human deletion demonstrating the redundancy of HS3 in β globin regulation.

P1012

FIRST EVIDENCE OF RENAL TUBULAR INJURY DURING SICKLE-CELL CRISIS

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Background: The pathophysiological mechanisms classically involved in sickle-cell nephropathy include endothelial dysfunction and vascular occlusion. Arguments demonstrating that ischemia-reperfusion injury-related kidney damage might coincide with vaso-occlusive crisis (VOC) are lacking.
Aims: We aimed to determine whether tubular cells and glomerular permeability might be altered during VOC.
Methods: Homozygous SCD patients, at least 18 years old with severe VOC requiring admission to our hospital, were eligible for inclusion. Exclusion criteria included VOC with parenteral hydration lasting >24 hours; blood transfusion during the previous month; acute chest syndrome or severe complication requiring a blood transfusion at inclusion; pregnancy and/or psychiatric disorder. Patients with preexisting chronic kidney disease, defined as a glomerular infiltration rate ≤60 mL/min/1.73 m^2 according to the modification of diet in renal disease (MDRD) formula, were excluded. Blood and urine parameters were analyzed the same day for each patient during VOC on hospitalization day 1 (D1) and D2 or D4, and at steady state (ST). ST was defined as a visit ≥1 months after an acute clinical event and ≥3 months after blood transfusion.
Results: Urine neutrophil gelatinase-associated lipocalin (NGAL) levels and albumin-excretion rates (AER) of 21 patients were evaluated prospectively during 26 VOC episodes compared to their ST. We observed significantly increased urine NGAL levels during VOC *versus* ST, while AER did not change significantly. The higher urine NGAL concentration was not associated with subsequent (24-48 hour) acute kidney injury. Univariate analysis identified a strong association only between urine NGAL level and white blood-cell count.
Summary and Conclusions: Our results demonstrated that tubular injury could be present during VOC and highlights the importance of hydroelectrolyte monitoring and correction during VOC.

P1013

INHIBITION OF ERYTHROPOIESIS THROUGH HEPCIDIN AND ROS IN TRANSFUSION-RELATED IRON OVERLOAD MICE

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Background: Some clinical observations that iron-chelating therapy for bone marrow failure syndrome patients with transfusion-related iron overload could improve cytopenia (especially anemia) were reported. They prompted us to consider that iron overload may have a negative impact on hematopoiesis. However, the precise mechanism remains to be elucidated.
Aims: The aim of this study was to determine theeffect of iron overload on hematopoiesis using forced iron-loaded mice.
Methods: While there are some model mice presenting hemochromatosis with mutant genes such as HFE and TfR2, they may show different pathology from transfusion-related iron overload patients. Therefore, we established iron overload model mice, which were six-week-old C57BL/6J mice injected with 5 mg of saccharated ferric oxide (FesinÒ) intra-peritoneally 10 times in two weeks. At 8 weeks after the last injection, the mice were sacrificed and analyzed. We used mice injected with saline instead of saccharated ferric oxide as controls. Peripheral blood cell count was performed by the standard procedure. Serum iron and unsaturated iron binding capacity (UIBC) were measured by colorimetric methods. Total iron binding capacity (TIBC) and transferrin saturation were calculated using serum iron and UIBC. We also analyzed the histology of liver and bone marrow in iron overload mice to assess iron deposition. Apoptosis and intra-cellular ROS production of immature or mature erythroid cells in bone marrow were analyzed by flow cytometry. Real-time quantification of mRNA transcripts of iron-related molecules such as hepcidin, IL6, IL1-beta, BMP6, and Id1 in liver was performed by quantitative RT-PCR.
Results: Iron overload mice showed higher serum iron concentration and higher transferrin saturation (serum iron 367±15 μg/mL, control 198±8 μg/mL, P<0.0001; transferring saturation 93.8±1.0%, control 48.1±1.2%, P<0.0001). These data are compatible with those of blood transfusion-related iron overload patients. Iron overload mice showed marked anemia, while the leukocyte count and platelet count did not differ from those of control mice (RBC 881±25×10^4/μl *vs.* 974±8×10^4/μl, P=0.002; Hb 13.7 g/dl *vs.* 14.6 g/dl, P=0.029; reticulocyte 20.36±0.91×10^4/μl *vs.* 27.67±1.68×10^4/μl, P=0.002; WBC 7038±1754/μl *vs.* 6556±444/μl, P=0.78; Plt 68.0±5.1×10^4/μl *vs.* 80.1±3.8×10^4/μl, P=0.07). We performed histological analysis of liver and bone marrow in iron overload mice. In these mice, diffuse iron deposition was clearly observed in hepatocytes. Interestingly and unexpectedly, iron deposition in bone marrow was observed on macrophages and vascular endothelial cells. Hematopoietic precursor cells in bone marrow had little iron deposition. We performed flow cytometric analy-

sis to determine the causes of anemia, which revealed that immature erythroid cells expressing both CD71 and Ter119 in bone marrow were significantly decreased in iron overload mice (17.1±0.7% *vs.* 20.7±1.1%, P=0.04). Intracellular ROS production in immature erythroid cells was increased compared with that in control mice. Next, we measured the expression of iron-related molecules such as hepcidin, IL6, IL1-beta, BMP6, and Id1 in liver by quantitative RT-PCR. We found that the expression of hepcidin, BMP6, and Id1 was significantly increased in iron overload mice compared with that in control, and also found thatthe expression of hepcidin was correlated with the expression of BMP6 and Id1.
Summary and Conclusions: These data suggest that iron overload may cause anemia through hepcidin production from hepatocytes and intracellular ROS production in immature erythroid cells.

P1014

CONGENITAL DYSERYTHROPOIETIC ANEMIA DUE TO A NOVEL GATA-1 MUTATION

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Background: Congenital Dyserythropoietic Anemias (CDA) are rare forms of bone marrow failure syndromes, characterized by ineffective erythropoiesis, with specific morphological changes in the bone marrow erythroblasts. There are three classical groups (CDA type I, II, III) whose genes have already been identified (*CDAI* and *SEC23B* for CDA type I and II respectively). Recently other rarer forms of CDA have been identified, like a new form that has been correlated to mutations in the gene (*GATA-1*, Xp11.23) encoding for the transcriptional factor GATA-1. Most of the *GATA-1* mutations are located in one of the two zinc finger domains and are associated with deregulation of erythroid and megakaryocyte lineages formation. The GATA-1 C-terminal finger is necessary for DNA binding and the N-terminal finger mediates interaction with FOG-1 (for friend of GATA-1), a cofactor of GATA-1.
Aims: To elucidate the etiology of a macrocitic anemia with dyserythropoiesis and mild thrombocytopenia.
Results: Clinical Case. A 12-year-old boy was referred to our consultation to elucidate the etiology of a congenital haemolytic anemia. At 3 months of age he presented Hb 5.9 g/dL, MCH 33 pg, MCV 95 fL, RDW 17.5%, reticulocytes 5.8%, normal platelet counts and a peripheral blood smear with anisocytosis, poikilocytosis and basophilic stippling. He received several blood transfusions during the first months of life. The bone marrow examination was reported as erythroid hyperplasia, dyserythropoiesis, with orthocromatic erythroblasts showing irregular nuclear contour and bi or multinucleated erythroblasts. Electronic microscopy identified vacuoles in the cytoplasm of the erythroblasts. Currently he has thrombocytopenia (platelet counts 73000), macrocytosis (MCV 104 fL), and hemoglobin ranging between 9 and 10 g/dl. Results. Molecular analysis of *CDAI*, *SEC23B*, *KLF1, HBB, HBA* genes was normal; *PKLR* gene presented the 1284delA mutation in the heterozygous state. *GATA-1* gene mutations screening (promoter and coding regions) was performed by PCR/sequencing and an A to G transition at nucleotide position 866 (c.866A>G) was identified in the hemizygous state. This novel mutation results in the substitution of histidine 289 for arginine in the protein C-terminal zinc finger. The patient inherited this mutation from his mother who is heterozygous for the mutation.
Summary and Conclusions: Congenital Dyserythropoietic Anemias are a heterogeneous group of hereditary disorders, both at clinical and genetic levels. GATA-1 is an important transcription factor in hematopoyesis regulation, in particular for erythroid and megakaryocyte lineages. The relative low number of mutations observed in this gene may indicate that the majority of the mutations are not compatible with life, confirming the central role of this transcription factor in mammalian erythroid development. This is the first report of a mutation in the *GATA-1* gene located in the C-terminal zinc finger domain of GATA1 protein that resulted in a Congenital Dyserythropoietic Anemia. This mutation is not described in the literature, but its location may justify the phenotype. Our data highlights the importance of analyzing both highly conserved zinc finger regions of GATA-1 in patients with Congenital Dyserythropoietic Anemias.

P1015

EFFECTS OF CHRONIC PSYCHOLOGICAL STRESS ON EXTRAMEDULLARY ERYTHROPOIESIS: INVOLVEMENT OF EPOR, GR, C-KIT AND BMP4 SIGNALING

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Background: Chronic stress is an increasingly important topic and its understanding requires an integrative approach. Unlike steady-state erythropoiesis in the bone marrow, under stress conditions spleen becomes a major site of red blood cell production. It has been suggested that spleen microenvironment provides a signal that induces cells to become stress erythroid progenitors. Although there is evidence that high erythropoietin level together with glucocorticoids, stem cell factor and bone morphogenetic protein 4 (BMP4) stimulates erythropoiesis during stress, very little information is available on expression of their receptors in the spleen.
Aims: To extend our previous observations that repeated restraint stress increased the expansion of erythroid progenitors, the purpose of this study was to investigate its effect on more mature erythroid cells and to determine involvement of the erythropoietin receptor (EpoR), glucocorticoid receptor (GR), stem cell factor receptor (c-KIT), as well as BMP4 and its receptors BMPRI and BMPRII in the regulation of spleen erythropoiesis during chronic stress.
Methods: Adult male CBA mice were subjected to 2 h daily restraint stress for 7 or 14 consecutive days. The level of plasma corticosterone was determined by RIA whereas the concentration of erythropoietin was measured by ELISA. In the spleen red pulp, the expression of TER119, a specific surface marker for erythroid differentiation from the early proerythroblast to mature erythrocyte, as well as EpoR, GR, c-KIT and BMP4 was assessed by immunohistochemistry. Additionally, the expression of EpoR and GR was evaluated using Western blot while BMPRI and BMPRII were analyzed by RT-PCR in splenic extracts.
Results: Repeated restraint stress elevated plasma levels of corticosterone and erythropoietin on days 7 and 14. In the red pulp of spleen, the number of TER119-immunoreactive (ir) cells was significantly increased following 14 days of exposure to restraint. Both Western blot and immunohistochemistry analyses revealed a significant decrease in the expression of both EpoR and GR in the spleen of mice restrained for 7 and 14 days. Further analysis showed that chronic restraint elicited a robust increase in the number of c-KIT-ir cells compared to controls in the red pulp of spleen after one and two weeks. Also, markedly increased expression of BMP4 and both BMPRI and BMPRII was found in the spleen of mice after repetitive restraint for 7 and 14 days.
Summary and Conclusions: After chronic stress, EpoR and GR levels in the spleen are decreased, while c-KIT, BMPRI and BMPRII are increased. Chronic restraint stress, as predominantly psychological stressor, enhances spleen erythropoiesis in a manner similar to that described in stress erythropoiesis-induced by anemia, implying involvement of elevated plasma levels of corticosterone and erythropoietin as well as c-KIT and BMP4-signaling in the complex regulation of this process. The repeated psychological stress may be a risk factor to a large number of diseases including hematological malignancies.

P1016

DOES THE OPTICAL MICROSCOPE TELL US THE COMPLETE STORY IN MEMBRANE-CYTOSKELETON DISORDERS? A STUDY OF RED BLOOD CELLS WITH ATOMIC-FORCE AND SCANNING-ELECTRON MICROSCOPES IN HEREDITARY SPHEROCYTOSIS

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Background: Hereditary spherocytosis (HS) is one of the most common genetic disorders of the red blood cell (RBC) membrane-cytoskeleton cohesion due to the abnormal expression of basic proteins either of the cytoskeleton (spectrin) or of its linkage with the upper lying lipid-lipid bilayer (ankyrin, band3, protein 4.2). As a consequence, there is a gradual loss of a significant part of the RBC membrane possibly in the form of vesicles.
Aims: To investigate these issues at the nanometer level and reveal information not only on the shape and morphology of the complete RBC but from specific areas of the modified cell membrane we employed advanced microscopes.
Methods: Intact and 0.9% NaCl-washed RBCs (iRBCs and wRBCs, respectively) produced from peripheral blood samples were investigated for the case of 3 HS patients in comparison with 3 healthy donors. The iRBCs and wRBCs single-layered smears were investigated in detail with conventional Optical Microscopy (OM) and the advanced imaging techniques Atomic-Force Microscopy (AFM) and Scanning-Electron Microscopy (SEM), both having spatial resolution at the nanometer level (1 nm=10^{-9} m).
Results: Regarding iRBCs, in one HS patient, macroscopic fission that sometimes was accompanied by a clear twisting of the two cell parts was observed by means of AFM and SEM (however not with OM). In all HS patients and healthy donors the iRBCs membrane presents *microscopic* circular morphological abnormalities (mCMAs) that reminisce of ulcers, with size in the range 200-2000 nm (see vertical-solid arrows in Figure 1). For the HS patients, in some cases the underlying spectrin network is observed by means of AFM at the interior of mCMAs (see vertical-solid arrow in lower Figure 1). Since the membrane mCMAs are observed in both groups they probably relate to the physiological aging of RBCs. Furthermore, the mCMAs incidence (number/top

RBC membrane) does not present statistically significant differences, 1.27±0.38 και 1.52±0.31 between the HS patients and healthy donors, respectively. On the contrary, the iRBCs of the HS patients exhibit an overpopulation of *nanoscopic* CMAs (nCMAs) of size 50-200 nm (see horizontal-dotted arrows in lower Figure 1) with a comparatively minor incidence in the healthy donors. Micro/nano-metric vesicles (mnVs) of *cylindrical* (length 0.5-7.0 μm, diameter 100-500 nm) and *toroidal* (diameter 0.5-1.5 μm) form were observed by means of both AFM and SEM for the HS patients, however without the observation of the corresponding snapshots referring to their releasing from the iRBCs. Regarding wRBCs, both AFM and SEM data revealed that for the HS patients they are extendedly degraded to 'ghosts' during the wash process with even 0.9% NaCl physiological saline, obviously due to their reduced osmotic resistance. Though the wRBCs 'ghosts' are cytoplasm depleted, they preserve the main morphological characteristics of iRBCs.

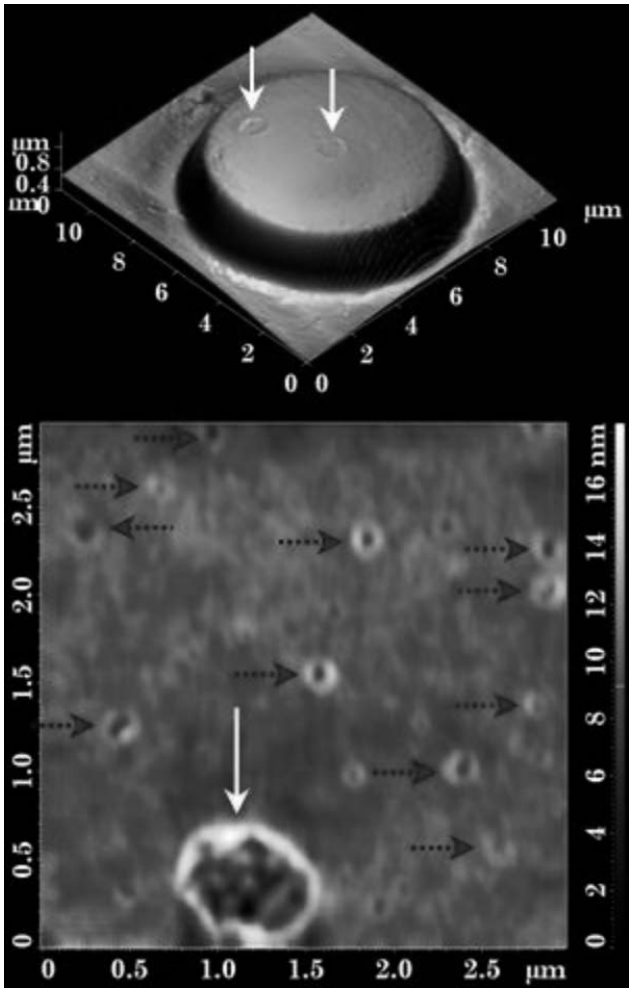

Figure 1.

Summary and Conclusions: The membrane of iRBCs of HS patients does not present differences in the mCMAs (size 200-2000 nm), in comparison to healthy donors. In contrast, the occurrence of a large population of nCMAs (size 50-200 nm) that are strongly increased in the HS patients when compared to the healthy donors, may relate to the significantly decreased osmotic resistance of the RBCs in the HS patients. Both mCMAs and nCMAs can be active sites from where cytoplasm is released to the extracellular space.
The incidence of *cylindrical* and *toroidal* mnVs without the observation of snapshots that document their releasing from RBCs suggests that either the specific mnVs originate from a different parent cell or they are truly released from RBCs during their passage from a parenchymal organ, most likely the spleen.

P1017

INVESTIGATION OF HEMORHEOLOGICAL PARAMETERS AT THE DIAGNOSIS AND THE FOLLOW-UP OF NUTRITIONAL VITAMIN B12 DEFICIENT CHILDREN

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Background: In vitamin B12 deficiency, tetrahydrofolate synthesis is impaired, leading to defective DNA synthesis and inhibition of cell division in the bone marrow, resulting in megloblastic anemia. Mean corpuscular volume (MCV) is increased, and in the peripheral blood smear the erythrocytes are large and mostly oval with hypersegmented neutrophils. In the literature it has been reported that, especially in hematological disorders which affect the shape of the erythrocytes, hemorheological parameters are altered variously. There are a limited number of studies investigating eythrocyte deformability in vitamin B12 deficient adults, and the only investigated hemorheological parameter was the eryhtrocyte deformability in these studies. Whereas, there is no study concerning the hemorheological changes in vitamin B12 deficient children.
Aims: The aim of this study was to investigate the hemorheological parameters as erythrocyte deformability, erythrocyte aggregation, whole blood and plasma viscosities in vitamin B12 deficient children, and to identify the possible alterations in these parameters in response to adequate vitamin B12 treatment, and to compare them with healthy controls.
Methods: The study enrolled 33 patients (17 female, 16 male) aged 2 months-16 years (mean age: 7±5.7 years), who were diagnosed as nutritional vitamin B12 deficiency, and 31 age and sex matched healthy controls (16 female, 15 male, mean age: 7.1±5.2 years). Informed consent form was obtained from the parents. Elongation index (EI) which is the indicator of erythrocyte deformability was measured at 9 different shear stresses between 0.3 and 30 Pa by an ectacytometer. Erythrocyte aggregation amplitude (AMP) was also determined by an ectacytometer. Plasma and whole blood viscosities were determined by a cone-plate rotational viscometer. The differences between patients and controls were compared. Hemorheological parameters were repeated in the patient group following two months of vitamin B12 treatment, and the results were compared with the initial results.
Results: In vitamin B12 deficiency, erythrocyte deformability was found to be significantly decreased compared with the controls, and after adequate vitamin B12 replacement it increased and came towards control values. On the other hand, eythrocyte aggregation was found to be significantly increased in the patient group before treatment compared with the controls. After treatment there was a significant decrease in erythrocyte aggregation, compared with the before treatmentvalues. Plasma viscosity was found to be decreased in deficiency compared with the controls but this decrease was not statistically significant, however it increased significantly after treatment and came towards control values. Whole blood viscosities measured at either autologous or standardized hematocrit (40%) were found to be significantly decreased in the patient group before treatment compared with the controls, and after treatment showed a significant increase. A negative correlation between the EI and MCV, and a positive correlation between AMP and MCV was found.
Summary and Conclusions: This study indicates that vitamin B12 deficiency has important effects on hemorheological parameters, and adequate treatment of deficiency not only corrects the hematological parameters, but also by helping to normalize the hemorheological parameters, may contribute to the regulation of microvascular perfusion.

P1018

X-LINKED SIDEROBLASTIC ANEMIA: A PROMOTER MUTATION IN THE ALAS2 GENE

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Background: Sideroblastic anemias (SA) are a heterogeneous group of disorders, congenital or acquired, associated with abnormal heme biosynthesis, characterized by: 1) microcytic hypochromic anemia, with dimorphism, 2) presence of ringed sideroblasts in the bone marrow, 3) important secondary hemosiderosis. X-Linked Sideroblastic Anemia (XLSA) is a hereditary disorder with a heterogeneous phenotypic expression in males. Female carriers can also have the disease, particularly after the fourth or fifth decade of life, due to unbalanced X chromosome lionization. Most frequently XLSA is diagnosed in males during the first years of life and is associated with mutations (primarily missense) in the erythroid-specific 5-aminolevulinic acid synthase gene, *ALAS2* (Xp11.21). In the less severe forms the diagnosis may be delayed to the first two decades of life or to the middle age, when the secondary iron overload is significant. Depending on the type and location of the *ALAS2* mutation, some patients may respond to oral pyridoxine treatment. Severe cases are transfusion dependent.
Aims: To elucidate the iron overloadetiology in a male patient with mild hypochromia and microcytosis.
Results: Clinical Case. A 54-year-old male was referred to elucidate the etiology of iron overload: serum ferritin 1600 ng/mL (normal 18-370), transferrin saturation 47.3%. He also presented hemoglobin in the lower normal range (13,7 g/dL), with hypochromia (MCH 26 pg), microcytosis (MCV 76 fL), RDW 14%, reticulocytes 160×10^3/mL, peripheral blood smear with elliptocytes, stomatocytes and a few microcytes; normal Hb A2 and Hb F. No secondary causes of iron overload were identified. He has been submitted to phlebotomies during the last 7 years, the hemoglobin varied between 11 and 12.5 g/dL. Results.

Molecular analysis of *HFE*, *SLC40A1* (*ferroportin)* and *TFR2* genes was normal; alpha globin genes presented a $-a^{3.7}$ deletion in the heterozygous state. Alpha thalassemia could justify the mild hypochromia and microcytosis, but not the iron overload. Even though the blood smear did not suggest a sideroblastic anemia, we sequenced the *ALAS2* gene promoter and coding regions. A C to G transversion at -258 from the ATG site was identified in the hemizygous state. This mutation, already described in a Welsh female and his son, is located in a transcription factor binding site. This is the second report of a regulatory mutation in the ALAS2 gene that resulted in a XLSA clinical phenotype.
Summary and Conclusions: In a 62 years old male patient with iron overload and mild hypochromic, microcytic anemia we identified a a^+ thalassemia and a XLSA. Considering that XLSA phenotype severity varies considerable in males, and can also be present in females, we suggest that all patients with no identified cause of iron overload should have their DNA examined for ALAS2 mutations, irrespective of their haematological parameters, sex and age. Identification of the ALAS2 mutation allows an early treatment with pyridoxine, prevention of iron overload and the identification of heterozygous women who will benefit from genetic counselling.

P1019

COMPARISON OF EARLY AND LATE GASTROINTESTINAL TRACT AND LIVER TOXICITY OF THE ORIGINATOR IRON POLYMALTOSE COMPLEX (IPC) AND AN IPC SIMILAR PREPARATION IN NON-ANEMIC RATS

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Background: Oral iron treatment of iron deficiency (anemia) [ID(A)] is often accompanied by poor tolerability and thus limited compliance. Ferrous compounds show poor gastrointestinal (GI) tolerability, possibly because the rapid release of iron and its uncontrolled uptake may trigger oxidative stress reactions [1]. The originator iron(III)-hydroxide polymaltose complex (IPC_{ORIG}, Maltofer®) exhibits good GI tolerability and negligible oxidative stress reactions [2], because its physico-chemical properties enable controlled, active absorption of iron [3]. The structure of IPC_{ORIG}, a polynuclear iron-carbohydrate complex, is largely defined by the manufacturing process [4,5]. A variety of IPC similar (IPCS) preparations are in use worldwide, but they often present with varying physico-chemical properties and, thus, different efficacy and toxicity *vs.* IPC_{ORIG} [2].
Aims: This nonclinical study in rats compared early (four weeks) and late toxicity (four months) of IPC_{ORIG} and an IPCS in the GI tract and liver.
Methods: Sixty rats were randomized to receive 280 mg Fe/kg body weight/day of IPC_{ORIG} or $IPCS_{HEMO}$ (Hemoval®, Laboratories Saval S.A., Santiago, Chile), or tap water (control). Thirty rats were sacrificed on day 29, and hematological variables and aspartate transferase (AST) were analyzed from blood. Early toxicity was assessed by microscopic evaluation of the villi/crypt ratio, and the number of Goblet cells and eosinophils per villi in the small intestine. Tissue ferritin in the liver and small intestine were assessed by immunohistochemistry. After 4 months, thirty rats were sacrificed and late toxicity was assessed by analyzing lipid peroxidation (malondialdehyde, MDA) and the activity of Cu,Zn-superoxide dismutase (Cu,Zn-SOD) in small intestine and liver homogenates.
Results: The $IPCS_{HEMO}$ group presented with significantly higher transferrin saturation (TSAT) and AST levels than the IPC_{ORIG} group. The villi/crypt ratio and the number of Goblet cells/villi were significantly lower and the number of eosinophils/villi significantly higher in the small intestine of the $IPCS_{HEMO}$ group *vs.* IPC_{ORIG} group. Ferritin immunostaining in the small intestine and liver and Prussian blue staining in the liver was significantly increased in the IPC_{ORIG} group *vs.* the IPC_{HEMO} group. The $IPCS_{HEMO}$ group showed a significant increase of lipid peroxidation and Cu,Zn-SOD activity in the small intestine and liver *vs.* IPC_{ORIG} after four months, indicating oxidative stress in the $IPCS_{HEMO}$ group (Table 1).

Table 1.

Mean ± SD (n= 10)	IPC_{ORIG}	$IPCS_{HEMO}$	Control
Hb (g/dL) (after 4 weeks)	15.9 ± 0.3	15.8 ± 0.2	15.5 ± 0.3
TSAT (%) (after 4 weeks)	39.0 ± 3.3*	51.5 ± 3.1#	33.7 ± 2.9
Early toxicity in the small intestine (after 4 weeks)			
Villi/crypt ratio	2.1 ± 0.1	1.8 ± 0.1*	2.3 ± 0.1
Goblet cells/villi	10.4 ± 0.9	8.8 ± 0.8*	11.8 ± 1.0
Eosinophils/villi	8.2 ± 0.9	13.5 ± 1.9*	7.7 ± 1.2
Ferritin score	3.0 ± 0.7*	1.4 ± 0.5#	0.4 ± 0.5
Early toxicity in the liver (after 4 weeks)			
AST (UI/L)	119.2 ± 11.0	144.5 ± 13.2†	112.9 ± 12.6
Ferritin (%/mm²)	10.5 ± 1.0*	6.3 ± 0.7#	0.6 ± 0.2
Late toxicity in the small intestine (after 4 months)			
MDA (nmol/mg protein)	1.1 ± 0.1	3.1 ± 0.2*	0.9 ± 0.2
CuZn-SOD (U/mg protein)	22.3 ± 4.0	56.3 ± 4.8*	18.3 ± 4.8
Late toxicity in the liver (after 4 months)			
MDA (nmol/mg protein)	1.0 ± 0.2	2.4 ± 0.3*	0.7 ± 0.3
CuZn-SOD (U/mg protein)	33.4 ± 4.2	63.8 ± 8.1*	29.2 ± 3.4

* $p < 0.01$ vs. all; # $p < 0.01$ vs. control; † $p < 0.05$ vs. all

Summary and Conclusions: Damage to the gastric epithelium in the small intestine was present to a variable degree in rats treated with $IPCS_{HEMO}$ but not with IPC_{ORIG}. This, together with higher levels of TSAT in the $IPCS_{HEMO}$ group, suggests the presence of weakly-bound or redox-active iron on the surface of $IPCS_{HEMO}$. Significantly lower levels of ferritin deposits and increased oxidative stress markers both in the liver and small intestine of the $IPCS_{HEMO}$ group indicate a less controlled iron utilization from $IPCS_{HEMO}$ than from IPC_{ORIG}. Indeed, saturation of the iron transport mechanisms may lead to uncontrolled iron uptake by various cells.

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Conflict of interest
JE Toblli has received research grants and consultancy fees from Vifor (International) Ltd. The other authors have no conflicts of interest to declare.

P1020

NEXT GENERATION SEQUENCING IN THE DIAGNOSIS OF RED BLOOD CELL MEMBRANE DISORDERS

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Background: Hereditary disorders of the red blood cell (RBC) membrane constitute a major cause of hereditary hemolytic anemia. It concerns a heterogeneous group of diseases with highly variable clinical expression. They are caused by mutations affecting structural proteins of the RBC cytoskeleton and its anchoring to the RBC membrane, thereby perturbing the cell's structure and function. Traditionally RBC membrane disorders are classified according to the morphological appearance on a blood smear. Hereditary spherocytosis (HS) and elliptocytosis (HE) are among the more common abnormalities, whereas hereditary pyropoikilocytosis (HPP) and stomatocytosis (HST) are more rare. Inheritance of these diseases is in most cases autosomal dominant.
Aims: Molecular analysis has long been hampered by the large and complex nature of the many different genes involved and the fact that most mutations are private ones. In this study we aimed to explore the molecular basis of various disorders of the red blood cell membrane by Next Generation Sequencing (NGS).
Methods: We implemented an NGS based test in our DNA diagnostics laboratory to sequence seven genes commonly associated with RBC membrane disorders: *SPTA1* (α-spectrin), *SPTB* (β-spectrin), *ANK1* (ankyrin), *SLC4A1* (band 3), *EPB41* (protein 4.1), *EPB42* (protein 4.2), and *RHAG* (Rhesus-associated glycoprotein). Twenty patients with defined membranopathies were selected for analysis (16 HS, 2 HPP, 2 HST). After enrichment of genomic DNA, using a custom Agilent SureSelectXT probe kit, protein coding and flanking intronic sequences were determined using a SOLID™-5500XL system. Variants were identified using an 'in house' developed NGS mapping and calling pipeline, and the Cartagenia BENCHlab NGS module was used for filtering and prioritization of possible pathogenic variants. Detected mutations were confirmed by conventional Sanger sequencing.
Results: Probable causative mutations were identified in 16 out of 20 (80%) of patients studied. As was expected, most of the detected mutations were novel (11 out of 15) and private. Most mutations concerned the substitution of a single amino acid and were located in *SPTA1* and *ANK1*. One particular mutation concerned a large deletion involving *SPTB*. Somewhat surprisingly, this small cohort showed no mutations in the gene encoding band 3 (*SLC4A1*), a frequent cause of HS.
Summary and Conclusions: The here presented approach represents a feasible and reliable diagnostic method to detect mutations in patients affected by various disorders of the RBC membrane. Establishing the molecular diagnosis will be particularly important in young children with congenital anaemia, transfusion-dependent patients, and in families with variable clinical expression or complex inheritance patterns. In addition, understanding the molecular mechanisms involved in disturbed RBC membrane function will contribute to a better understanding of normal RBC physiology.

P1021

CARDIOPULMONARY BYPASS PROCEDURE INDUCES EXTRACELLULAR RED BLOOD CELL VESICLE FORMATION

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Background: Extracellular vesicles are produced and released by most cells and help to maintain homeostasis under physiological conditions. They play an important role in cell-to-cell communication and have been identified in different body fluids. They are released in a higher extent during cellular activation and stress. Use of a heart-lung machine in patients, also called cardio-pulmonary bypass procedure (CPB), is hypothesized to be major stressor for erythrocytes and associated with hemolysis. During CPB blood transfusion is often inevitable. Our hypothesis is that the diffuse inflammation during cardiopulmonary bypass is, at least partly, mediated by vesicles formed in (transfused) blood as these vesicles show avid interaction with endothelium, can activate complement and induce thrombosis.
Aims: Aim of this study is to investigate if contact with exogenous surfaces, pumps, and buffer solutions in CPB induces blood cell vesiculation.
Methods: In the clinical set-up, patients' CPB residue collected from the heart-lung machine at the end of the operation was anonymously processed in experiments. In the *ex vivo* set-up, blood from healthy volunteers, obtained after informed consent, was subjected to a neonatal CPB set-up mimicking the procedure without a patient. Extracellular vesicles and red blood cells were isolated by (ultra)centrifugation procedures and characterized with Nanosight regarding size and number. The interaction of red blood cell vesicles with endothelium was quantified using a pseudoperoxidase assay (measuring hemoglobin) and visualized after labelling of vesicles with PKH67. mRNA expression levels of selected proteins involved in iron homeostasis were quantified by qPCR.
Results: Surprisingly, vesicle formation could not be detected in CPB residue from patients undergoing CPB. This could either mean that vesicles are not formed during the procedure or that their clearance rate surpasses the formation rate. By subjecting whole blood to an *ex vivo* CPB set-up detached from a patient, clearance of vesicles could be eliminated. In this set-up, vesicle formation was prominent and dependent on duration of the procedure. Judging by the red color of the vesicle-pellet, the predominant source of vesicles was red blood cells. Apart from clearance by macrophages, these vesicles could be cleared by other cell types and induce phenotypic changes. When we incubate red blood cell vesicles with activated endothelium cells we observed uptake and processing of vesicles. Uptake was enhanced in the presence of the phosphatidylserine-binding opsonin lactadherin. Uptake led to upregulation of iron processing-enzymes such as heme-oxygenase-1, ferritin and ferroportin.
Summary and Conclusions: Our results show that extracellular vesicles are formed during CPB, but these vesicles are rapidly cleared in patients. Apart from macrophages, extracellular vesicles can be taken up by other cell types and induce phenotypic changes that could potentially contribute to the diffuse inflammatory state after CPB in patients.

Granulocytes

P1022

IMMUNOGENICITY OF LIPEGFILGRASTIM AND PEGFILGRASTIM IN BREAST CANCER PATIENTS RECEIVING CHEMOTHERAPY: INTEGRATED ANALYSIS FROM PHASE II AND III STUDIES

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Background: Lipegfilgrastim is a novel, long-acting, fixed-dose, glycoPEGylated recombinant granulocyte-colony stimulating factor (rG-CSF) under review for neutropenia prevention in patients receiving myelosuppressive chemotherapy (CTx) for cancer. Like any biological product, lipegfilgrastim could potentially elicit an anti-drug antibody (ADA) response, which could cause adverse events (AEs) or lack of efficacy.
Aims: To assess the immunogenicity of lipegfilgrastim compared with pegfilgrastim using data from a Phase II and a Phase III study in patients receiving CTx for breast cancer, and to correlate ADA test results with clinical events.
Methods: In the Phase II study, breast cancer patients undergoing CTx received a subcutaneous (s.c.) injection of lipegfilgrastim3, 4.5, or 6 mg or pegfilgrastim 6 mg on Day 2 of each of four CTx cycles. In the Phase III study, breast cancer patients undergoing CTx received an s.c. injection of 6 mg lipegfilgrastim or pegfilgrastim on Day 2 of each of four cycles. In both studies, serum samples were taken before each cycle, at the end of the study (Day 85), and on Day 180 and Day 360 during the 360-day follow-up period. Samples were analyzed using multi-tiered ADA assays based on Mesoscale Discovery technology. Screening-reactive samples were confirmed by immunocompetition. All confirmed ADA positive (ADA+) study samples were measured for titer and analyzed with the NFS-60 cell proliferation assay for neutralizing activity against lipegfilgrastim or pegfilgrastim and endogenous G-CSF. The confirmed ADA+ samples from lipegfilgrastim-administered patients were also characterized for ADA specificity towards XM21 (G-CSF moiety of lipegfilgrastim) and PEG moiety, as well as cross-reactivity to endogenous G-CSF by competition with XM21, PEG, and glycosylated G-CSF, respectively.
Results: The incidence of ADAs is summarized in the Table 1. In the Phase II study, only two subjects had antibodies related to lipegfilgrastim treatment. Among them, one subject had antibodies binding to the PEG moiety, and titer for anti-lipegfilgrastim antibodies was found to be 0.6 on Day 85 only. Another subject had antibody titer of 0 and the antibodies did not bind to either the protein or PEG moiety, indicating a weak or possibly false-positive response. In pegfilgrastim-treated patients, only one subject was found to have antibody response to the treatment. Antibody titer and binding specificity were not investigated. In the Phase III study, only one subject had antibodies related to lipegfilgrastim treatment, with the titer value being 1.2 and 2.1 on Days 180 and 360, respectively. Antibody specificity characterization showed that the antibodies bind to the protein moiety of lipegfilgrastim. In pegfilgrastim-treated patients, only one subject was found to have antibody response to the treatment. Antibody titer and binding specificity were not investigated. ADA+ samples did not exhibit neutralizing activity against lipegfilgrastim, pegfilgrastim, or endogenous G-CSF. Unexpected AEs or lack of efficacy were not observed for patients with confirmed ADA+.

Table 1. Incidence of confirmed anti-drug antibodies.

Drug/study	Percent patients with confirmed ADA+ responses (Number of patients with confirmed response/total patients)	
	Phase II (n/N)	Phase III (n/N)
Lipegfilgrastim	1.3% (2/154)	1% (1/100)
Pegfilgrastim	1.85% (1/54)	1% (1/101)

Summary and Conclusions: Immune response following lipegfilgrastim administration was comparable to pegfilgrastim and was not considered clinically significant. Serum ADA status did not appear to impact the clinical safety and efficacy of lipegfilgrastim.

P1024

LIPEGFILGRASTIM–A LONG-ACTING, ONCE-PER-CYCLE, GLYCOPEGYLATED RECOMBINANT HUMAN FILGRASTIM CREATED WITH SITE-SPECIFIC GLYCOPEGYLATION TECHNOLOGY

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Background: Lipegfilgrastim is a once-per-cycle, fixed-dose glycoPEGylated granulocyte-colony stimulating factor (G-CSF) under development for the prevention of severe neutropenia in cancer patients receiving chemotherapy (CTx). Traditional PEGylation of biologic molecules has been used successfully for more than 10 years to extend the half-life in the body, requiring less frequent dosing and allowing for the administration of G-CSF once per CTx cycle, making treatment potentially less expensive and enhancing patient compliance and safety. However, the traditional PEGylation use of chemical conjugation through reactive groups on amino acids can result in multiple PEGylated isoforms with reduced protein activity, as well as non-uniform chemical and pharmaceutical properties.
Aims: To describe a highly site-specific glycoPEGylation technology for site-directed PEGylation and the implications of the technology with regard to improved outcomes, and to summarize preclinical findings for lipegfilgrastim compared with pegfilgrastim (Neulasta®).
Methods: Glycosylation sequon scanning was used to identify the glycoPEGylation site with the least impact on protein activity. Unlike endogenous G-CSF, recombinant G-CSF, expressed in *Escherichia coli*, is not *O*-glycosylated. *E. coli*-expressed G-CSF was selectively glycosylated at the natural *O*-glycosylation site, and a polyethylene glycol (PEG) sialic acid derivative was attached using a sialyltransferase (glycoPEGylation technology). Ligand binding affinity was assessed using the BIACORE 3000 system. Biologic activity of lipegfilgrastim was assessed in an NFS-60 cell line proliferation assay *versus* filgrastim and pegfilgrastim. Pharmacokinetic (PK) and pharmacodynamic properties were studied in healthy and neutropenic animal models.
Results: The use of the naturally present glycosylation site to achieve enzymatic coupling of an activated PEG-sugar conjugate to the filgrastim polypeptide produces a glycoPEGylated, long-acting G-CSF which is similar in structure to the native glycosylated protein. Lipegfilgrastim also showed improved *in vivo* PK profiles in animal models. *In vitro*, lipegfilgrastim had binding affinity and specific activity comparable to pegfilgrastim, while both were slightly lower than non-PEGylated filgrastim. Comparable increases in leukocytes, neutrophilic granulocytes, and monocytes were seen with lipegfilgrastim and pegfilgrastim in rats and monkeys and were consistent with the effects expected for a long-lasting G-CSF. No adverse effects on organ systems were reported after a dose 100 times higher than the recommended human dose.
Summary and Conclusions: The glycoPEGylation technology platform described here is used to produce lipegfilgrastim, a novel, biologically active G-CSF with greater structural homogeneity and comparable pharmacologic properties to conventionally PEGylated G-CSFs, thus enabling a safe and effective once-per-cycle fixed dosing regimen.

P1025

SAFETY AND TOLERABILITY OF LIPEGFILGRASTIM IN BREAST CANCER PATIENTS RECEIVING CHEMOTHERAPY: AN INTEGRATED ANALYSIS OF PHASE II AND III STUDIES

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Background: Myelosuppresive chemotherapy (CTx) frequently causes neutropenia, leading to an increased risk of infections and delays in subsequent chemotherapy treatments. Covalent attachment of polyethylene glycol (PEG) has been shown to prolong the half-life of therapeutic proteins, allowing for less frequent dosing, improved patient compliance, and potentially lower production costs. However, traditional PEGylation methods rely on chemical conjugation through amino acid reactive groups, which may reduce protein activity and result in non-uniform chemical, pharmaceutical, and tolerability profiles. Lipegfilgrastim is a highly homogenous once-per-cycle, fixed-dose glycoPEGylated granulocyte-colony stimulating factor (G-CSF) created with a highly site-specific glycoPEGylation technology for site-directed PEGylation, which is under review for the prevention of neutropenia in cancer patients receiving CTx. Non-inferiority of lipegfilgrastim *versus* pegfilgrastim (Neulasta®) was previously demonstrated in a Phase III trial conducted in CTx-naïve breast cancer patients.[1]
Aims: To report the safety and tolerability findings from an integrated analysis of two Phase II/III studies of lipegfilgrastim *versus* pegfilgrastim in CTx-naïve breast cancer patients.
Methods: All patients who received full-dose CTx (doxorubicin 60 mg/m^2+docetaxel 75 mg/m^2) and either a single 6 mg subcutaneous injection of lipegfilgrastim or pegfilgrastim were included in this analysis. The incidence and severity of treatment-emergent adverse events (TEAEs) and those the investigator deemed related to study drug (TEADRs) over all four cycles were compared.
Results: TEAEs were experienced by 94.7% of patients in the lipegfilgrastim group (N=151) and 89% of patients in the pegfilgrastim group (N=155). The most common TEAEs were consistent with the effects of CTx and the underlying disease and were not necessarily due to G-CSF study medication. Frequencies of TEAEs were comparable between treatment groups. As expected, there was a time-dependent trend across cycles within each treatment group; *i.e.* the frequencies of TEADRs decreased with each subsequent CTx cycle. Severe TEADRs were reported in one lipegfilgrastim patient and two pegfilgrastim patients. There were no deaths as a result of study drug treatment in either group. TEADRs were consistent with the underlying medical condition and administration of CTx. Type, incidence, and severity of TEADRs are provided in the Table 1.

Table 1. Type, incidence, and severity of TEADRs.

Adverse event category, n (%)	Lipegfilgrastim 6 mg (N=151)	Pegfilgrastim 6 mg (N=155)
Any TEAE	143 (94.7)	138 (89.0)
TEADR	46 (30.5)	49 (31.6)
Serious TEADR	2 (1.3)	1 (0.6)
Severe TEADR	1 (0.7)	2 (1.3)
Death due to TEADR	0 (0.0)	0 (0.0)
Most frequent TEADRs*		
Bone pain	19 (12.6)	19 (12.3)
Myalgia	8 (5.3)	6 (3.9)
Erythema	6 (4.0)	3 (1.9)
Arthralgia	4 (2.6)	2 (1.3)
Nausea	5 (3.3)	8 (5.2)

*Preferred term ≥3% in any cohort

Summary and Conclusions: Lipegfilgrastim has a favorable safety profile consistent with the G-CSF class of molecules and is an acceptable alternative to pegfilgrastim for the prevention of neutropenia in cancer patients receiving chemotherapy.

Reference

1. Bondarenko I *et al. J Clin Oncol*. 2012;30 (suppl; abstract e19587).

P1026

CARDIAC INVOLVEMENT IN TYPE 3 EGYPTIAN GAUCHER DISEASE PATIENTS: A REPORT OF 7 CASES AND A NEWLY REPORTED ANOMALY

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Background: Gaucher disease(GD) is due to deficiency of B- glucocerebrosidase leading to depodtion of lipid laden macrophages(Gaucher cells) typically in the liver, spleen, bone marrow, lymp nodes, lungs, brain and very rarely the heart usually associated with D409H/D409H mutation.Cardiac involvement includes constrictive pericarditis, pericardial calcification, infiltration of the myocarduim leading to cardiomyopathy and calcification of the mitral, aortic valves and aortic root.
Aims: To report cardiac findings in 7 Egyptian type 3 GD
Methods: Four D409H/D409H patients, a 20 year old male with typical phenotype of D409H/D409H and 2 L444P/L444P children with cardiomeagly on x-ray were assessed for cardiac anomalies.The D409H/D409H patients did Multislice CT angiography, CT brain and ophthalmologic assessment.
Results: The 3 female D409H/D409H siblings(19.17.11 years old showed mitral regurge(MR); aortic regurge(AR), aortic root calcificationand ectatic proximal coronaries; thickened aortic valve, increased echogenicity of aortic root and MR respectively. They also had increased reflectivity of cornea, apraxia and hydrocephalic changes,The 15 year old female with cardiac symptoms showed MR, MS, AR and calcification of the aorta extending to the coronaries as well as apraxia and hydrocepahlic changes.The fifth patient had severe valvular affection, corneal opacities, apraxia and died postopeartively.The 2 L444P/L444P children had dilated cardiomyopathy and coarctation that showed improvement.
Summary and Conclusions: This is the first report of coronary ectasia in GD.Cardiac affection in GD can invlove other mutations than D409H/D409H which should be screened routinely to pickup anomalies early.

P1027

A FEASIBILITY STUDY OF HOME MONITORING OF BLOOD NEUTROPHIL COUNTS IN PATIENTS WITH CHRONIC NEUTROPENIA

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Background: Currently patients with chronic neutropenia and other hematological disorders must go to a doctor's office, medical laboratory or hospital for monitoring of their hematological status. For some chronic diseases, *e.g.*, cyclic and congenital neutropenia, an accurate diagnosis depends on obtaining a series of daily or every-other-day counts over several weeks, but many patients find it difficult to comply with the number of laboratory visits that are required. It would be far more convenient if their blood cell counts could be monitored at home. Home monitoring may prove to be useful for monitoring blood counts for patients at risk of developing febrile neutropenia, for reducing number of costly visits to medical laboratories and for reducing patients' exposure to new pathogens outside the home environment.
Aims: To determine if patients can learn to use a small home monitoring device to measure their own blood cell counts on a daily basis and to compare the accuracy of patient testing *versus* testing by a skilled laboratory technician.
Methods: Ten patients with cyclic and idiopathic neutropenia currently enrolled in the Severe Chronic Neutropenia International Registry (SCNIR) will be enrolled. Initially the patients will make three visits to the laboratory to learn to collect blood using sterile finger puncture techniques and to use the counting device (XBC, Philips, Eindhoven, NL). At each visit the patient and the technician perform counts using the XBC device and the technician makes a control count using a venous blood sample and a reference electronic counter (BC, Beckman Coulter, Miami, FL). After the third laboratory visit, the patient takes the XBC device home to monitor his/her own counts on a daily basis for 28 days. Patients return to the laboratory each week of the study period for follow-up in-laboratory counts by the patient and technician.
Results: Initially we tested three normal donors, six tests on each of three XBCs and 4 repeat tests of each sample on BC and observed no significant differences (two-way ANOVA).
We have enrolled four patients, three patients were receiving granulocyte colony stimulating factor (G-CSF) and one was being treated with prednisone. The patients and technicians both performed finger sticks and both have performed tests with XBC. The technician has performed counts on venous blood using the BC device as the reference control. Teaching and learning proceeded smoothly and easily. Comparison of counts performed in the laboratory with the XBC device *vs* the BC counter are shown in Table 1. There were no significant differences in results for the two instruments (two-way ANOVA or Students t-test). Other comparisons were: patients *vs.* technician in lab with XBC and patients using XBC in lab or at home. There were no significant differences.
For the two patients who have completed the 28 day study, compliance rates for daily counts for were: 75% and 86%, the lower percentage for the first patient due to running out of testing cassettes. Error rates requiring repeat counts were 26% and 17% and both patients regularly repeated the finger-stick sampling and counting on the second try. Patients 3 and 4 have quickly learned counting techniques, are maintaining their records and will soon complete the study. The discomfort of the finger sticks has not limited the study. The six additional patients will be studied in the spring 2013.

Table 1. Comparison of blood neutrophil count for four patients using test (XBC) *vs* reference (CB) instrument.

	Patient 1 (n=5 tests)		Patient 2 (n=5 tests)		Patient 3 (n=5 tests)		Patient 4 (n=2 tests)	
Instrument	XBC	BC	XBC	BC	XBC	BC	XBC	BC
Mean ANC ($x10^9$ /L)	11.0	10.2	1.8	2.1	1.9	1.7	1.4	1.6
SEM	2.9	1.4	0.2	0.2	0.5	0.4	0.05	0.25

Summary and Conclusions: Based on this initial experience, we believe that the XBC counter is reliable and accurate for measurement of WBC and ANC. We find that patients can quickly learn to use this device to monitor their blood leukocyte and differential counts at home. We believe that home monitoring is a promising strategy for diagnostic testing and evaluation of treatment responses in patients with neutropenia.

P1028

GRANULOCYTE FUNCTION IS NOT IMPAIRED BY ARGININE DEFICIENCY

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Background: Arginine deficiency is often encountered in cancer patients and has all the characteristics of a double-edged sword: on the one hand, it is induced by arginase expressing myeloid cells (neutrophil granulocytes or myeloid-derived suppressor cells) in the context of cancer-related inflammation. The ensuing arginase-mediated arginine deficiency may profoundly suppress the adaptive immune response by inhibiting T cell proliferation and cytokine synthesis. On the other hand, arginine depletion might have anti-tumoral potential, especially against tumors that are unable to resynthesize their own arginine from the precursor amino acid citrulline via argininosuccinate synthase. Pharmacological arginine depletion via pegylated arginine deiminase (ADI-PEG20) is already in clinical phase 1-3 human cancer trials.
Aims: Given the profound suppressive effect of arginine deficiency on adaptive immunity, we now studied the function of granulocytes in the context of arginine depletion.
Methods: Human primary granulocytes were purified from healthy donors and important granulocyte effector functions were measured in cell culture medium in the presence or in the absence of arginine. Arginine deficiency itself was induced either by using arginine-free preformulated medium or by subjecting normal cell culture medium or whole blood to sonicates of human granulocytes with a defined arginase activity, leading to the enzymatic depletion of arginine. The following granulocyte functions were measured *in vitro*: viability (PI/annexin V), expression of various cell membrane marker proteins, activation-induced shedding of CD62L (flow cytometry), phagocytosis (FITC-coupled *E. coli*: Phagotest®), microbicidal activity (XTT viability of *C. albicans*), generation of reactive oxygen species (cytochrome C reduction and oxydation of dihydrorhodamine 123: Phagoburst®), chemotaxis (human serum-induced migration in Boyden chamber), activation-induced synthesis of IL-8 (ELISA) and various other gene products (Real Time PCR). Granulocyte function was also analysed *in vivo* in a murine model of *Aspergillus fumigatus* pneumonia and arginine depletion *in vivo* was induced by injection of ADI-PEG20.
Results: Arginine deficiency had no influence on human granulocyte viability, expression of activation markers and constitutive cell membrane proteins, shedding of CD62L, phagocytosis, generation of reactive oxygen species, fungicidal activity, chemotaxis and induced synthesis of IL-8 or any other gene products on mRNA level. Also, profound pharmacological arginine depletion *in vivo* via ADI-PEG20 did not inhibit murine granulocyte functions: successful granulocyte-dependent clearance of *Aspergillus fumigatus* and survival of mice were not impaired.
Summary and Conclusions: Granulocyte-based innate immune function is not inhibited by arginine deficiency. This novel finding adds to a better understanding of immunity during cancer inflammation-associated arginine depletion and is also important for the development of therapeutic arginine depletion as antimetabolic tumor therapy. Our results are also consistent with the lack of co-existent infections in humans treated with ADI-PEG20.

P1029

LANGERHANS CELL HISTEOCYTOSIS (LCH) IN EGYPTIAN CHILDREN. A SINGLE CENTER EXPERIENCE.

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Background: Langerhans cell histiocytosis (LCH) is a rare immune multisystem disease that behaves from spontaneous regression to rapid progression and death or repeated reactivations especially upon end of treatment. Localized monostotic disease (skin, bone or lymph node) have a good prognosis without treatment. In contrast, multiple, especially high risk organs (liver, spleen, hemopoietic system) have a poor outcome and may need chemo reinforcement.
Aims: To evaluate children cancer hospital Egypt experience in pediatric Langerhans cell Histeocytosis
Methods: In the period between 12/7/2007 and 9/12/2011, 80 LCH patients were treated at Children Cancer Hospital (CCHE) according to the LCH III protocol. M/F: 43 (46%)/37 (54%) with a mean age of 4 $^{2/12}$ years (3 months–13 $^{3/12}$y). Patients were divided into 4 groups: I monostotic lesion n=14 (17.5%), II multisystem with no risk organs RO- n=29 (36%), III multisystem with risk organs RO+: n=19 (24%) and IV unisystem multifocal lesions +/- special sites: n=18 (22.5%). Group I did not receive systemic treatment. Vinblastine (VBL) and Prednisone (PRED) were given as one +/- 2nd induction in Group II and IV, and were associated to methotrexate(MTX)in group III. Maintenance included 6 m to 1 year VBL, PRED for group IV and II respectively. Group III had a 1 year VBL, PRED, MTX and 6 mercaptopurine (6MP).
Results: *Survival* After a minimum 1 year and up to 5.5 year follow up, 70 patients are better (87.5%) in No Active Disease (NAD) or Active Disease Regressive (ADR), 3 patients are intermediate(4%) in Active Disease Stable (ADS), 3 patients are worse (4%)in Active Disease Progressive (ADP), 3 patients (4%) died (all in RO+): disease progression n=2 and viral pneumonia n=1. The Kaplan Meier 5 year

Overall Survival (OS) is (96.3%), the 5 y Event Free Survival (EFS) is (54.7%): Group I (93%), Group II (52%), Group III (47%), Group IV (83%) p0.013. The EFS is worse if age <2 years old (38%) *vs* (71%) (2-10 y), *vs* 90% (>10 y) p0.001. EFS is worse in patients received a 2nd induction (48,7%) *vs* (80.5%) p0.009. *Response*The 6 week evaluation from diagnosis revealed (75%) better. This percentage rose to 89% at W12 with or without induction(s). *Progression /Reactivation* Reactivation occurred n=25 (38%), while progression post induction n=3 (4.5%). The probability of reactivation or progression is 47%. Reactivation is less in prolonged maintenance as 37 patients non reactivated with a mean 10 m maintenance duration *vs* 25 reactivated with a 8 m treatment duration p0.043.

Summary and Conclusions: In Egyptian LCH children, response to induction is satisfactory. Although a greater incidence of reactivation, survival remains the rule. Low age and slow responder carry a worse prognosis. With RO+ methotrexate, 6MP reinforcement, EFS is nearly equivalent to RO-. However mortality are exclusively RO+.

P1030

PEGFILGRASTIM AS PROPHYLAXIS OF FEBRILE NEUTROPENIA DURING CHEMOTHERAPY OF RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Background: Patients receiving cancer chemotherapy are at an increased risk of neutropenia, leading to an increased risk of infections and delays in subsequent chemotherapy treatments. Recombinant granulocyte colony stimulating factors (G-CSFs) have been developed to stimulate proliferation and differentiation of neutrophils in patients receiving chemotherapy. Pegfilgrastim is a pegylated long-acting recombinant form of G-CSF that extends the half-life and allows for once-per-cycle dosing, requiring less frequent dosing than non-pegylated G-CSF. Multiple Myeloma (MM) in advanced phases of disease may be managed by regimens combining agents not frequently employed in early phases of treatment (*e.g.* Anthracyclines, Alkylating agents, etc), but myelotoxicity is the main expected side effect.In this context, G-CSFs are often necessary to counteract the risks of febrile neutropenia: their use is bound to a frequent evaluation of neutrophil counts that may not be easy for patients in home-care. Avoiding severe neutropenia by prophylactic pegfilgrastim seems particularly useful in these cases.

Aims: The objective of this study was to compare the efficacy and safety of pegfilgrastim in patients affected by Multiple Myeloma in advanced phase of disease. In order to determine whether a single subcutaneous injection of pegfilgrastim is as effective as daily injections of standard filgrastim, in terms of haematological toxicity, febrile neutropenic episodes, antibiotic usage and hospedalization duration.

Methods: We have enrolled in our study 18 patients (10 male and 8 female) with a median age of 62 years (range 54-82) affected by multiple myeloma, all relapsed and refractory to a median of 6 lines of therapy (range 4-8), all previously exposed to Bortezomib, Lenalidomide, Melphalan and all relapsed after auBMT, treated with different chemotherapy regimens combining Bortezomib, Lenalidomide, Bendamustine, Melphalan, Doxorubicine.

Results: Since first course, received in our out-patient department, patients performed blood counts twice weekly and received, from day 8 to day 19, prophylactic oral chinolonic antibiotics and anti-fungal drugs. Filgrastim (5 µgr/kg/day for 3 days) was given if neutrophils count was <1500×10^9 cells/L. Median number of filgrastim administrations was 4.5, r. 3-6; nadir neutropenia was registered after a median of 11 days (r. 8-14); median of nadir neutrophil count was 1.27×10^9 cells/L (range 0.4–1.8×10^9 cells/L), with maximum duration of 10 days. After the first course of chemotherapy, patients received prophylactic pegfilgrastim (6 mg), injected subcutaneously with a single administration on day 2 after last dose of chemotherapy, independently from the neutrophil count at that time. Primary endpoint was the duration of neutropenia (neutrophil count <1.5×10^9 cells/L), which was never longer than 10 days. Median nadir neutrophil count, registered 11 days after the end of the therapy (median, r. 9-15) was 1.576 (range 0.63-2.25×10^9 cells/L); two patients (11%) needed a second administration of pegfilgrastim one week after the first (day +9,+10 and +11, respectively) and also a supplement of 3 administrations of filgrastim. During pegfilgrastim, neutropenia lasted less than 10 days. Pegfilgrastim was well tolerated in all patients: main side effects in our patients were fever and bone pain, (3/18 patients,16%)

Summary and Conclusions: In conclusions, in patients affected by MM exposed to myelosuppressive agents in advanced phases of myeloma disease, pegfilgrastim seems to reduce the incidence of neutropenia and may increase the possibility to maintain the scheduled time of treatment.

P1031

CHRONIC BENIGN NEUTROPENIA IN ADULTS: LABORATORY AND CLINICAL PARAMETERS OF A 6-YEAR FOLLOW-UP

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Background: Chronic benign neutropenia (CBN) is a rare hematological condition, characterized by an absolute neutrophil counts (ANCs) lower than 1800/µl in white and 1500/µl in black people for more than 3 months. This condition can be congenital or acquired, idiopathic or secondary and may be characterized by the presence of anti-neutrophils antibodies. While congenital and acquired forms of infancy and childhood are largely studied, with particular attention to the infective diathesis, less is known about adult neutropenia.

Aims: To evaluate clinical and laboratory features of chronic benign neutropenia in adult patients, focusing on ANCs variations, on the positivity for anti-neutrophil auto-antibodies and on the incidence of infectious episodes.

Methods: Complete blood counts and physical examination were performed every 3-4 months in the first 2 years, then at least once a year. Anti-neutrophil antibodies were determined by direct and indirect granulocyte immunofluorescence test (GIFT method). Infectious episodes were defined according to Common Terminology Criteria for Adverse Events (Version 4.0 http://evs.nci. nih.gov).

Results: 47 patients (17 males and 30 females, median age 55 years, range 25-86 years) were followed up for a median time of 47 months (range 6-240 months). As Figure 1 shows, mean ANCs at enrolment and over time displayed a great variability, both inter and intra-subject (>500/µl in 74% and >1000/µl in 25% of patients). Considering the severity of neutropenia, we observed 23 patients (49%) with neutrophils lower than 1000/µl in at least one observation (median number of 470/µl neutrophils, range 100-969/µl). Finally, the mean ANCs observed during the follow up was significantly lower in males than in females (610/µl, range 100-1380/µl *versus* 1070/µl, range 100-1750/µl, respectively; P=0,02). Anti-neutrophil antibodies were detected in 18/45 patients (40%), and mean ANCs were significantly lower in positive *versus* negative cases (P=0,021 for 6 and 12 months time). Bone marrow evaluation showed features of dismyelopoiesis in 14 cases (56%), hypocellularity in 3 (12%) and normal morphology in 8 (32%). Flow cytometry demonstrated increased Natural Killer cells in 13 patients (28%), Cytogenetic was normal 22 cases (88%), while in 3 male patients karyotype was 45, X0 (7, 6 and 3 metaphases respectively). Finally, monocyte counts were higher than 600/µl in 8 patients (17%), and 5 of them (62%) showed an NK marrow infiltrate (P=0,015). Mild splenomegaly was present in 10 patients (21%) with a mean maximal diameter of11,4 cm by ultrasonography. These cases displayed lower ANCs compared with cases without splenomegaly (835/µl, 140-1400, *versus* 1380/µl, 200-3239, at 3 months; P=0,03). During the study 11 patients (23%) had an infection needing oral antibiotic or antiviral therapy (7 upper respiratory tract infections, 3 Herpes Zoster Virus infections and 1 urinary tract infection); no relationship was found between the occurrence of infections and the patient's mean ANCs value, the nadir of ANCs, and the presence of anti-neutrophil antibodies.

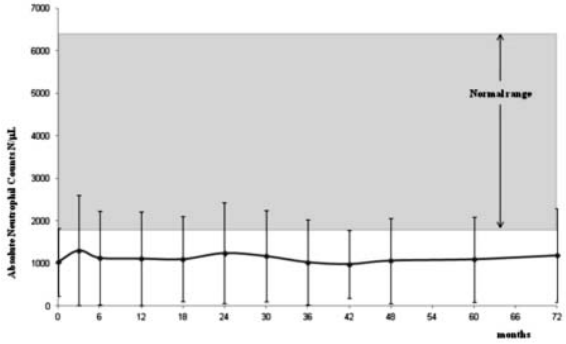

Figure 1. Mean ANCs values during follow up (mean±SD).

Summary and Conclusions: In spite of the alarm that produces in the general practitioner, CBN in adults is a benign disease, often incidentally diagnosed, with an infectious rate comparable to that of general population and frequent spontaneous ANCs variations. Bone marrow evaluation shows abnormal findings in a half of patients, without reaching the criteria for clonal hematological diseases, but suggesting that this condition deserves clinical follow up.

P1032

NEUTROPHIL RESPIRATORY BURST ACTIVITY COULD SERVE AS A PROGNOSTIC MARKER IN SEPSIS PATIENTS, AND NEUTROPHIL CD10 AND CD16 EXPRESSIONS MIGHT BE BIOMARKERS FOR SEPSIS SEVERITY

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Background: Respiratory burst activity (RBA) is important for the killing of

microorganisms; however, it may be also detrimental to the body due to tissue damage. The role of neutrophils in septic shock has not been well investigated. Recently, changes were reported in the neutrophil surface antigens CD64 (high affinity receptor for IgG), CD10 (neutral endopeptidase), and CD16 (low affinity Fc receptor) during sepsis and DIC.
Aims: The aim of this study was to evaluate RBA and surface antigen expressions of neutrophils in septoc patients to determine their clinical significance.
Methods: EDTA peripheral blood was drawn from 105 healthy controls and consecutive 71 patients (52 males and 19 females; median age, 67 years; range, 29–92 years) with severe sepsis (16 patients) and septic shock (55 patients) from January 2011 to October 2012. Using flow cytometry, RBA was measured in nonstimulated and PMA-stimulated states by the change of nonfluorescent DCF-DA to green fluorescent DCF with the production of hydrogen peroxide. The surface expression of CD64, CD10, and CD16 on neutrophils were measured by the binding of fluorescence-conjugated anti-CD64, anti-CD10, or anti-CD16 antibodies.
Results: Compared with control neutrophils, patients' neutrophils with sepsis showed increased levels with non-stimulated RBA and CD64 expression, and decreased values in CD10 and CD16 expression (P<0.001 for all biomarkers). Expressions of CD10 and CD16 were lower in patients with septic shock than patients with severe sepsis (P=0.031, 0.002, respectively). When the patients were subdivided into 2 groups according to the mortality within 28-days, PMA stimulated RBA of the patient's neutrophils was higher in survivor group (P=0.002). CD64 expression was also elevated in survivor group, but there was no statistical significance (P=0.071).
Summary and Conclusions: During sepsis, neutrophils are highly activated, and the increased neutrophil RBA by PMA stimulation is associated with better prognosis. Neutrophil RBA could serve as a prognostic marker in patients with sepsis, and CD10 and CD16 expressions of neutrophils might be biomarkers for sepsis severity.

P1033

THE ULTRASOUND EXAMINATION OF SPLEEN CAN STRONGLY RAISE THE SUSPICION OF THE GAUCHER DISEASE.
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Background: The Gaucher disease (GD) is a lipid storage disease inherited as an autosomal recessive trait. It results from an inherited deficiency of acid β- glucosidase leading to accumulation of glucosilceramide in tissue macrophages, causing hematologic, visceral organs, and skeletal abnormalities.
Aims: To provide clinical and laboratorical data from GD patients and to try to find any special features that raise suspicions of diagnosis prior to the bone marrow examination, or enzyme estimation.
Methods: 1996-2012 We analyzed the observational data from 11 GD patients diagnosed for the first time in our clinic and we tried to find any correlation betweem them and GD diagnosis before BM examination.
Results: Mean age was 30.7 years (16-65). 81% (9/11) of patients were diagnosed before age 35. Only one was 65 years old. Three patients were from the same family, two brothers and one sister. Ratio M/F 54% (6/5). At diagnosis, anemia, HB<11gr/dl, was present in 72% (8/11), thrombocytopenia PL<100000/μL in 63% (7/11), and leucopenia WBC<4000/μL in 54% (6/11). Albuminuria was reported in 63% (7/11) of the patients. Bone marrow examination revealed the presence of Gaucher cells in all cases. Hemoglobin electrophoresis was normal in all cases except2, in which minor thalassemia was observed. Hepatomegaly was reported in all patients. Bone pain was reported in 45% (5/11) of patients.Splenomegaly was reported in all patients at 100% (11/11). Spleen size varied from 5-20 cm under the costal arch. In 63% of pts. the spleen was larger than 10 cm under costal arch. The most surprising thing was the strong correlation between spleen size and the view of the ultrasound examination (USE) of the spleen. The USE of the spleens showed hypoechodens areas in 63% (7/11) of the pts. Hypoechodens areas are prescribed by some "brave" examiners as areas that suggest spleen lymphomas or spleen malignancy, but these were not associated with signs and symptoms of malignancies such as temperature, sweats, weigh loose, etc. These Hypoechodens areas are observed in all cases (7/7) with splenomegaly>10 cm under costal arch.
Summary and Conclusions: Splenomegaly was among the most common signs of GD patients. The hypoechodens areas reported in USE of the spleen, mainly in the large spleen, which are not associated with signs and symptoms of malignancy, may strongly generate suspicions and lead to the GD diagnosis.

P1034

LANGERHANS CELL HISTIOCYTOSIS: ONE CENTER EXPERIENCE BETWEEN 1990-2012
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Background: Langerhanscell histiocytosis(LCH) isa disease seen in children especially.
Aims: Diagnosis andfollow-upof Langerhanscell histiocytosis(LCH)patientsat our center were evaluated in this study
Methods: Retrospectively the files of the LCH patients who were treated at our center were analyzed
Results: During the 23 year of follow up, 80 patients (56 boys and 24 girls) were diagnosed with LCH. The complains of the patients were; local swelling (n:36, 45%), bone pain (n: 24, 30%), skin eruption (n:16, 20%), discharge from the ear (n:6, 8%), polydipsia (n:6, 8%), fewer (n:3, 4%), cough-respiratory distress (n:3, 4%) jaundice (n:2, 2%), abdominal distention (n:2, 2%), tooth loose (n:2, 2%) and diarrhea (n:1, 1%). One of the patients were diagnosed with an X-Ray taken after trauma. The patients were evaluated at 4 different groups; One system one organ involvement (n:28), one system multifocal involvement (n: 15), multisystem low risk (n:21), multisystem high risk (n: 16). The mostly involved area was bone; (one system one organ involvement n:22, one system multifocal involvement n:14, multisystem low risk n:14). Mostly involved organ at multisystem risk group were; liver (n:14), skin (n:17), bone (n:6), bone marrow (n: 6), spleen (n: 5), lungs (n:5), lymph node (n:3), soft tissue (n:1) and intestine (n:1). Ten of the 80 patients were transferred to other centers after diagnosis. The remaining 70 patients were treated at our center. Fourteen patients with one system one organ involvement and 4 patients with one system multifocal involvement were treated with curretage-excision of the tumour. Only one of these patients had a local relapse and treated succesfully with re-excision of the tumour. Three patients received systemic chemotherapy (prednisolone and etoposide) after curretage because lesion could not be removed completely. No relapse occured at any of these patients. Five of the patients with one bone involvement received radiotherapy to the involved side; and 3 of these patients relapsed and all had gone into remission with treatment (1 patient recieved systemic chemotherapy an 2 had excision of the lesion). Remission was achieved at patients with soft tissue involvement with local intralesionel steroid thereapy (n:4.) One of the patients had regression of the tumour spontaneously. The chemothereapy protocole was LCH-I before 1996, LCH-II between 1997 and 2002 and LCH-III after 2002. Twenty seven of the patients with systemic involvement received prednisolone+vinblastine, 13 received prednisolone+vinblastine+etoposide and 3 patients received prednisolone+vinblastine+methotraxate. Respectively 7, 4 and 1 of these patients had reccurence. Fifty four of 70 patients achieved remission after treatment and did not have any relapse. Twelve of patients had (17%) had relapse. Six of these patients had 2, 4 of these patients had 3 and 2 of these patients had 4 times of relapses. One of these patients died after the second relapse. The rest 11 patients are in remission. Four patients with multi system involvement died during treatment due to progression of the disease. These patients were not in remission and died with an avarage of two months after the diagnosis.
Summary and Conclusions: Langerhans cell histiocytosis is a disease that involves multiples organs. Systemic or local treatment has a good response but 20-25% of patients might have relapses.

P1035

A NEW TYPE OF CHEDIAK-HIGASHI SYNDROME? CASE REPORT OF A BLACK HAIRED GIRL WITHOUT OCULOCUTANEUS ALBINISM PRESENTED WITH EARLY ONSET HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS
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Background: Chediak-Higashi Syndrome(CHS) is a rare, autosomal recessive disorder characterized by variable degrees of oculocutaneus albinism, severe immune deficiency, lymphoproliferative syndrome and intracytoplasmic giant granules in leukocytes. Giant granules may be present in Imphocytes, monocytes and melanocytes.
Aims: In this article, we report a case of CHS with neither oculocutaneus albinism nor silvery hair presented with early onset hemophagocytic lymphohistiocytosis(HLH).
Methods: Case report
A five months old girl presented with high fever. She had splenomegaly, pancytopenia, hipertrigliceridemia and high ferritin levels. Hemophagocytosis was observed on bone marrow aspiration smear. She was diagnosed with HLH. HLH-2004 treatment protocol including dexametasone and etoposide was administrated. Any known perforin gene defect was not determined. On both bone marrow aspiration and peripheral blood smears intracytoplasmic giant granules on lymphocytes were observed. She had neither oculocutaneus albinism nor silvery hair but analysis of the hair shaft with ligth microscope was consistent with CHS (photograph). Thus she was diagnosed with CHS.
Results: CHS is usually known as a disease characterized by oculocutaneus albinism and silvery hair but our patient does not have oculocutaneus albinism and she is black haired. Patients with CHS may have a variable clinical presentation due to a different mutations in the lysosomal trafficking regulator gene (LYST), other genetic factors and an exposure to other different pathogens. Nonsense and frameshift mutations of the LYST gene are asssosiated with severe early-onset

childhood CHS and characterized by fatal infections and HLH. Whereas missense mutations of the same gene are assosiated with milder, late-onset CHS with slowly progressive neurological impairment or an adolescent form with infections but no HLH. Also there is a correlation between the absence of cytotoxic T lymphocyte citotoxicity and risk of developing HLH. Probably there may be a mutation of the LYST gene that is assosiated with normal phenotype and the specific findings of the CHS on peripheral blood smear, bone marrow aspiration smear and light microscopic image of the hair shaft but however causes to an early-onset HLH. In our case there was no specific treatment for CHS. Intraveneus antibiotherapy and in accordance with HLH 2004 treatment protocol dexamethasone and etoposide were used and the patient has been positively responsive to chemotherapy. Stem cell transplantation has been planned (Figure 1).

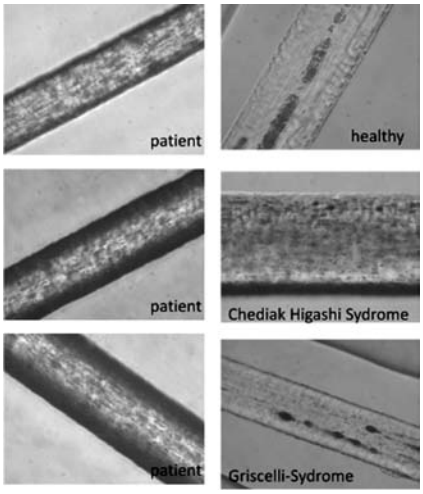

Figure 1.

Summary and Conclusions: Our case is suggestive of that it is not necessary to find oculocutaneus albinism and silvery hair in all patients to diagnose CHS. Peripheral blood smear and light microscopic image of the hair shaft findings may be sufficient to diagnose in some cases. And it is more cost effective than genetical analysis even for prenatal diagnosis.

P1036

CHEMOTHERAPY-ASSOCIATED TREATMENT BURDEN IN BREAST CANCER PATIENTS RECEIVING LIPEGFILGRASTIM OR PEGFILGRASTIM: SECONDARY EFFICACY DATA FROM A PHASE III STUDY

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Background: Myelosuppressive chemotherapy (CTx) frequently causes neutropenia, leading to an increased risk of infections and delays in subsequent CTx treatments. PEGylation of the granulocyte-colony stimulating factor (G-CSF) molecule extends its half-life in the body, requiring less frequent dosing than traditional non-PEGylated G-CSFs, potentially making treatment less expensive while enhancing patient compliance and safety. Lipegfilgrastim is a long-acting, once-per-cycle glycoPEGylated G-CSF in development for the prevention of neutropenia in cancer patients receiving CTx. Noninferiority of lipegfilgrastim *versus* pegfilgrastim (Neulasta®)was demonstrated in a Phase III trial conducted in CTx-naïve breast cancer patients.[1]

Aims: To present secondary data on the incidence of hospitalization and density and intensity of CTx in lipegfilgrastim- and pegfilgrastim-treated patients.

Methods: Patients with high-risk Stage II, III, or IV breast cancer were randomized to a single 6 mg subcutaneous injection of lipegfilgrastim (n=101) or pegfilgrastim (n=101) on Day 2 of each CTx cycle. Full-dose CTx (doxorubicin 60 mg/m^2+docetaxel 75 mg/m^2) was started on Day 1 of each cycle (day 22 of the previous cycle) for up to four consecutive cycles. The primary endpoint was duration of severe neutropenia. Secondary endpoints included days in the hospital or intensive care unit (ICU) and incidence of treatment with intravenous antibiotics because of febrile neutropenia (FN) or related infections; actual *versus* scheduled cumulative CTx dose per patient; reduced, omitted, or delayed CTx doses; and duration of CTx delays.

Results: Key secondary endpoints are listed in the Table 1. In the intent-to-treat population, two pegfilgrastim-treated patients and one lipegfilgrastim-treated patient were hospitalized in Cycle 1 because of FN or associated infection. The two pegfilgrastim-treated patients were hospitalized for FN for 5 and 6 days, respectively, and the lipegfilgrastim-treated patient spent 1 day in the ICU for FN and a fungal infection. All hospitalized patients received antibiotics; the lipegfilgrastim-treated patient also received antipyretics. An additional pegfilgrastim-treated patient received antibiotics but was not hospitalized. The frequency and duration of CTx dose delays were low and comparable between groups; <40% of patients had CTx dose delays. The majority of patients received CTx as scheduled, with the mean percent of planned doxorubicin and docetaxel doses reaching over 98% in all cycles. Thirty-four patients in the lipegfilgrastim group and 41 patients in the pegfilgrastim group received delayed CTx in Cycles 2−4. There were no dose omissions or reductions in the lipegfilgrastim group and eight in the pegfilgrastim group in Cycles 2−4.

Table 1. Key secondary endpoints.

	Pegfilgrastim 6 mg				Lipegfilgrastim 6 mg			
Cycle	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
n	101	100	98	98	100	99	98	95
Hospitalized, n (%)	2 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
CTx delay, n (%)	N/A	15 (15.0)	17 (17.3)	9 (9.2)	N/A	16 (16.2)	14 (14.3)	4 (4.2)
CTx dose reduced/ omitted, n (%)	N/A	4 (4.0)	2 (2.0)	2 (2.0)	N/A	0 (0.0)	0 (0.0)	0 (0.0)
Actual CTx dose applied, (% of planned dose)	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
• Doxorubicin	99.3	98.2	98.3	98.4	99.1*	98.7	98.8	98.9
• Docetaxel	99.2	98.2	98.3	98.3	99.3*	98.9	98.9	98.9

*N=101

Summary and Conclusions: The burden of treatment associated with myelosuppressive CTx was similar in breast cancer patients treated with lipegfilgrastim or pegfilgrastim, with no clinically relevant differences in density and intensity of CTx.

Reference

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Platelets 2

P1037

USE OF ROMIPLOSTIM IN PATIENTS WITH CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA (CITP) DURING PERI-OPERATIVE PERIOD

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Background: Conventional treatments for managing cITP in the peri-operative period include corticosteroids or IVIG and immunosuppressive therapies. Each of these therapies has its own drawbacks.[1-3]Romiplostim, a thrombopoiesis stimulating peptibody is currently indicated for use weekly for long-term management of patients with prolonged very low platelet counts (<20/nL) or low platelet counts with bleeding (20-30/nL). Cessation of therapy after periods >10 weeks can be associated with marked, unremitting rebound thrombocytopenia[4]. Its use in the peri-operative period is not well established. Patients who are refractory to steroids and IVIG can be a challenge to manage peri-operatively. Safety data on use of thrombopoietin agonists is limited with little or no published data exists on short-term use, such as during the peri-operative period.
Aims: The aim of this cohort study was to evaluate the safety and effectiveness of Romiplostim in managing the bleeding risk of cITP patients peri-operatively.
Methods: Patients with cITP requiring surgery who had proven refractory to IVIG and steroids were trialled on Romiplostim to determine its effectiveness at managing their bleeding risk peri-operatively. Patients were given up to three doses starting 2 weeks prior to their surgery with a starting dose of 3µg/kg. Platelet counts were monitored and subsequent doses were adjusted as necessary.
Results: Thirteen surgical procedures were performed after using this protocol and none of the patients treated with this protocol experienced any bleeding complications peri-operatively (Table 1). One patient experienced mild headaches associated with Romiplostim usage which resolved once therapy was ceased. One patient experienced mild rebound thrombocytopenia which resolved to baseline spontaneously after 4 weeks. One patient had a delayed response to treatment. One patient achieved sub-optimal response but no surgical complications. Possible platelet dysfunction did not appear to contribute to any bleeding complications.

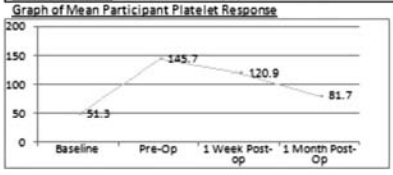
Table 1.

Summary of Results

Demographics	Type of Surgery	Baseline Plts	Pre-Op Plts	1 Week Post-Op Plts	1 Month Post-Op Plts	Platelet Function	Complications
Female/ 63 years old	Mitral valve replacement	65	139	174	146	Abnormal	Nil
Male/ 65 years old	Mitral valve replacement	35	100	70	40	Abnormal	Nil
Male/ 52 years old	Liver biopsy	32	114	80	45	Abnormal	Headache
Male/ 56 years old	Thyroidectomy	35	145	164	97	Abnormal	Nil
Male/ 52 years old	Aortic valve replacement	68	215	128	166	Abnormal	Nil
Female/ 46 years old	Mucosectomy	39	122	139	86	Normal	Nil
Male 69 years old	Coronary artery bypass graft	51	104	52	57	N/A	Nil
Male/ 56 years old	Cystoscopy and prostate enucleation	75	207	200	114	Abnormal	Nil
Male/ 45 years old	Thyroidectomy and neck dissection	64	420	42	62	Normal	Rebound thrombocytopenia
Male/ 75 years old	Spinal surgery	62	104	247	73	Abnormal	Nil
Female/ 59 years old	Colonoscopy/ gastroscopy	54	50	134	51	N/A	Had a delayed response to therapy.
Female/ 58 years old	Liver Biopsy	47	104	102	89	N/A	Nil
Male/ 64 years old	Colon Polypectomy	39	70	40	36	Abnormal	Platelet count only 70 pre-op, surgeon happy to go ahead.

Abbreviations used: Plts = platelet count (nL)

Graph of Mean Participant Platelet Response



Summary and Conclusions: Romiplostim so far appears to be effective in managing bleeding risk in the peri-operative period. It eliminates the need for platelet transfusions or immunoglobulin infusions, saving this resource. In addition, it is relatively convenient for patients when compared to intravenous therapies, in terms of time required, invasiveness and incidence and severity of adverse events. The platelet response is maintained beyond one month in some cases, and rebound thrombocytopenia does not appear to be a concern in our experience. Our data supports use of Romiplostim as a safe and effective therapy for management of bleeding risk for patients with cITP, during the peri-operative period.

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P1038

CIRCULATING FOLLICULAR HELPER T CELLS IN CHILDREN WITH IMMUNE THROMBOCYTOPENIC PURPURA

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Background: Immune Thrombocytopenic Purpura (ITP) is an autoimmune disorder characterised by low platelet count and mucocutaneous bleeding. Immunological tolerance among T cells is of paramount importance for the control of autoimmune antibody specificities. Recent studies examined the T-cell repertoire in patients with ITP and found differences compared with healthy individuals, lending further credence to T cells being at the heart of the pathogenesis of the disease. T follicular helper cells (TFH) are the effector T helper that regulates the steP-wise development of antigen-specific B cell immunity *in vivo*. It is likely that TFH cells provide inappropriate helper signals to self-reactive B cells in cases of antibody-mediated autoimmune diseases. Recent studies raised the possibility that dysregulated TFH cell activity may contribute to SLE in humans.
Aims: The aim of the study is to analyze the TFH cells in children with ITP.
Methods: Thirty-one pediatric ITP patients, 15 males and 16 females, with a median age of 5 years (range 1-15), were enrolled in the study. In13, TFH analysis was performed at diagnosis, prior to any drug treatment (acute ITP). Eighteen patients were enrolled in the chronic phase of the disease (ITP lasting for more than 12 months); at the time of sample collection, patients were at least one month off-treatment. Twelve healthy children were enrolled as control group.T cells were analyzed by flow cytometry from peripheral blood mononuclear cells (MNC). Immunofluorescence was performed using the following monoclonal antibodies (Becton Dikinson): FITC anti-CD45RO, PE anti-CXCR5, Per-CP anti-CD3, APC anti- CD4. Cells were analyzed by using aBD FACS Canto II equipment and were gated for lymphocytes on the basis of their forward scatter and side scatter profile. A minimum of10,000 events of the MNC fraction was collected. Statystical analysis was performed with the Wilcoxon's rank sum test and the t-student test. Spearman's rank correlation test was used for correlation analysis.
Results: The median percentage of circulating TFH cells resulted significantly lower in acute ITP patients than in chronic ITP patients (4,23*versus*8,60; P=0.0001) and in acute ITP patients than in controls (4,23*versus*7,79; P=0.0004). No significant correlations were detected between TFH cells and platelet count, between TFH cells and gender, and between TFH cells and anamnestic data (family history for autoimmune disease, recent history for infection or vaccination).
Summary and Conclusions: Our results suggest that dysregulated TFH may have a prognostic value in ITP.

P1039

A MULTICENTER OBSERVATIONAL STUDY FOR EARLY DIAGNOSIS OF GAUCHER DISEASE IN PATIENTS WITH SPLENOMEGALY AND/OR THROMBOCYTOPENIA

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Background: Gaucher disease (GD) is an autosomal recessive lysosomal storage disorder resulting from deficiency of beta-glucosidase and the accumulation of glucocerebroside in the reticuloendothelial cells. Prevalence of GD is elevated in Ashkenazi Jewish population (1/450-1/1000), and rare in the non-Ashkenazi (1/40000-1/60000). GD is a multisystemic disease; cytopenias and splenomegaly are frequently the presenting symptoms leading to hematological evaluation. Data from the Gaucher Registry 2008 show that splenomegaly and thrombocytopenia are present at diagnosis in more than 5000 patients (respectively 86% and 60%). Because of the non-specific presenting symptoms, diagnostic delays are frequent, leading to severe complications including hematological malignancies.Enzyme replacement therapy is available and-

effective in reversing or preventing many manifestations, including hepatosplenomegaly, marrow infiltration, cytopenias and osteopenia (Weinreb 2002). A global survey among 406 Hematology-Oncology specialists demonstrated that only 20% consider GD in the differential diagnosis of cytopenia, hepatosplenomegaly, and bone pain (Mistry 2007). It is clear that a different approach based on a specific diagnostic algorithm is necessary to avoid underdiagnosis (Mistry PK 2010).

Aims: The aim of this multicenter observational study is to evaluate the prevalence of GD in a selected population presenting to hematological clinic with at least one of the two including criteria: 1) splenomegaly, 2) thrombocytopenia associated to at least one of the following symptoms: anemia (Hb<11 g/dl for women, and Hb<12 g/dl for men), MGUS, policlonal gammopathy in patient younger than 30 yo, splenectomy or history of bone pain. Exclusion criteria include: a) splenomegaly due to portal hypertension in cirrhosis, b) hematological malignancy, c) hemoglobinopathies or other hemolitic anemias.

Methods: Thirty five Italian Hematologic Centers participate in this study. According to a preliminary survey, 18% of all hematologic first evaluations are positive for splenomegaly and/or thrombocytopenia, among them 11% did not received an appropriate diagnosis. According to these data 762 patients are expected to be tested every year (mean of 1100 first evaluations/year for each center). Patients fulfilling including criteria who have given their informed consent are recruited into the study and tested for beta-glucosidase enzyme activity on Dried Blood Spot (DBS). All the analysis are centralized and performed by the Laboratory of diagnosis of Metabolic Diseases Ospedale Gaslini, Genova. Results can show normal, decrease or borderline beta-glucosidase activity. In the last case, DBS must be repeated to confirm the result. Beta-glucosidase deficiency and GD diagnosismust be subsequently confirmed dosing the enzyme activity in the leukocytes from fresh blood and by DNA analysis. The expected duration of the study was 24 months, starting from September 2010, subsequently extended up to the enrollment of 500 patients (recruitment still active at present).

Results: Starting from September 2010 153 patients (45 female, 108 male) have been enrolled. All the patients are non-Ashkenazi, among them 61% had splenomegaly, 4% thrombocytopenia and 35% both of them. Six patients have been diagnosed with GD.

Summary and Conclusions: Our results are clinically relevant, showing that the use of a simple diagnostic algorithm helps identify patients presenting to hematologists with a early diagnosis of GD, leading to an appropriate and prompt therapy to prevent the development of complications.

P1040

A RETROSPECTIVE ANALYSIS OF EPSILON-AMINO-CAPROIC ACID THERAPY IN HYPOPROLIFERATIVE THROMBCYTOPENIA

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Background: We performed a retrospective analysis of epsilon-amino-caproic acid (EACA) use in patients with hypoproliferative thrombocytopenia from January 2006 to July 2012. 221 patients, 18 years or older, received EACA on 260 separate occasions. EACA use increased from 13 courses in 2006 to 57 during Jan-July 2012. Of the 160 patients reviewed to date, 101 were treated with at least two doses of EACA and were included in this analysis. Data were collected on indications for EACA therapy (to treat bleeding *vs.* prophylaxis), WHO bleeding grade before and during therapy, platelets and red blood cells transfused for 3 days prior to and up to the first 7 days after starting therapy, and thrombotic events for 2 weeks after beginning therapy. We used a modification of the WHO bleeding scale to identify patients with grade 2a bleeding, defined as WHO grade 2 excluding skin bleeding.

Aims: To review the efficacy of EACA therapy for prevention and treatment of bleeding in hypoproliferative thrombocytopenia.

Results: Of the 101 patients evaluated, 92 were treated with chemotherapy for hematologic malignancy, and of those 32 were post stem cell transplant. The other 9 patients had aplastic anemia, immune thrombocytopenia or drug induced marrow aplasia. All 101 patients received EACA in hospital; 12 were also treated as outpatients. Twenty-two patients were treated with 3 to 8 grams of EACA prophylactically [14 (63%) were platelet refractory], and none developed bleeding. Seventy-nine patients were treated with 2 to 24 grams of EACA daily for treatment of bleeding [43 (54%) were platelet refractory]. Among the 79 bleeding patients, the highest grade of bleeding was grade 1 in 12 patients (15%), 2a in 29 (37%), grade 3 in 26 (33%) and grade 4 in 12 (15%). Bleeding improved in 58 (74%) patients, was unchanged in 14 (18%), and worsened in 7 (8%). Improvement in bleeding grade was seen in 7 of the 12 patients with grade 1 bleeding, 24 of 29 patients with grade 2a bleeding, 21 of 26 patients with grade 3 bleeding, and 6 of the 12 patients with grade 4 bleeding. The average platelet and red blood cell transfusion rate decreased substantially while on EACA therapy (Table 1).

None of the patients treated with EACA prophylactically had a thrombotic event. Of the bleeding patients, 4 patients developed thrombotic events: 1 patient had an incidental sub-segmental pulmonary embolus identified on a CT chest exam; 1 patient developed atrial fibrillation 3 days after stopping EACA and had an upper extremity central line related deep venous thrombosis and a sub-segmental pulmonary embolism; 1 patient developed a central line associated internal jugular vein thrombus; and 1 patient had hypoxia secondary to circulatory overload and an incidental finding of a sub-segmental pulmonary embolus. None of the 101 treated patients developed sinusoidal obstructive syndrome (veno-occlusive disease). The overall incidence of thrombotic events in all patients treated with EACA was 4/101 patients (3.9%), not unlike the incidence previously reported in similar patients not treated with EACA.

Table 1. Transfusion frequency before and after EACA treatment.

	Before EACA Treatment*		During EACA Treatment**	
All Patients (n=101)	*Red Blood Cells*	*Platelets*	*Red Blood Cells*	*Platelets*
Units per patient /day	0.87	1.16	0.51 (-37%)	0.95 (-22%)
	Before EACA Treatment*		During EACA Treatment**	
Bleeding Patients (n=78)	*Red Blood Cells*	*Platelets*	*Red Blood Cells*	*Platelets*
Units per patient/day	0.98	1.24	0.56 (-43%)	1.04 (-21%)
	Before EACA Treatment*		During EACA Treatment**	
Clinically significant Bleeding * (n=67)**	*Red Blood Cells*	*Platelets*	*Red Blood Cells*	*Platelets*
Units per patient/day	1.09	1.31	0.60 (-49%)	1.12 (-19%)

*Three days of observations before EACA treatment
**Seven days of observations after EACA treatment
***Bleeding grade 2a or greater

Summary and Conclusions: Our data support the hypothesis that EACA therapy decreases bleeding and reduces transfusion requirements in patients with hypoproliferative thrombocytopenia.

P1041

THE USE OF MYCOPHENOLATE MOFETIL IN THE TREATMENT OF CHRONIC REFRACTORY IMMUNE THROMBOCYTOPENIA PURPURA

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Background: Immune thrombocytopenia purpura (ITP) is an autoimmune disorder frequently encountered in clinical practice. It is characterized by an isolated thrombocytopenia without an obvious precipitant.

There is a general consensus that first-line treatment includes steroids and/or immunoglobulin. Second-line treatment options are more variable and less evidence-based. Failure to sustain a response to standard first line therapies (steroids and IVIg) frequently presents a clinical challenge. Thrombopoietin agonists are not yet universally available, and so more commonly available drugs may be useful interim measures.

Aims: We present 25 chronic or refractory ITP patients treated with mycophenolate mofetil (MMF), in order to investigate whether this is a useful treatment modality in this difficult disease.

Methods: Retrospective data was collected and a total of 25 patients were identified as having been treated with MMF. We collected demographic information, co-morbidities, previous treatment strategies, when MMF was commenced, type and time to response, duration of response, dose of MMF required to maintain a response, intercurrent therapies (eg steroids), side effects and the current status of all patients.

Results: The 25 patients were aged 20 to 93 years old (median 45 years), 14 men and 11 women. Previous treatment strategies included IVIg (n=20), steroids (n=20), rituximab (n=10), splenectomy (n=2), azathioprine (n=4), anti-D (n=4) and eltrombopag (n=1). Comorbidites were varied and included hepatitis C, HIV, diabetes, systemic lupus erythematosus, liver cirrhosis and schizophrenia. These influenced treatment options and outcome. 12 of 25 patients (48%, 5 men and 7 women), responded to MMF, defined as a platelet count of $>30\times10^9$/L and absence of bleeding, most on an initial dose of 1gram BD. Responders were aged 19 to 85 years (median 43 years). 4 patients showed a sustained complete response (platelets $>100\times10^9$/L); 2 men and 2 women aged between 21 and 67 years of age. Time to response was on average 2 to 5 weeks. and sustained at a median dose of 500mg BD MMF.

7 responders had a sustained platelet count of 50-100 × 10^9/L; 3 men and 4 women aged 19 to 85 years. Time to response was 4 weeks, and sustained at 500mg BD (range 2–52 months). There was 1 partial responder, with a sustained platelet count of 30- 50 × 10^9/L on 500mg BD MMF and no bleeding, in a 43-year-old woman whom had failed to respond to multiple therapies including TPO agonists. No significant side effects were documented. The 13 non-responders (5 men and 8 women) included 2 patients who discontinued MMF before any response was seen, due to gastrointestinal and dermatological side effects. Age range was 30 to 93 years. 4 patients were HIV or hepatitis C (HCV) positive. 4 of the non-MMF responders subsequently responded to a TPO agonist.

Summary and Conclusions: From our case series, we conclude that MMF is a useful steroid-sparing immunosuppressant in resistant, non-viral induced ITP. There were response rates in 48% of cases and 20% achieved a complete response. There was an acceptable side effect profile in patients who previously had refractory immune thrombocytopenia.

P1042

SELF-ADMINISTRATION OF ROMIPLOSTIM BY PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA DOES NOT ADVERSELY IMPACT EFFICACY OR SAFETY COMPARED WITH ADMINISTRATION BY A HEALTHCARE PROVIDER

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Background: Romiplostim is a subcutaneously administered thrombopoietin-receptor agonist that significantly increases and maintains platelet counts in patients with chronic immune thrombocytopenia (ITP). Romiplostim was initially approved in the EU and US for administration by a healthcare provider; however, self-administration has recently been approved by the European Medicines Agency.

Aims: To evaluate the impact of self-administration on efficacy, safety, and maintenance of therapy in adult patients who received romiplostim by self-administration in the home setting (SA group) compared with those who received romiplostim by a healthcare provider in a clinical setting (HCP group).

Methods: Outcomes were retrospectively compared between SA and HCP groups using data from 2 phase III randomized controlled trials and 1 open-label trial of romiplostim in adult patients with ITP in which self-administration was allowed. Patients achieving a stable romiplostim dose for ≥3 consecutive weeks could begin SA. The start of SA was used as the first week of analysis for the SA group. The HCP group included patients who had all romiplostim doses administered by a HCP and who stayed in the study for at least8,6, and 12 weeks (representing the first weeks of analysis in studies1,2, and3, respectively, and the median time of initiation of SA). Evaluations of efficacy, safety, and maintenance of therapy were conducted for 12-week treatment intervals. Endpoints for this analysis included maintenance of self-administration, platelet counts, and percentage of weeks with a platelet response. Duration-adjusted rates of adverse events (AEs), serious adverse events (SAEs), and treatment-related AEs, were also summarized.

Results: There were 563 patients in the SA groups and 241 patients in the HCP groups (Table 1). Baseline characteristics (eg, age and prior/concurrent ITP therapy in study 1; lower baseline platelet count, history of splenectomy, and concurrent ITP therapy in study 2; and history of splenectomy in study 3) suggested more severe disease in the HCP groups than in the SA groups. Greater proportions of patients in the SA groups achieved the target platelet range, and patients in the SA groups maintained platelet counts within the target range for a greater proportion of the treatment period than patients in the HCP groups (75.1–88.3% of weeks for the SA groups *vs* 46.9–76.3% of weeks for the HCP groups; Table 1). The rate of discontinuation of romiplostim was 2- to 4-fold lower in the SA groups than the HCP groups, with AEs and requirement for alternative therapy being common reasons for discontinuation. Rates of duration-adjusted AEs, SAEs, and treatment-related AEs were lower in the SA groups than the HCP groups.

Summary and Conclusions: In adult patients with ITP receiving romiplostim, self-administration did not reduce efficacy or compromise safety profiles as compared with healthcare provider administration. Indeed, compared with administration by a healthcare provider, a greater percentage of patients who self-administered romiplostim maintained target platelet counts and for longer time periods, and had lower rates of discontinuation. Duration-adjusted AEs were generally lower in the SA group than in the HCP group. However, patients were not randomized to SA/HCP groups, and the imbalance of the baseline characteristics may have confounded the results. Nonetheless, these results suggest that self-administration of romiplostim is a feasible option for certain patients with ITP.

Table 1. Summary of results.

Parameter	Study 1[1]		Study 2[2]		Study 3[3]	
	HCP (n=42)	SA (n=109)	HCP (n=48)	SA (n=239)	HCP (n=151)	SA (n=215)
Mean (SD) baseline PLT, 10^9/L	27.9 (14.2)	29.9 (13.1)	61.6 (83.1)	75.9 (85.4)	18.6 (23.1)	17.2 (12.5)
Patients (when n≥20) with PLT in target range* for all 12-wk intervals, %	54.5%–58.3%	57.7%–65.7%	45.2%–68.2%	61.8%–68.7%	37.0%–58.6%	58.2%–67.6%
Mean (SD) % of wk with PLT in target range*	52.3 (28.0)	58.4 (29.6)	46.4 (29.5)	54.7 (28.6)	40.4 (32.2)	56.0 (29.5)
Mean (SD) % of wk with PLT response†	76.2 (35.2)	88.3 (22.1)	60.8 (39.8)	75.1 (32.1)	46.9 (39.5)	78.7 (28.7)
Discontinued treatment, no (%)	19 (45.2)	10 (9.2)	31 (64.6)	56 (23.4)	56 (37.1)	33 (15.3)
Exposure (total), pt-wk	1479.6	4157	3309.7	24,642	4246.7	9227
AEs‡	20.8	15.7	31.4	18.3	29.3	12.6
SAEs‡	1.6	0.5	2.3	0.8	2.2	0.9
Treatment-related AEs‡	2.2	1.5	1.2	0.9	1.8	1.6

PLT=platelet count; pt-wk=patient-weeks; *≥50 to ≤200×10^9/L. †Platelet response defined as PLT >50×10^9/L (Study 1), PLT ≥50×109/L (Study 2), and doubling of baseline PLT and PLT ≥50×109/L (Study 3). ‡Events per 100 pt-wk.

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P1043

DURATION OF BLEEDING SYMPTOMS >14 DAYS BEFORE DIAGNOSIS IS A STRONG PREDICTOR FOR DEVELOPMENT OF CHRONICITY IN CHILDHOOD PRIMARY IMMUNE THROMBOCYTOPENIA (ITP)

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Background: Immune thrombocytopenia is the commonest cause of bleeding diathesis in children. Most of the patients run a mild course with complete remission usually within three months from diagnosis. It still remains challenging though to predict which children will develop chronic thrombocytopenia.

Aims: The aim of the present study is to identify clinical and/or laboratory findings at presentation predicting a chronic course for childhood immune thrombocytopenia (ITP).

Methods: This study included 53 patients diagnosed with immune thrombocytopenia, 17 of which were known patients with chronic ITP and 36/53 were prospectively recruited between March 2008 and August 2010 and were followed up for at least 12 months post recruitment for study purposes. Data such as age, duration of bleeding symptoms before suspicion of ill health, bleeding stage according to Provan *et al.*, 2010 and platelet count at diagnosis were collected in all patients either prospectively or retrospectively in chronic patients (using medical notes and patient/parent interviews). Age at diagnosis, age >10yrs at diagnosis, platelet count of >10000/μL at diagnosis, bleeding stage 3+4 at diagnosis and duration of symptoms >14 days at diagnosis were associated with the course of the disease, using student's t–test, Mann–Whitney U test and Fisher's exact test accordingly.

Results: The cohort consisted of 26 boys and 27 girls with a mean age of 5.95±3.88 years (range: 0.375–14.83 years).Thirty–two out of 53 patients ran an acute/persistent course (duration <12 months) and 21 followed a chronic course of the disease. The percentage of children with duration of bleeding symptoms >14 days before suspicion of the disease was significantly higher between the children who developed chronic ITP (75% *vs* 3% in the group with acute/persistent course). None of the other parameters studied proved to be statistically significant.

Summary and Conclusions: Insidious onset of disease defined as duration of symptoms >14 days before diagnosis of childhood primary immune thrombocytopenia is a strong predictor for development of chronic ITP. This information is important for management approach and parent/patient consultations.

P1044

PLATELET ACTIVATION MARKERS IN PATIENTS WITH INCREASED CARDIOVASCULAR RISK

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Background: Platelet activation is considered a significant factor contributing to the increased incidence of arterial thrombotic complications observed in patients with increased cardiovascular risk, such as patients with essential

hypertension. Although a variety of platelet activation markers have been proposed, their usefulness in the clinical setting has been questioned.
Aims: The aim of this study was to estimate the degree of platelet activation at rest and after acute high-intensity exercise in a group of naïve, never treated patients with essential hypertension (UH) as compared to normotensive (NT) individuals, using the most reliable flow cytometric markers of platelet activation (circulating monocyte-platelet aggregates or MPA and platelet P-selectin). Secondly, we investigated whether antihypertensive treatment reduces exercise-induced platelet activation in essential hypertension.
Methods: We studied 15 NT and 30 UH individuals, 17 of which were followed-up after a 3-month period of monotherapy with antagonists of the renin-angiotensin system (RAS). All subjects underwent a treadmill exercise test. Circulating monocyte- MPA levels and platelet P-selectin were measured using flow cytometry at baseline, at maximal exercise and at10, 30 and 90 minutes later.
Results: Maximal platelet activation was observed at 10 minutes post-peak exercise in both groups. In UH, MPA levels remained increased at 30 minutes post-peak exercise, despite BP fall to baseline levels. MPA levels were significantly higher in UH than NT at maximal exercise, and at 10 and 30 minutes of recovery. P-selectin expression also increased during exercise in both groups, with no significant differences between UH and NT. Post-treatment MPA levels increased significantly only at 10 minutes into recovery. Post-treatment MPA and P-selectin values at each time point were similar to those of NT individuals.
Summary and Conclusions: Circulating MPA levels represent a sensitive and reproducible marker of platelet activation in the clinical setting, potentially useful in the evaluation and management of patients with increased cardiovascular risk. Platelet activation in response to acute high-intensity exercise is exaggerated and prolonged in UH compared to NT individuals. RAS blockade with adequate BP control greatly improves exercise-induced platelet activation in essential hypertension. Further studies are needed to clarify whether this phenomenon depends purely on BP lowering or benefits also from the pleiotropic effects of RAS antagonists.

P1045

FC GAMMA RECEPTOR POLYMORPHISMS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenia (ITP) is an autoimmune blood disease with unknown etiology. Both genetic and environmental factors are thought to play role in the development of the disease. The aim of our study was to investigate a possible role of two polymorphisms in the Fc gamma receptor 2A and 3A (FCGR2A and FCGR3A) in the development of primary ITP.FCGR2A is polymorphic and has two alleles, FCGR2A-H131 and FCGR2A-R131. This polymorphic variation of FCGR2A is due to a single base substitution at nucleotide adenine for guanine in position 494. The allele FCGR2A-H131 has a higher affinity for human IgG2, comparing to FCGR2A-R131. The gene for FCGR3A has also two polymorphic variant alleles: 158 valine (V158) and phenylalanine (F158) due to single base substitution of timidine to guanine at nucleotide 559. This polymorphism influences ligand binding. FCGR3A-158V allele variant has higher affinity for Fc fragment of IgG1 and IgG3 than 158F variant. These Fc gamma receptor polymorphisms may influence antibody-mediated phagocytosis and antigen presentation activity.
Aims: The aim of our study was to investigate a possible role of FCGR2A and FCGR3A polymorphisms in the development of primary immune thrombocytopenia.
Methods: We have analyzed 125 unrelated adult patients with ITP (35 men and 90 women), with median age of 47(range 14-83) and 120 healthy matched controls. The median follow up of the patients was 44 months (12-384). All 125 patients were initially treated with corticosteroids, 38 were splenectomized. Forty two (34%) patients had refractory or unresponsive form of disease, according to the definition of the International Working group for ITP. Refractory ITP was present in 14 (11%) of patients. Twenty eight (22%) patients had "unresponsive to one or more treatments" form of ITP. Genotyping was performed by using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. The distribution of genotypes and allele frequencies were compared between patients and controls using a chi-squared test or Fisher's exact test.
Results: Our results have shown significantly higher frequency of high affinity FCGR3A-158V allele in patients with ITP comparing with control subjects (47.2% *versus* 37.5%; P=0.037). We did not found significant differences in the genotype distribution or allele frequencies for FCGR2A-131H/R between patients with ITP and controls, P=0.652 and P=0.478. In the group of patients with unresponsive and responsive ITP we found significantly different genotype distribution and allele frequencies for FCGR3A, P=0.036 and P=0.008 respectively. There was no significant difference in genotype and allele frequencies for FCGR2A between these two groups of patients. We did not found significant differences in genotype and allele frequencies for both gene polymorphisms between splenectomized and unsplenectomized ITP patients.Our results confirmed that, the combination of high affinity FCGR2A-131H and FCGR3A-158V allele was more common in patients with ITP than in control subjects (55% *versus* 40%; P=0.024).
Summary and Conclusions: Results of this study, suggest possible role of FCGR3A polymorphism in the etiology, development and clinical outcome of immune thrombocytopenia, but further larger prospective studies are necessary to confirm these results.

P1046

THROMBOCYTOPENIA PREDICT MORTALITY IN CRITICALLY ILL PATIENTS

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Background: Thrombocytopenia is a common laboratory abnormality seen in ICU patients. There are retrospective studies suggesting that thrombocytopenia in medical ICU patients is associated with adverse outcomes like prolonged hospital stay and decreased survival.
Aims: In this prospective study we investigated the prognostic significance of thrombocytopenia in critically ill patients.
Methods: We have included a cohort of 548 consecutive patients admitted to Çukurova University Medical School Hospital Intensive Care Unit which is a tertiary referral center in southern region of Turkey for a population of approximately two million people, between July 2011 and August 2012. Of these, 165 (30.1%) patients met the inclusion criteria. Patients were stratified according to the degree of thrombocytopenia and APACHE II score. Primary cause necessitating hospitalization of the patient, comorbidities and exposure to drugs were also recorded. APACHE II score was used in order to measure the severity of the disease in ICU patients. Higher scores correspond to a more severe disease and a higher risk of death in general with this scoring system.
Results: Among 165 patients, 104 (63%) were male and the median age was 57.2 yrs. While 143 (81.2%) of the patients had thrombocytopenia on admission, 31 (18.8%) of the patients developed thrombocytopenia during their ICU stay. Sepsis and DIC (Disseminated intravascular coagulation) were the most common causes for thrombocytopenia in medical ICU patients. Totally 115 (69.7%) of the 165 thrombocytopenic patients and 173 (45.1%) of the 383 non-thrombocytopenic patients were deceased while on ICU stay (P=0.02). Mortality rate was significantly higher in thrombocytopenic patients with lower counts and a similar APACHE II scores (P=0.002). Thrombocytopenia was also a poor prognostic factor for thrombocytopenic patients who developed thrombocytopenia after admission compared to patients with existing thrombocytopenia on admission (P=0.001).
Summary and Conclusions: In conclusion, sepsis and DIC were the most frequent causes for thrombocytopenia in medical ICU patients. Thrombocytopenia seem to be an independent risk factor for survival in critically ill ICU patients.

P1047

IDIOPATHIC THROMBOCYTOPENIC PURPURA IN CHILDREN: A 20-YEAR NATURAL COURSE AT A SINGLE INSTITUTION

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Background: Idiopathic thrombocytopenic purpura is an hematologic disorder with a variable clinical course. However, there is paucity of data on long term probability of remission in chronic idiopathic thrombocytopenic purpura.
Aims: This study reviewed the clinical manifestation and response to therapy in patients with ITP, in order to identify natural course of chronic ITP.
Methods: The study included all patients diagnosed as ITP between January 1992 and December 2011.
Results: Two hundred sixty four patients were identified (48% male, 52% female). Median age at diagnosis was 4.8 years (range: 1 month-17 years). The mean platelet count was12,000/uL (range:3,000-135,000/uL). There are no seasonal variation of presentation. Twenty-six patients (10%) improved without any specific treatment. Intravenous immunoglobulin (IVIG) were used as a first-line treatment in 114 patients and showed a total response of 70% compared to 78% for steroids treated 14 patients. 10 children received anti-D immunoglobulin (Response rate 60%). Chronic ITP was noted in 41 patients (15.5%). Chronic patients presented at an older age (6.6 *vs* 3.9 years, $P<0.05$) and with higher platelet count (22,000/Ul *vs*7,000/uL, $P<0.05$) at diagnosis. In 80% of them, remission was observed between 13 month and 6 year 2 month. The probabilities of remission for chronic ITP were 22%, 44% and 51% at 1 year, 2 year and 3 year respectively. Splenectomy was performed in four patients with chronic disease. Threre are no severe bleeding such as intracranial hemorrhage.
Summary and Conclusions: ITP patients have a benign course in the majotiry of cases. The predicted spontaneous remission rate with chronic ITP was 22% and 80% at 1 year years, respectively.

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ABSOLUTE LYMPHOCYTE COUNT AND RISK OF SHORT-TERM INFECTION IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: Patients with ITP may have increased risk of infection for that steroid and other immunosuppressive agents have been the mainstay treatment for ITP.
Aims: This study aimed to characterize risk of infection events within 6 months after diagnosis of ITP and its impact on short-term outcome of these patients.
Methods: We retrospectively evaluated 239 patients (107 men, 132 women; median age: 61 yr) diagnosed between Jan1, 1997 to Aug 31, 2011. Every patient received at least steroid treatment according to platelet count or bleeding symptoms after diagnosis. Patients who were found to have malignancies, systemic autoimmune diseases, lymphoproliferative disorders or other secondary thrombocytopenia within 3 months after ITP diagnosis was excluded.
Results: 62 patients (25.9%) had a total of 73 infection events within 6 months after ITP diagnosis and treatment, including 10 patients had 2 events and 1 patient had 3 events. Among these infection events, 32 (43.9%) was pneumonia, 13 (17.8%) was UTI, 9 (12.3%) was herpes zoster, 8 (10.9%) was cellulitis, and 11 (15.1%) were other infections, including oral candidiasis, cholangitis, fungemia, bacteremia, intra-abdominal abscess, facial abscess, osteomyelitis, and pseudomembranous colitis Using univariate analysis, ALC $<1\times10^9$/l (P=0.008, OR 2.263), old age (age>65) (P=0.004, OR 2.416), and at least one comorbidity (P=0.050, OR 1.794) were identified as a risk factor for infection within the 6 months after ITP diagnosis. Multivariate analysis revealed that low ALC was the most significant risk factor (P=0.039, 95% CI 1.033 to 3.599, OR 1.928) associated with short term infection in patients with ITP. A total of 11 patients died during follow-up after ITP diagnosis, among whom 10 patients died of infectious disease and the remaining 1 died of bleeding complication. 9 patients died within 1 year after ITP diagnosis. Kaplan-Meier survival curve showed that patient had infection within the first 6 months after ITP treatment had poorer one-year survival than those without infection (P=0.001) (Figure 1).

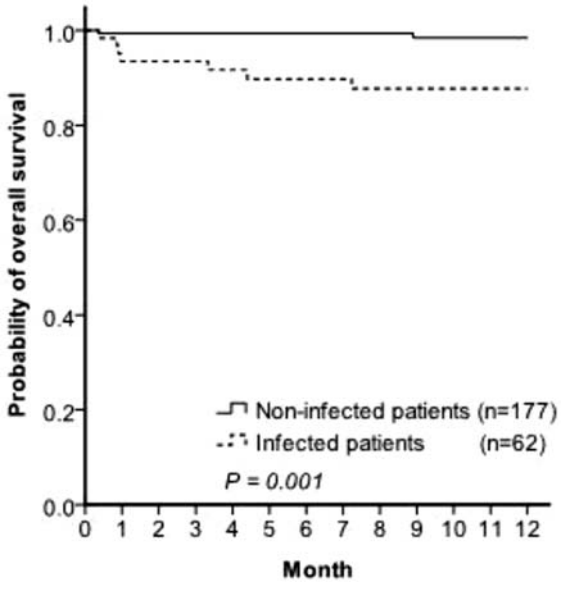

Figure 1.

Summary and Conclusions: In conclusion, ITP patients with infection occurring within 6 months post diagnosis had inferior one-year survival, and a low ALC may be associated with increased risk of infection within the 6 months of diagnosis. Apart from attention to bleeding risks, individualized management with cautious monitoring and surveillance for infection complications was also needed for better outcome.

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THE IMMATURE PLATELET FRACTION IS SENSITIVE TO THE PLATELET SIZE AND A USEFUL PARAMETER FOR SCREENING FOR MACROTHROMBOCYTOPENIA

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Background: The immature platelet fraction (IPF) is a useful parameter indicating thrombopoietic activity to differentiate the causes of thrombocytopenia. We previously reported that the percentage of IPF (%IPF) is negatively correlated to the platelet count among ITP patients, and not among myelodysplastic syndrome (MDS) patients. We also noticed that some MDS patients exhibited extremely high%IPF values, which were dissociated from the percentages of reticulated platelets (%RP) measured by flow cytometry. Such discrepancies were also observed in hereditary macrothrombocytopenias, which are sometimes difficult to be distinguished from ITP, because ITP also exhibits increased number of reticulated platelets in a slightly larger size. Once misdiagnosed, a hereditary macrothrombocytopenia patient might be subjected to an invasive treatment such as splenectomy. In order to avoid such mistreatments, a clear marker to differentiate macrothrombocytopenia is desperately needed.
Aims: To elucidate the mechanisms of the aberrant increase of IPF among macrothrombocytpenic patients, and search for a useful parameter to distinguish macrothrombocytopenia from ITP
Methods: The IPF values and other platelet indices of various hereditary macrothrombocytopenia were determined using Sysmex XE-2100 automatic hematology analyser in the blood samples. Platelet count and other parameters of platelet, such as MPV, PDW and P-LCR were measured simultaneously. Phosphorylation of myosin light chain was also examined to estimate activation status. Sixteen individuals from twelve families of hereditary macrothrombocytopenia were enroled in this study. We also monitored the IPF during EDTA-induced aggregation, platelet agglutination by macroglobulinemia and cold-storage. The morphological changes of platelets were also examined on the blood film.
Results: The IPF values were about 5 times higher in MYH9 disorders (%IPF 48.0±1.8) and about 1.5 times higher in other macrothrombocytopenias (%IPF 17.0±2.2) than immune thrombocytopenic patients with similar platelet counts (%IPF 9.3±0.4). These results suggested that the platelet size affect the IPF value. However it still remains the possibility that some factors other than the size might make an influence on the IPF. No one knows whether large platelets are functionally identical to normal platelets except for the size. In order to exclude the possibility, we next examined the changes of IPF values during EDTA aggregation, agglutination by macroglobulinemia and cold-storage. The IPF was significantly increased under these conditions in a time dependent manner along with forming platelet clumps. Durnig cold-storage, each platelet increased in size with fewer granules probably due to degranulation, and a couple of platelets stuck to each other to form a few tiny clumps. The IPF was strongly influenced by a few tiny platelet aggregates rather than other platelet indices, such as mean platelet volume (MPV), platelet-large cell ratio (P-LCR) and platelet distribution width (PDW).
Summary and Conclusions: IPF is susceptible to the platelet size, and could be a useful parameter for screening of macrothrombocytopenia from ITP.

P1050

THE INCIDENCE OF THROMBOCYTOPENIA AMONG OLDER PRIMARY MDS PATIENTS: A SEER-MEDICARE ANALYSIS

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Background: Myelodysplastic syndromes (MDS) represent a heterogeneous group of hematological malignancies, affecting predominantly the elderly. Although MDS have been studied recently, the epidemiology of MDS is still not fully characterized, especially the occurrence of thrombocytopenia among MDS patients.
Aims: To estimate the incidence of thrombocytopenia among older MDS patients, we used the linked Surveillance, Epidemiology, and End Result and Medicare (SEER-Medicare) database.
Methods: The SEER-Medicare database consists of the SEER cancer registries, the authoritative source of cancer epidemiology information in the U.S. linked with Medicare claims data in people aged 65+ years. SEER data represent a diversity of geographic areas across the U.S. and thus population-based reflecting 'real world' practice. We included MDS patients 65+ years who were newly diagnosed between January1, 2001 and December 31, 2002, and who were enrolled in Part A and Part B Medicare coverage. MDS was defined using ICD-O 3 codes where the primary site was bone marrow (C42.1), histology code (9980, 9982—9986, 9989, 9945), and behavior code was malignant neoplasm (3). All MDS diagnoses were histologically confirmed. Subtypes of MDS were classified according to two classification systems: FAB and WHO. Thrombocytopenia was defined using ICD-9 codes (287.3, 287.4, 287.5). Descriptive statistics were calculated and Cochran-Armitage trend test was used to test for trend.
Results: During the two years,2,734 patients with primary diagnosis of MDS were identified. Fifty four percent were males and 46% females. The majority of patients were Caucasians (88%) and 47% were 80+ years. The overall frequency of thrombocytopenia among the primary MDS patients was 53% (n=1,449). With increasing age groups, the incidence decreased: 65-69 years (59.5%), 70-74 years (58.9%), 75-79 years (53.5%), 80-84 years (50.8%), 85+ (46.4%). This trend was statistically significant (P<0.001). The incidence of

thrombocytopenia was statistically significantly higher among males (55.5%) *vs* females (50.1%) (P<0.01). Among1,382 FAB MDS subtypes, thrombocytopenia was more common among patients diagnosed with RAEB type (216 of 323; 66.9%), RAEB-T (26 of 39; 66.7%), CMML (182 of 288; 63.2%), followed by RA (191 of 441; 43.3%) and RARS (96 of 291; 33.0%). Of2,407 patients with WHO MDS subtypes, the greatest proportion with thrombocytopenia were observed among those with RAEB subtype (216 of 323; 66.9%), MDS-U (677 of1,234; 54.9%), RCMD (38 of 72; 52.8%), followed by 5Q (23 of 46; 50.0%), RA (191 of 441; 43.3%), and RARS (96 of 291; 33.0%).

Summary and Conclusions: Overall, thrombocytopenia is a very frequent symptom that occurs among patients with MDS. The study brings new data on incidence of thrombocytopenia among older patients with MDS in the U.S., which will help in the development of new therapies to manage the disease. Moreover, the high incidence data gathered in this study are similar with that observed in other studies of thrombocytopenia in MDS patients and confirms that thrombocytopenia continues to be a frequent clinical problem in older patients with MDS.

P1051

MEAN PLATELET VOLUME AND LABORATORY MARKERS FOR CARDIAC DISEASE: A LINK BETWEEN PLATELET SIZE AND HS-CRP

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Background: Mean platelet volume (MPV) is a parameter generated by fully-automated blood count analyzers as part of a routine complete blood count (CBC). It is also a useful platelet function index that can show platelet activation and its production rate in bone marrow. Other than just hematologic diseases, MPV has been studied in various disease groups such as cardiovascular and rheumatic diseases as well. Recently we have also studied this platelet index, MPV in various conditions (Platelets. 2013;24(1):75-6., Platelets. 2012;23(8):648-9., Platelets. 2013;24(2):164-5., Thromb Res. 2012 Sep;130(3):557-8.).

Aims: In this study, we investigated MPV in Korean patients who have cardiovascular diseases to analyze the association between this platelet index and various laboratory markers for cardiac diseases such as hs-CRP, TnI and apolipoprotein.

Methods: This study included1,059 retrospective results with increased hs-CRP level above the upper reference limit (>3.9 mg/L) for the patients group, at Kyung Hee Medical Center, a tertiary teaching hospital, between January 2011 and April 2012. For the control group, 143 subjects for medical check-ups were enrolled from the same hospital. Individuals with a history of high blood pressure and diabetes were excluded from the control group. Blood sampling was performed through venepuncture and MPV was measured using EDTA-containing tubes in Advia 2120 (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) within 2 hours. hs-CRP was measured in BN™ II analyzer(Siemens Healthcare Diagnostics, Marburg, Germany) with CardioPhase® High Sensitivity C-Reactive Protein (Siemens Healthcare Diagnostics, Marburg, Germany) and TnI was estimated by Access II (Beckman Coulter Inc., Fullerton, CA, USA) with AccuTnI reagent (Beckman Coulter Inc., Fullerton, CA, USA). Independent t-test and Pearson's correlation analysis were done to evaluate the diagnostic performance and to examine the relationship between variables. Statistical analyses were performed with MedCalc v11.6 (MedCalc Software, Mariakerke, Belgium) and Excel 2007 (Microsoft corporation, Redmond, WA). *P* values <0.05 were considered statistically significant.

Results: In the patients group, the mean age was 57.84 (median 63.0, range 0-90 yr) and the male to female ratio was 542:517. The mean of serum hs-CRP was 15.88 mg/L. Mean MPV levels did not show a significant difference between the patients and the control group, which were 8.06 fl in the patients group and 7.96 fl in the control group, respectively (*P*<0.001). Moreover, MPV showed a positive correlation with hs-CRP in the patients group (correlation coefficient *r*=0.07656, *P*=0.0127). Out of 269 patients with the TnI test results, this positive correlation grew stronger for 79 patients whose TnI increased more than 0.04 (correlation coefficient *r*=0.444, *P*=0.0001). However, there was no significant correlation between MPV and pro-BNP. Finally, there was a strong negative correlation between MPV and HDL (*r*=-0.1511, *P*<0.0001).

Summary and Conclusions: This study showed a positive correlation between MPV and hs-CRP in the patients group with increased hs-CRP levels. MPV has been regarded as an hematologic index for platelet reactivity and hs-CRP has been known to a sensitive biochemical maker for the presence of vascular lesions. The meaningful relationship in these two markers reconfirms the close link between endothelial damage and platelet activation. It also sheds new light on the interesting connection between hematologic and biochemical maker in the field of vascular pathology. Further study should be conducted to understand underlying mechanisms on the relationship of MPV and hs-CRP.

P1052

OVERVIEW OF RUSSIAN PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) REGISTRY

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Background: Clinical presentation of ITP is variable from no symptoms to serious bleeding manifestations. There are very few precise data about course features of ITP and treatment methods of patients with ITP in Russia.

Aims: We aim to evaluate disease characteristics and treatment practices of ITP in Russia.

Methods: Clinical and laboratory data of 415 patients (363 adults and 52 children) were extracted from ongoing multicentre prospective observational cohort study (Russian ITP registry). Using web based data transfer investigators from major Russian haematological centres registered all patients with ITP visited him/her as out-patients or admitted to hospital and fulfilling inclusion/exclusion criteria. Inclusion criteria: primary diagnosis of ITP, signed informed consent. Exclusion criteria: secondary ITP. Primary objectives: to describe the features of clinical course, outcomes and current treatment options for ITP patients in Russia. The results of first planned interim analysis are presented below. Descriptive statistics was used.

Results: At the moment of the first interim analysis mean follow up period was6,9±2,6 months (0,3-12,6) for adults and5,47±2,7 (1,3-9,64) for children. Newly diagnosed, persistent and chronic ITP were reported in 34,6%,11,5% and 51,9% of children and in16,8%, 19,8% and 63,4% of adults. Mean lowest platelet count (min, max) was 22,1 (1-72) × 10^9/L in children and 22,2 (0-119) × 10^9/L in adults. Bleeding history was reported in 98,1% of children and 92,6% of adults, WHO grade 3-4 bleeding at the onset was observed in5,8% of children and14,9% of adults. History of intracranial hemorrhage, GI bleedings and haematuria were observed in1,5%, 0%, 0% of children and in 0,6%,4,7%,2,8% of adults. Splenectomy was performed in 63 (17,4%) adults patients with complete response in 39,7% of these pts, objective response in 19%, no response in3,2%, loss of response in 36,5%. Refractory ITP (resistance to medical therapy in splenectomized patients) developed in15,9% of adults. Patients receiving ≥2nd line of therapy were reported in 23,3% of children and 27,7% of adult population. Treatment with rituximab and TPO-R agonists were reported in1,9% and11,5% of adults. Resistance to ≥2nd line of therapy was observed in1,9% of children and in12,9% of adults.

Summary and Conclusions: The interim analysis of the first in Russia prospective ITP Registry has shown a variable clinical course of the disease. Serious bleeding complications were rare, 36,5% of patients lost response on splenectomy, 1/3 of patients developed a resistance to medical therapy and received a multi drug treatment. The study is ongoing and further data are essential for planning of future trials including targeted therapy with TPO-R agonists and for developing new treatment approaches for ITP patients in Russia.

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P1053

DIFFERENTIAL DIAGNOSIS OF THROMBOTIC MICROANGIOPATHY: DATA FROM PADOVA TTP CASE SERIES

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare but fatal disease. Differential diagnosis is often difficult, due to similarity in presentation features with others thrombotic microangiopathies (TMA), including hemolytic uremic syndrome (HUS).

Aims: To investigate differences in clinical and laboratory features (including ADAMTS13 activity and IgG anti-ADAMTS13 levels) between TTP, HUS and other TMA in our case series.

Methods: Patients admitted to our court with a first clinical diagnosis of acute TMA were included. Clinical and laboratory data were retrospectively analyzed. TTP and HUS diagnosis were clinically made as reported in the literature. All patients not fitting these criteria were included in the other TMA group. ADAMTS13 activity and IgG anti-ADAMTS13 were assessed using commercially available FRETS-VWF73 and ELISA assays respectively. Severe protease activity deficiency was considered as ≤10%, as reported in the literature; however two subgroups were made (protease activity ≤5% and 6-10%) to compare different threshold of the assay. IgG anti-ADAMTS13 was considered present for title ≥17 U/mL, according to the manufacturers instructions. Chi-square test and Anova analysis were used for groups comparison.
Results: 119 patients between June 2005 and November 2012 were collected (M/F=44/85; mean age 44±17, range 3-82): 88 were TTP, 9 HUS and 22 other TMA patients. Half of the TTP patients showed an associated condition, namely autoimmune disease (25%), infectious disease (15%, including 1 patient with HIV infection), cancer (3%), drugs (3%), pregnancy (2%) and obesity (9%). Patients clinical characteristics are shown in Table 1. TTP patients more often presented with neurological signs as compared with HUS and other TMA patients (p 0.006). Acute renal failure was always present in HUS patients, while observed in 28% of TTP and 50% of other TMA patients (P<0.001). Bleeding prevalence was significantly different between groups too (p 0.014). Mean platelet count at diagnosis was lower in TTP as compared with HUS and other TMA patients (P<0.001). Mean levels of creatinine at diagnosis was higher in HUS respect to TTP and other TMA patients (P<0.001). ADAMTS13 activity assay and the presence of IgG anti-ADAMTS13 were tested in 106 patients; these parameters showed a significant difference between the three groups (P<0.001); in fact none of TTP patients had normal values of ADAMTS13 activity, while none of HUS or other TMA patients had severely reduced levels of the protease at diagnosis. Comparison of ADAMTS13 activity between HUS and other TMA groups didn't show any difference. Most of TTP patients were positive when tested for the presence of IgG anti-ADAMTS13, while most of patients in the two other groups were negative.

Table 1. Clinical and laboratory features of patients with acute TMA (Padova case series, June 2005-November 2012).

	TTP (88 pts)	HUS (9 pts)	Other TMA (22 pts)	P value
Fever	36 (41%)	5 (56%)	10 (45%)	n.s.
Neurological impairment	57 (65%)	3 (33%)	7 (32%)	0.006
Acute renal failure	25 (28%)	9 (100%)	11 (50%)	< 0.001
Bleeding	68 (77%)	3 (33%)	15 (68%)	0.014
		53 ± 36	48 ± 28	< 0.001
Plts (G/L, mean ± SD)	18 ± 17	94 ± 21	83 ± 16	n.s.
Hb (g/L, mean ± SD)	87 ± 21	2320 ±	2141 ±	n.s.
LDH (U/L, mean ± SD)	1721 ± 1151	1555	2684	< 0.001
Creatinine (umol/L, mean ± SD)	101 ± 63	448 ± 363	157 ± 155	
ADAMTS13 activity (%)				
- ≤ 5%	68 (77%)	0	0	
- 6-10%	5 (6%)	0	0	< 0.001
- 11-64%	3 (3%)	4 (44%)	14 (64%)	
- ≥ 65%	0	4 (44%)	8 (36%)	
-Untested	12 (14%)	1 (12%)	0	
Anti-ADAMTS13 IgG (%)				
- negative	6 (7%)	6 (66%)	20 (91%)	< 0.001
- positive	70 (79%)	2 (22%)	2 (9%)	
- Untested	12 (14%)	1 (12%)	0	

TTP, thrombotic thrombocytopenic purpura; HUS, hemolytic uremic syndrome; TMA, thrombotic microangiopathy; pts, patients; plts, platelets; Hb, hemoglobin; LDH, lactic dehydrogenase; n.s., not significant.

Summary and Conclusions: Clinical features at presentation can guide differential diagnosis when TMA is present; in particular a low level of platelet count and predominant neurological impairment orient toward a diagnosis of TTP, while the presence of acute renal failure with high levels of creatinine can be more specific for HUS diagnosis. Very low levels of ADAMTS13 activity are specific for TTP diagnosis. As a consequence, when protease activity is >10% at presentation, the diagnosis of TTP can be reasonably excluded. There seems to be no difference when choosing a threshold of 5 or 10% activity to define a severe reduction for FRETS-VWF73 assay. ADAMTS13 activity, however, seems not useful for the distinction between HUS and other TMA.

P1055

EXTREME THROMBOCYTOSIS (>1000×10^9 /L) IN 336 PATIENTS- REPORT OF A FOUR-YEAR EXPERIENCE

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Background: Extreme thrombocytosis (EXT), defined as a platelet count >1,000×10^9/L, is quite rare, as only 2–5.8% of patients demonstrate this degree of thrombocytosis.
Aims: There are limited data on the etiology and frequency of EXT, we aim to explore retrospectively the causes and complications of 336 serial cases of EXT.
Methods: During 2006- 2010, we evaluated 793525 complete blood counts of 188914 patients. Among those, 336 patients (0.18%), with a median age of 45 years (0.1-100), were identified with EXT. Medical records of all 336 patients were examined concerning the etiology of thrombocytosis, peak of platelet count and complications such as bleeding tendency or thrombosis.
Results: Exploring the etiology of EXT, 294 cases (87.5%) had reactive EXT and 42 patients (12.5%) had clonal thrombocytosis, due to MPNs. The RT group included post surgical and orthopedic conditions and oncologic malignancies, that were the most common causes of RT (23.13% and 22.11%, respectively). Infection (10.20%) was also a frequent RT cause, accounting mainly younger age groups. Among MPNs, Essential Thrombocytosis was the predominant cause of EXT (85.72%), followed by Polycythemia Vera (9.52%) and Myelofibrosis (4.76%). Concerning age distribution of EXT, MPN EXT appears by the 3rd decade, and shows increasing rates at older age groups, achieving its' highest percentage (23.53%) at patients older than 70 years. As a result, there was statistically significant difference between the median age of RT and MPN patients, P=0.044. Focusing on children of the first decade of life, 65.17% of EXT cases, were attributed to infection, with respiratory infection being high on the list of causes. Children younger than 8 years old account the majority of patients (70%) with infection induced EXT. Mean PLT Max of MPN patients (1396.36×10^9/L) was significantly higher (P=0.017) than Mean PLT Max of RT patients (1186.01×10^9\L). Exploring admission PLT count of the patients we found out that Mean PLT count at the time of admission of the MPN group (1088.29×10^9\L) was significantly higher (P<0.005) compared to RT group (642.28×10^9/L). Moreover, 75.17% patients of the RT group had PLT count <1000×10^9/L by the time they were admitted at the hospital. However, significantly lower number of patients (30.95%) at the MPN had PLT count <1000×10^9/L (P<0.001). EXT complications, thrombosis or bleeding, were observed at 4.76%. The incidence of EXT complications was statistically significantly higher among MPN patients- 30.95%>than among RT patients- 1.02% (P<0.005). At the MPN group both thrombosis (23.81%) and bleeding (7.14%) were present. Although Mean PLT Max of patients who suffered bleeding (2625.67×10^9/L) was much higher than thrombosis patients (1596.10×10^9/L), it didn't reach significance levels, P=0.069.
Summary and Conclusions: Based on our study and previously published data, bleeding or thrombotic complications at RT EXT are quite infrequent, so RT EXT could remain untreated, and followed up. However, there are cases that the underlying condition requires PLT anti- aggregation agents, such as Kawasaki syndrome.

Bleeding and thrombosis

P1056

ANTIPLASMIN, BUT NOT AMILORIDE, PREVENTS SYNOVITIS AND CARTILAGE DESTRUCTION FOLLOWING HEMARTHROSIS IN HEMOPHILIC MICE

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Background: Blood-induced joint damage in hemophilia is characterized by synovitis and cartilage destruction. Recently, we demonstrated that a joint bleeding in hemophilic mice results in alterations in the fibrinolytic system with an increase in synovial cells expressing urokinase-type plasminogen activator (uPA), an increase in synovial levels of uPA and plasmin, and in plasmin-mediated cartilage destruction.
Aims: In this study, we evaluate the alterations in synovitis and cartilage destruction following the induction of a joint bleeding in hemophilic mice treated with placebo, amiloride (a specific inhibitor of uPA), or antiplasmin.
Methods: The right knees of hemophilic mice were punctured to induce hemarthrosis. Subsequently, mice were randomized between daily oral treatment with amiloride or control, while other mice were randomized between weekly intra-articular treatment of the right knee joint with amiloride, antiplasmin, or control. After five weeks of treatment, the mice were sacrificed, knee joints were isolated, sectioned for histology, and stained with hematoxylin-eosin (synovitis) or safranin O (cartilage destruction). Hemophilic synovitis and cartilage destruction were determined by two blinded observers according to Valentino (score 0-10) and Glasson (score 0-6). An increase in Valentino and Glasson score represents an increase in hemophilic synovitis and cartilage destruction, respectively. Treatment with amiloride or antiplasmin was compared with control. Categorical data were analyzed by loglinear analysis and Pearson Chi-Square.
Results: No significant alterations in synovitis and cartilage destruction were found when comparing the oral amiloride group with the oral control group, and when comparing the intra-articular amiloride group with the intra-articular control group. In contrast, intra-articular treatment with antiplasmin resulted in a statistical significant (P<0.01) reduction in synovitis, as assessed by the Valentino score, when comparing the intra-articular control group to the intra-articular antiplasmin group: 1 (0% *vs.* 11.1%), 2 (4.2% *vs.* 11.1%), 3 (16.7% *vs.* 61.1%), 4 (29.2% *vs.* 5.6%), 5 (20.8% *vs.* 11.1%), 6 (8.3% *vs.* 7.7%), 7 (8.3% *vs.* 0%), and 8 (12.5% *vs.* 0%). In addition, treatment with intra-articular antiplasmin resulted in a statistically significant (P<0.01) reduction in cartilage destruction, as assessed by the Glasson score, when comparing the intra-articular control group to the intra-articular antiplasmin group: 2 (8.3% *vs.* 10%), 3 (12.5% *vs.* 50%), 4 (33.3% *vs.* 30%), 5 (33.3% *vs.* 10%), and 6 (16.7% *vs.* 0%).
Summary and Conclusions: Intra-articular treatment with antiplasmin following the induction of a joint bleeding prevented synovitis and cartilage destruction in hemophilic mice. Oral and intra-articular treatment with amiloride failed to attenuate synovitis and cartilage destruction. Given that complete prevention of joint bleeds in hemophilia is not feasible at the moment despite the prophylactic use of factor replacement, the data presented herein offer promise for the use of antiplasmin as a new therapeutic intervention.

P1057

A VERY RARE SIMULTANEOUS PRESENCE OF A RING CHROMOSOME 13 AND A SPLICING SITE MUTATION ON FACTOR X GENE

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Background: Factor X (FX) coagulation disorder is a rare autosomal recessive disease with an incidence of 1 in10^6 in the general population. Patients affected with the severe form of this defect tend to be the most seriously affected among those with rare bleeding disorders. The bleeding tendency may appear at any age, although the more severely affected patients present early in life with umbilical-stump or central nervous system (CNS) bleeding. The *F10* gene is located on chromosome 13q34 and spans 27 kb; to date, more than 100 mutations were reported to cause FX deficiency and most of them (80%) are missense mutations located at the catalytic domain.
Aims: Molecular characterization of a two-years-old boy with severe FX deficiency (FX:C <2%) associated with severe clinical symptoms such as spontaneous CNS subdural haemorrhages.
Methods: Direct sequencing analysis of the *F10* gene by ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Milan, Italy); analysis of 7 highly polymorphic short tandem repeats (STRs) selected from the Genethon human linkage map (ABI PRISM Linkage Mapping Set MD10) to verify the presence of parental alleles; investigation of aberrant splicing mechanisms by expression of FX minigenes in mammalian cells; FISH on metaphase chromosomes using Vysiss LSI probe for 13q34 and Vysis TelVysion probe for 13q (Abbott Molecular, Hoofddorp, The Netherlands).
Results: The proband resulted to be homozygous for the c.370+2T>C mutation occurring at the conserved GT dinucleotide of the donor splice site (5'ss) of intron 4. This genetic change is predicted to significantly reduce the splicing efficiency from a score of 39 to undetectable. Expression studies with minigenes constructs indicated that the mutation c.370+2T>C abolishes the canonical 5'ss and activates a cryptic 5'ss in intron 4 (position +55), leading to the insertion of an intronic sequence with a premature stop codon, therefore preventing synthesis of a functional protein. However, while the proband's father confirmed to be heterozygous for the same mutation, the mother was wild type. The presence of a gross deletion on the second allele of the proband or an uniparental disomy (UPD) were then hypothesized. Six out of 7 analyzed STRs indicated that the proband had both paternal and maternal alleles; the remaining STR (D13S1265) indicated the possibility of a reduction to homozygosity in the proband due to a partial UPD or of a partial deletion of chromosome 13q34. A further cytogenetic analysis on 80 cells showed 46 chromosomes including a small ring chromosome 13 with breakpoints at p13 and q34. The karyotype was 46, *XY*, r(13)(p11q34). The karyotypes of both parents were normal and confirm the absence of subtelomere 13q and 13q34 region.
Summary and Conclusions: The molecular characterization in this family identified a very rare simultaneous presence of a ring chromosome 13 causing the loss of the entire FX gene associated with a splicing site mutation resulting in an aberrant splicing event. These abnormalities led to a severe case of FX deficiency who required regular prophylaxis.

P1058

MOLECULAR DIAGNOSTIC IN 47 UNRELATED FAMILIES WITH HAEMOPHILIA A IN ARAGON (SPAIN)

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Background: Haemophilia A (HA) is an X-linked bleeding disorder caused by deficiency or absence of coagulation factor VIII which is required for blood coagulation. HA is one of the most common coagulation disorders with an incidence of 1 in5,000 males worldwide. Haemophilia severity is defined by depending on the activity level of coagulation F8 (FVIII:C) in plasma. The disease is defined as severe (<1%), moderate (1%>5%) and mild (>5%><40%). F8 is located at Xq28, spans 186kb and comprises 26 exons. The cDNA is approximately 9kb in size and encodes a mature protein of 2332 amino acids.
Aims: In this study we analyze the type and distribution of molecular events in HA families referred to our center, located in Aragon (middle-west region of Spain).
Methods: DNA samples were extracted from peripheral blood leucocytes from 47 unrelated families (65 patients) with HA. Initially, intron 22 and intron 1 inversions were detected in patients with severe HA using a long range PCR. The mutations in patients with mild/moderate and non-inversion severe patients were identified by PCR and DNA sequencing. A presumed mutation was validated on a second independent PCR product.
Results: HA causative mutation was identified in 63 patients from 46 families. We were not able to identify it in one family (2%). HA phenotype of the studied families was 49% mild, 4% moderate and 47% severe. In 14 families we detected Intron 22 and intron 1 inversions (28% and 2% of the total mutations, respectively). Besides the recurrent gene inversion, 30 different F8 mutations were detected (see attached Table 1). Severe non-inversion mutations were associated with 5 mutations predicted to cause a truncated protein, 3 nonsense (7%) and 2 frameshift (4%). Severe phenotype was related also to 1 missense, 1 splicing (2%) and 1 deletion in-frame (2%). We detected 25 families with missense mutations (53%). All moderate and mild cases with a causative mutation identifiedwere missense.Recurrent missense mutation p.K2136E was found in three unrelated families. Five novel missense mutations were found, representing 11% of the mutations in the entire group. Four of them were associated with mild phenotype: p.D82E; p.K123N; p.D569E (c.1764C>G) and p.I1852S. By contrast, the mutation p.W208C was related to severe phenotype. Among reported mutations p.R531H is well known by the discrepancy between FVIII:C results in one-stage and two-stage which has clinical importance in mild HA. In our study not only found this discrepancy in both methods, but also we identified a different behavior with distinct APTT reagents. F8 inhibitors were not found in this study except one in three patients from the family with p.R593C mutation. It has been shown to have an increased risk of developing F8 inhibitory antibody in mild HA. However, it seems to others non genetic factors could have an important role. F8 mutations in this study were located along all the FVIII domains. A2, A3 and A1 held the major mutations with 27%, 21% and 18% of the total non-inversion mutations. B domain contained the least number of mutations (3%).

Table 1.

Exon/ Intron	Nt.Change (c.)	Aa.Change (p.)	Exon/ Intron	Nt.Change (c.)	Aa.Change (p.)
2	205-206delCT	L50VfsX13	14	2215G>A	E720K
3	303T>A	D82E	14	2945dupA	N963KfsX9
4	426A>T	K123N	14	5123G>A	R1689H
6	681G>T	W208C	15	5286T>A	F1743L
7	854T>C	V266A	IVS15	5374-1G>C	
7	904C>T	Q283X	16	5531C>T	P1825L
8	1180G>T	A375S	17	5612T>G	I1852S
11	1649G>A	R531H	18	5879G>A	R1941Q
12	1764C>G	D569E	18	5936G>A	G1960E
12	1804C>T	R583X	18	5938C>T	H1961Y
12	1834C>T	R593C	22	6385A>T	K2110X
13	1916A>G	Y620C	23	6463A>G	K2136E
13	2014_16delTTC	F653del	23	6545G>A	R2163H
13	2103G>A	M682I	24	6686T>C	L2210P
14	2149C>T	R698W	25	6857A>G	A2267G

Summary and Conclusions: The results from this study suggest that we were able to identify the causative mutation in 98% of the studied families what shows that our protocol is a useful tool to find F8 gene defects. The characterization of these mutations is important to avoid misdiagnosis in some cases of mild haemophilia, to prevent the development of inhibitors in mutations with high risk and to determine the carrier status in female relatives with prenatal diagnosis if necessary.

P1059

IMPAIRED PROPLATELET FORMATION IN A NOVEL GLANZMANN VARIANT MACROTHROMBOCYTOPENIA

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Background: Glanzmann thrombasthenia (GT) is an autosomal recessive bleeding disorder caused by a defect in the expression or the function of integrin αIIbβ3. Although the majority of patients with mutations in *ITGA2B* and *ITGB3* have a normal platelet size and count, it has been recently demonstrated that variant-type GT patients with a mutaion in the cytoplasmic domain residues αIIbArg995 and β3Asp723, which form a salt bridge, manifest moderate thrombocytopenia and large platelets, and the mutated integrins were shown to be partially activated.
Aims: The aims of this study is to characterize a novel missense mutation (S175P) in αIIb associated with a Glanzmann variant macrothrombocytopenia.
Methods: We investigated the genetic relevance of large platelets among the patient's family members. Protein expression of integrin αIIbβ3 was determined with flowcytometry, and megakaryocytopoiesis and proplatelet formation in this congenital macrothrombocytopenia was studied using peripheral blood CD34+cells.
Results: The case is a 39yo Japanese female with a novel congenital macrothrombocytopenia associated with compound heterozygous mutations (p.S175P and p.Y238X; c.523T>C and c.713_714 insA) of the *ITA2B* gene. The surface expression of platelet αIIbβ3 was decreased. The mean fluorescence intensities of αIIb, β3 and αIIbβ3 in flow cytometric analyses complex were decreased to 36.4%, 52.8% and 15.7% compared to the control respectively. Spontaneous PAC-1 binding to the resting platelets wasn't observed. Both mRNA and protein from Y238X mutant were not detected. Her father has a heterozygous mutation of S175P, her mother and sons have a heterozygous Y238X, and neither of them manifested giant platelets, even though αIIbβ3 expression was impaired in their platelets. Megakaryocytes derived from CD34+ cells of the patient's peripheral blood extended a reduced number of proplatelets and the tips were significantly decreased in number and larger in size compared with control.
Summary and Conclusions: The mutation S175P is located in β-propeller domain of the αIIb and impaired the normal function. The mechanisms of producing giant platelets could be different from αIIb R995W, who manifests constitutive sub-maximal activation. These data may provide new insight on the role of the αIIbβ3 in giant platelet formation.

P1060

MUTATION ANALYSIS IN TURKISH RARE BLOOD DISORDERS

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Background: Rare bleeding disorders (RBDs) are autosomal recessive diseases including the inherited deficiencies of coagulation factors such as fibrinogen, factor (F) II, FV, FV+FVIII, FVII, FX, FXI, FXIII, and multiple deficiency of vitamin K-dependent factors, with clinical manifestations ranging from mild to severe. The molecular diagnosis of RBDs is based on the mutation search in the genes encoding the corresponding coagulation factors.
Aims: In our study, we performed mutation analysis of ERGIC, MCFD2, MYH9, GpIIB, GpIIIA, FGB genes in order to analyse Combined FV-FVIII Deficiency, Macrothrombocytopenia, Glanzmann Thrombasthenia and Afibrinogenemia patients of Turkish origin respectively.
Methods: DNA was isolated from by proteinase K and phenol/chloroform extraction. All of the exons of the genes screened. Exons were amplified by polymerase chain reaction (PCR). Patient sample was sequenced, using a DNA sequencer (Beckman Coulter DNA Sequencer,USA)
Results: Direct PCR analysis and sequencing revealed novel mutations at these genes. In our study, we detected two novel mutations, a novel polymorphism at ERGIC-53 gene; four novel missense mutations at MYH9 gene; five novel mutations at GpIIB gene and a novel mutation at FGB gene.
Summary and Conclusions: All of these novel mutations were not defined Human Gene Mutation Database (HGMD) and not reported previously. (Table 1).

Table 1.

Gene	Disorder	Mutations	Aminoacid change
ERGIC53	Combined FV+FVIII Deficiency	Exon4 /c.157 delT	
		Exon 9/ c. 1105C>T	p. R202X
MYH9	Macrothrombocytopenia	Exon 1 /c.197 G>C	p. L64A
		Exon 1/ c.286 T>G	p.S96A
		Exon 26/ c. 3756 C>A	p. L1176M
		Exon 26/ c. 3762 G>A	p. E1182K
GpIIB	Glanzmann Thrombasthenia	Exon 4/ c.4466 G>T	p.G159V
		Exon 4/ c.4420 T>G	p. V147G
		Exon 13 / c. 9092 G>T	p. V420L
		Exon 13/ c. 9179 T>A	p.P448Y
		Exon 19/c. 11453 A>G	p.T646A
FGB	Afibrinogenemia	Exon 9/ c. 5321 T>C	p. L198P

(Aminoacid symbols: R: Arg, X: Stop, L: Leu, A:Ala, S: Ser, M: Met, E:Glu, K: Lys, G: Gly, Y: Tyr, T: Thr, P: Pro)

P1061

CONGENITAL FXI DEFICIENCY: EVALUATION OF BLEEDING PHENOTYPE AND CORRELATION WITH FXI ACTIVITY (FXI:ACT).

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Background: Bleeding phenotype in FXI deficiency is variable and generally related to surgery/trauma. Moreover, there is a poor correlation between bleeding and baseline FXI:Act.
Aims: To retrospectively describe the hemorrhagic phenotype of our FXI deficient population and to relate the phenotype with FXI:Act.
Methods: Since 1973, we have been following 94 FXI deficient patients from 65 different families: 43 F, 51 M; diagnosis median age: 28.7 years (0.9-83.9); median follow-up: 0.9 years (0.1-36.2); median FXI:Act of all patients: 38% (range 0.5-69%; normal values: 70-140); FXI:Act ≤1% in 5 patients, >1≤5% in12, >5≤10 in3, >10≤20 in6, >20≤70 in 68. Excessive bleeding is reported as described in the medical records.
Results: Fifty six patients experienced bleeding episodes not surgery-related: ecchimoses in 27, hematomas in2, epistaxes in 23, gastrointestinal hemorrhages in14, meno-methrorrhagia in2, hematuria in4, post-traumatic intracranial hemorrhage in1, gum bleeding in1, pulmonary bleeding in 1. Prior to diagnosis, 64 patients underwent 133 surgeries (93 major, 40 minor). Prophylactic treatment was administered in 3/133 procedures: tranexamic acid (TA) in1,

fresh frozen plasma (FFP) in 2. Twenty eight/133 (21%) post-surgery hemorrhages were reported in 19 patients; in 12/28 cases, transfusional therapy (FFP and/or red blood cells units) was needed. Median FXI:Act of bleeding patients was 28% (0.5-53%). Twenty nine spontaneous deliveries (SD) and 8 cesarian sections (CS) were performed without prophylaxis: 4 post-partum hemorrhages occurred (patients FXI:Act:2,6, 27, 52.3%, respectively). In 3 cases, transfusional therapy was necessary. Forty nine patients underwent dental surgeries without prophylaxis: 17 experienced (bleeders median FXI:Act: 11% [1-57%]). In one case, FFP was necessary to stop the bleeding. After diagnosis, 23 patients underwent 34 surgeries (14 major, 20 minor). Prophylactic treatment was administered in 23/34 procedures: TA in7, FFP in2, desmopressin in4, FFP+TA in8, desmopressin+TA in 2. The only bleeding reported (1/23, 4%) was after an emergency appendectomy performed under TA administration in a patient whose FXI:Act was 2.8%. In 2/11 surgeries performed without prophylaxis, an excessive bleeding was reported but transfusional therapy was not necessary; FXI:Act was 29% for both bleeding patients. Four SD and 5 CS were performed with prophylaxis: FFP in4, TA in2, desmopressin in 3. No post-partum hemorrhages occurred. Sixteen patients underwent 20 dental surgeries; prophylactic treatment was administered in 15/20 procedures without any bleeding complications: TA in6, FFP+TA in4, FFP in4, desmopressin+TA in 1. During two dental procedures performed without prophylaxis, patients bled excessively; their FXI:Act was 6% and 45%, respectively.

Summary and Conclusions: We confirm the broad variability in bleeding phenotype in FXI deficient patients, not related to the baseline FXI:Act levels. Moreover, we highlight that a good management of prophylaxis treatment dramatically reduces the percentage of bleedings in case of surgery (21% *vs* 4%), deliveries and dental procedures. Because of the low correlation between FXI:Act and the phenotype, we underline the need of laboratory-based prognostic factors for a better management of these patients.

P1062

HOW DO WE ENCOUNTER RARE FACTOR DEFICIENCIES IN CHILDREN? SINGLE CENTER RESULTS

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Background: Rare factor deficiencies are autosomal recessively inherited coagulation factor deficiencies encountered at a frequency of between one in 500 thousand and one in 2 million.

Aims: 192 rare factor deficiency cases followed and treated at our clinic between 1990 and 2012 were retrospectively evaluated in our study.

Methods: We retrieved from patient records and from the records contained in the dataprocessing environment introduced in 2005.

Results: One hundred and thirty of the cases were boys and 62 were girls. The age range was between two week-24 years and the ages at the admission of 192 patients were between 2 weeks-16 years. The rate of familial consanguinity was 49.4%. From the 192 cases, 10 suffered from Fibrinogen, one from Factor II, 6 from Factor V, 2 from FV+FVIII, 142 from Factor VII, 15 from Factor X, 14 from Factor XI, and 2 from Factor XIII deficiencies. FVII deficiencies had the largest share in our patient group with a rate of 74%. The F:C levels of patients were <5% in 48 patients, between 5 and 30% in 40 patients, and between 30 and 50% in 104 patients. Eighty eight of our patients were asymptomatic (45.8%) and 104 were symptomatic (54.2%). Asymptomatic patients were diagnosed by family histories (39.8%), preoperative laboratory studies (54.6%) and operational bleeding (5.6%). Sixty eight of our symptomatic patients have grade II (65.4%) and 36 have grade III bleeding symptoms (34.6%). The ages at the admission of symptomatic patients were between 2 weeks-16 years (mean: 1.12±0.8 year). Forty two of symptomatic cases were diagnosed before the age of one (40.4%). When all patients were taken into consideration, first bleeding regions were skin bleedings 32.7%, epistaxis 27.8%, Central Nervous System (CNS) bleedings 15.4%, oral cavity bleedings 10.4%, haematomas 5.7%, haemarthrosis 2.8%, hematuria 1.9%, Gastrointestinal System (GIS) bleedings 1.9%, respectively. The bleeding prevalence rates of our cases are listed as epistaxis 62.5%, skin bleedings 53%, oral cavity bleedings 28.8%, haematoma 18.3%, CNS bleedings 17.3%, haemarthrosis 14.4%, GIS bleedings 3.8%, menorrhagia 2.9%, haematuria 1.9%, bleedings due to operations 1.9%, ileopsoas bleedings 1.9%. CNS bleedings (47.3%) take the first place among the serious bleedings of our cases, followed by haemarthrosis (40%), Gastrointestinal System (GIS) bleedings (9.1%) and ileopsoas bleedings(3.6%). Prophylaxy was applied to 8 patients (five patients with FVII and one each with Fibrinogen, FV, and FX deficiency).

Summary and Conclusions: The characteristics, clinical manifestations, bleeding attacks, and treatments in our rare factor deficiency cases as well as prophylactic approaches are discussed in our article.

P1063

CLINICAL AVAILABILITY OF REPORTING PROTHROMBIN TIME AS INTERNATIONAL NORMALIZED RATIO FOR PATIENTS WITH CHRONIC LIVER DISEASE

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Background: The international normalized ratio (INR), which standardizes prothrombin time (PT) during oral anticoagulation, has been extended to standardize PT in liver diseases and is included in prognostic models such as the Model for End-stage Liver Disease (MELD). However, PT prolongation mechanisms in liver diseases differ from those in oral anticoagulation, and thromboplastin sensitivities to these 2 mechanisms differ.

Aims: We aimed to establish liver patient-specific international sensitivity index (ISI_{liver}) and INR (INR_{liver}) values with thromboplastins and to evaluate the effect of their use in chronic liver disease patients.

Methods: Sixty cirrhosis patients were selected and their PTs measured with 5 thromboplastins. Twenty healthy subjects were involved to assign the mean normal PT (MNPT) and ISI. Each thromboplastin was also assigned an ISI_{liver} by substituting plasma from vitamin K antagonist patients with that from cirrhosis patients in the calibration. INR, INR_{liver}, MELD, and $MELD_{liver}$ values for individual patients were calculated using the ISI and the ISI_{liver}, respectively.

Results: The INR obtained with the 5 thromboplastins were significantly different (P=0.049). Conversely, the INR_{liver} were not. The mean INR differences between the thromboplastins were significantly larger than that of INR_{liver} (P=0.000). The mean values were 0.12 and 0.06, respectively. The mean INR CV between the 5 thromboplastins were significantly larger than that of INR_{liver} (P=0.000). The mean values were 5.4% and 3%, respectively. Though there was no significant difference in MELD scores obtained with the 5 thromboplastins (P=0.871) or $MELD_{liver}$ (P=0.100), the mean MELD differences between the 5 thromboplastins were significantly larger than that of $MELD_{liver}$ (P=0.000). The mean values were 0.80 and 0.45, respectively.

Summary and Conclusions: Alternative thromboplastin calibration using plasma from cirrhosis patients instead of vitamin K antagonist patients is feasible and may resolve the variability of INR measurement. However, further studies are needed to clarify the clinical impact.

P1064

THE HAEMOCARE PROTOCOL–A COMPOSITE METHOD TO MEASURE THE DISEASE BURDEN FROM HAEMOPHILIA IN DEVELOPING COUNTRIES

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Background: Burden of disease has immense impact on the suffering of individuals, the family, society and the nation. Measuring disease burden, so vital for policy planning and interaction for improving outcomes, is a complex issue ranging from estimation of macro-level 'global burden of diseases' to micro-level assessments of a given disease in a given society/region within a given healthcare environment. No single measure of disease burden is applicable to all diseases or to all countries. The issue is even more complex and amorphous for rare diseases such as haemophilia, an X-linked recessive bleeding disorder.

Aims: To develop a protocol for studying the disease burden from haemophilia in developing countries.

Methods: Based on the cross-sectional HAEMOCARE study of haemophilia in five developing countries–Algeria, India, Morocco, Oman and South Africa–we propose a composite methodology for assessing disease burden from haemophilia in developing countries. This methodology captures study parameters relating to the three different aspects of disease burden–epidemiology of musculoskeletal morbidity, quality of life and economic costs–in 282 male patients with previously confirmed severe haemophilia (A or B) alone. Participants were aged ≥6 years and receiving on-demand treatment with anti-haemophilia factors (AHF). Patients were stratified by presence or absence of inhibitors, and were free of hepatitis C or HIV infection.

Results: Clinical morbidity parameters included the standard Haemophilia Joint Health score (HJHS) and Pettersson score. Secondary assessments related to different aspects of haemophilia management, including bleeding

episodes, requirement for AHF, use of adjunctive treatments, and access to specialist care. Quality of life was assessed using the standard EQ-5D-3L questionnaire to study index scores for mobility, self-care, usual activities, pain/discomfort and anxiety/depression. In addition to the direct cost of AHF treatment, indirect costs were studied in terms of transportation costs/distances, and schooldays missed or workdays lost. All economic costs were computed across time in this cross-sectional study. Overlapping costs from other healthcare facilities were not computed separately. Some opportunity costs could be deduced indirectly from the protocol. From these practical yet comprehensive parameters, a composite measure with possible index score may be generated by assigning weights in accordance with the demographic, ethnic, sociocultural, and healthcare environment as applicable to our developing countries. Though epidemiological prevalence of haemophilia was not studied, the percentage of the expected cases diagnosed in a given country (a predictable occurrence) may be accounted for in a realistic assessment at any given timepoint. Preliminary results have shown musculoskeletal problems in 84% of the study population.
Summary and Conclusions: Based on our HAEMOCARE study, a methodology and protocol for a composite assessment of disease burden from haemophilia in developing countries is proposed, in which different weights may be assigned to the study parameters to produce meaningful index scores for local use. Such composite measures and index scores may be valuable tools for making uniform comparisons and for assessment of future/intervention benefits in developing regions of the world.

P1065

DIAGNOSIS AND MANAGEMENT OF ACQUIRED HEMOPHILIA A (AHA) PATIENTS: EXPERIENCE OF A SINGLE CENTER.

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Background: AHA is a rare bleeding disorder with an incidence of 1.5/million/year. Mortality rate is high (9-22%), if the diagnosis is delayed and treatment is not promptly implemented.
Aims: A retrospective evaluation of AHA affected patients diagnosed and followed at our Institution. Patients characteristics, bleeding symptoms and treatment, eradication therapy and response, occurrence of possibly related diseases have been considered.
Methods: Between 1984 and 2012, 34 patients (M, 14; F, 20) were diagnosed with AHA. Diagnosis median age: 70.2 years (25-89); median time from the first bleeding symptoms and diagnosis: 48.5 days (4-264); median follow-up (FU): 19.7 months (2.6-215.4).
Results: At diagnosis, bleeding symptoms were present in 33/34 patients (97%) (28 muscle hematomas, 3 hematuria, 2 post-partum hemorrhages, 1 GI bleeding, 1 hemoperitoneum, 1 hemarthrosis, 2 post-surgerybleedings); the median inhibitor titer was 9.6 BU/mL (2.5-138) and the median FVIII:C level 2% (0.01-21); in 16 patients, the FVIII:C level was <1%. Clinical conditions capable of triggering the inhibitor appearance were present in 17 patients (50%): a previous delivery in9, autoimmune diseases in4, cancer in 4. First-line eradication therapy was prescribed in all patients: prednisone (PDN) (median dose 1 mg/kg day, range 0.5-2) for 4 weeks in 23 (68%), dexamethasone (DXM) (median dose 24 mg/day, range 24-40) for 4-day courses in 7 (20%), azathioprine (AZA) 100 mg/day for 3 months in 2 (6%), cyclophosphamide (CTX) (0.5 and 1.5 mg/kg/day) plus PDN (standard doses) for 2 months in 2 (6%). Thirty-one patients are evaluable for response, 27 treated with PDN/DXM, 4 with other immune suppressive drugs. An inhibitor eradication (undetectable inhibitor, normal FVIII:C levels, absence of bleedings) was obtained in 24/31 patients (77%) (22/27 [81%] on PDN/DXM, 2/4 [50%] on other immune suppressants). Second-line therapy was administered to 4/7 non-responder patients: CTX+PDN in2, DXM in1, PDN in 1. Inhibitor eradication was obtained in 1. Third-line therapy was performed in 2/3 second-line non-responders: 1 CTX+PDN, 1 AZA+PDN. No responses were observed. One of these 2 patients was then treated with Rituximab, which allowed to obtain a persistent inhibitor eradication. Three patients relapsed after first-line treatment (3/24, 12.5%). At the last control, 23 patients have maintained a persistent inhibitor eradication at a median FU of 25.9 months (2.6-150.9). Bypassing agents (rFVIIa or FEIBA) were used in 21 patients with a high efficacy to control bleeding symptoms: FEIBA in11, rFVIIa in7, either FEIBA or rFVIIa in 3. A severe bleeder, non-responsive to eradication therapy, is on FEIBA prophylaxis 3 times weekly with a good control of hemorrhagic events. During the FU, occurrence of diseases possibly related to the inhibitor presence (cancer) was recorded in 2/17 idiopathic cases (11.7%); 5/34 deaths, not bleeding-related, were recorded. No relapses were observed in two post-partum inhibitor affected patients who underwent 3 deliveries after a period of 2, 3.6 and 7.3 years, respectively, after inhibitor eradication.
Summary and Conclusions: We confirm that the prevalence of idiopathic AHA is about 50% of cases. We observed a high response rate after steroid administration (81%). Bypassing agents were efficacious in all treated patients. The relapse rate was relatively low (12.5%). A good and prompt management of AHA reduces the bleeding-related mortality risk.

P1066

ACQUIRED VON WILLEBRAND SYNDROME AND MONOCLONAL GAMMOPATHY: CLINICAL CHARACTERIZATION AND RECOVERY TO INFUSION OF VWF-CONTAINING CONCENTRATES

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Background: Acquired von Willebrand syndrome (AVWS) is a rare bleeding disorder caused by structural or functional defects of von Willebrand factor. It has been described as secondary to autoimmune, hematologic, malignant or cardiovascular disorders. Diagnosis is generally underestimated because of the presence of moderate bleeding tendency in diseases that may be associated with drug related impaired haemostasis. The suspicion of AVWS is supported by the detection of both lone low plasma levels of vWF:RCo alone and in association with low vWF:Ag and FVIII:C levels, in absence familial or personal bleeding history and with previously normal clotting screening tests. Diagnosis is supported by impairment in recovery of infused plasma derived concentrates rich in vWF/FVIII. This study reports the recovery in five patients with monoclonal gammopathy and low levels of vWF/FVIII.
Aims: The evaluation of recovery after infusion of vWF-containing concentrates and its role to support the diagnosis of acquired coagulopathy.
Methods: Activated partial thromboplastin time (aPTT) and vWF:RCo, vWF:Ag and FVIII:C levels have been detected before the infusion of plasma derived vWF-containing concentrates (30 U/Kg bw) and after 30, 60, 120, 180 minutes, 6 and 24 hours.
Results: Five patients were evaluated from 2006 to 2012. All patients presented prolonged aPTT, low levels of vWF:Ag and vWF:RCo and no family history of bleeding disorders. Four out to five patients had not prior personal history of bleeding. Patient 1. 69 years old man, affected by Waldenstrom Macroglobulinemia (WM, IgMk). He suffered from acute severe epistaxis. After infusion of concentrates he had temporary uncomplete recovery of vWF:RCo. Patient 2. 73 years old man, with biclonal gammopathy (IgGk and IgMk); he presented melena due to acute intestinal microhemorrhages. After the infusion of concentrates he presented a temporary good recovery of vWF:RCo. Patient 3. 75 years old man, with monoclonal gammopathy of undetermined significance (MGUS, IgGk). He had an acute severe mucocutaneous hemorrhage after minor surgery and epistaxis. There was not significative recovery of vWF:RCO after infusion of concentrates. Patient 4. 40 years old woman, with MGUS (IgGk). She presented acute menorrhagia and severe anemia, needing for blood transfusions. The infusion tests showed only mild and temporary increase without any normalization of the parameters. Patient 5. 65 years old woman, with WM (IgMk) and frequent epistaxis, menorrhagia before menopause and mucocutaneous bleeding tendency. She had complete and sustained recovery, with normalization of vWF/FVIII levels over 6 hours after infusion of concentrates. In this case personal history and results of the infusion test may be consistent with a coincidental presence of congenital von Willebrand disease and the lymphoproliferative disorder.
Summary and Conclusions: To distinguish AVWS and von Willebrand disease (vWD) important features are the late onset of bleeding and a negative family history for hemorrhagic disorders but it's also known that mild vWD can be asymptomatic for decades and lack of a significative family history may be due to its low penetrance. Monoclonal gammopathy can coexist with congenital vWD. Studying the recovery to vWF-containing concentrates may be helpful to differentiate AVWS from vWD.

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P1067

VENOUS THROMBOEMBOLISM (VTE) PROPHYLAXIS IN HOSPITALIZED OBSTETRIC PATIENTS: A MULTICENTRE CROSS-SECTIONAL STUDY

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Background: VTE complicates 1 - 2/1000 pregnancies, and the risk increases with age, mode of delivery, and presence of co-morbidities. It continues to be one of the leading causes of maternal death. Prophylaxis with low molecular weight heparin (LMWH) is safe. Despite having a population of only 4.5 million, Ireland has 20 maternity hospitals. Given this large number, it is difficult to standardize practice with international best practice.
Aims: To assess the prevalence of VTE risk in pregnant women in the hospital setting and to determine the proportion of at-risk patients who receive effective prophylaxis.
Methods: The study period was September 2011 to November 2012. All patients admitted to the participating hospitals on the day of investigation were assessed for risk of VTE on the basis of hospital chart review. Risk was assessed in accordance with the 2009 Royal College of Obstetricians and Gynaecologist Guidelines. Patients undergoing procedures or on the labour ward at the time of review were excluded. Ethical approval was obtained from the ethics committees governing all centres.
Results: 540 pregnancies were reviewed across 16 centres. The average age of was 31+/- 5.65yrs (Range 16-47), with 21.87% (117/535) aged over 35. 22% (118/535) had a parity of 3 or more. The average weight was 71.51kg (Range 42-134kg, SD 14.482kg). Data on BMI was available for 77% - 34% were overweight and 21% were obese. 1% (6/420) had a BMI>40. 31% (168/540) were antenatal and 69% (372/540) were postnatal. 63% (105) of antenatal patients were low risk (<2 risk factors), 35% (59) were intermediate risk (2 or more risk factors, prophylaxis should be considered) and 2% (4) were high risk. All the high risk patients were on prophylaxis at an appropriate dose. 4% (6) of the low risk patients were on prophylaxis unnecessarily. Only 7% (4/59) of the intermediate risk patients were on prophylaxis (3/4 were on too low a dose) Among postnatal patients, 41% (153) were low risk (<2 risk factors), 58% (217) were intermediate risk (2 or more risk factors, require prophylaxis) and <1% (2) were high risk. 80% (296) were appropriately risk stratified and put on LMWH if necessary. 59% (219) of patients should have been on LMWH but only 42% (157) were (92% Tinzaparin and 8% Enoxoparin). This included 8 patients who were on LMWH unnecessarily. 38% (59/157) were on too low a dose.
Summary and Conclusions: VTE prophylaxis is an important issue in obstetrics given its prominent role in maternal morbidity and mortality and the increasing prevalence of risk factors such as obesity and increasing maternal age. It is clear that while there is good awareness of the risk in the postnatal period, there is less emphasis on risk assessment in antenatal patients where prophylaxis is rarely used. Those on prophylaxis are also likely to be on too low a dose. Given the number of maternity hospitals in Ireland, there is a role for a national guideline to standardize care for all pregnant women.

P1068

INVESTIGATION TO IMPROVE DETECTION OF LUPUS ANTICOAGULANT IN A LOCAL POPULATION EXPERIENCING RECURRENT MISCARRIAGE AND INTRAUTERINE DEATH

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Background: It is widely reported that antiphospholipid syndrome (APS) is detectable in approximately 15% of women with recurrent miscarriage in the absence of any other abnormalities. The detection rate in our laboratory is much lower, at 1-2%. All screening is carried out according to international guidelines published by the Scientific Standards Committee of the International Society for Thrombosis and Haemostasis which caused us to question why the detection rate is so low. As lupus anticoagulant (LA) detection is the most complicated part of the APS screening process, with numerous tests and interpretation methods available, this became the focus of our investigation.
Aims: To investigate the use of alternative interpretation methods for dilute Russell's viper venom (dRVVt)-based screening tests, to investigate the use of integrated screening and confirmatory testing methods and to determine the usefulness of factor assays in LA screening, with the ultimate aim of improving detection of LA in our laboratory.
Methods: Factor assays for VIII, IX, XI and XII were carried out at serial dilutions- 1:10, 1:20, 1:40 and 1:80 on 50 patients. The results were than analysed for a pattern of inhibitory activity, *i.e.* presence of LA; and compared to the PTT-LA results from the routine LA screen. dRVVt and dRVV Confirm assays were carried out on 80 samples and interpreted using 3 different methods, namely the dRVVt/Confirm ratio, the normalised ratio and the calculation for the percentage correction of clotting time. The use of the dRVVt and Confirm together as an integrated test was also compared to the dRVVt alone as a screening test. All results were compared using Chi-Square analysis where a $P<0.05$ was considered statistically significant.
Results: Factor assays were found to be of no additional benefit to the LA screening process, as was the percentage correction of clotting time calculation for interpretation of the dRVVt and Confirm tests; both methods were shown to increase equivocal results. Integrated testing using the normalised ratio as the interpretation method of choice showed an improvement in sensitivity detecting three new weak-titre LAs. A more significant development occurred on further analysis of the data when it was noted that 15% of patients showed increased factor VIII activity, indicative of an acute phase, which one would expect to witness in pregnancy; the mean dRVVt was also lower for this study population than for the general population. This study population, though no longer pregnant appear to share characteristics with pregnant women in relation to their coagulation assays. If this is the case then these patients should not be compared to reference ranges derived from the general population but to reference ranges relevant to their own specific population. To investigate this discovery further new study population reference ranges were generated. Comparing our results to the new reference ranges produced one weak positive from the three previously detected. More significantly, the dRVVt for this sample was actually prolonged using the new reference range, therefore this LA would be detected without the use of integrated testing.
Summary and Conclusions: This study has shown for the first time that population-specific reference ranges increase the sensitivity of LA screening assays and may provide a more specific alternative to integrated testing, as illustrated by the dRVVt results.

P1069

PRACTICAL EXPERIENCE OF THROMBOPROPHYLAXIS AFTER LOWER LIMB REPLACEMENT–RIVAROXABAN VERSUS ENOXAPARIN

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Background: Since October 2010, rivaroxaban has been approved by Korean government to be covered by national health insurance reimbursement system as thromboprophylaxis after total hip arthroplasty and total knee arthroplasty. However, there is no available data on outcomes of rivaroxaban as thromboprophylaxis in Korea.
Aims: We performed a retrospective study to compare the efficacy and safety of venous thromboembolism prophylaxis with enoxaparin or rivaroxaban in 195 consecutive patients undergoing major orthopaedic surgery at our center.
Methods: This study retrospectively reviewed the medical records of the 195 patients who underwent a total hip replacement arthroplasty or total knee replacement arthroplasty and received thromboprophoylaxis with enoxaparin or rivaroxaban at Soonchunhyang University Hospital between March 2009 and May 2012. Each patient's medical records included information on age, sex, comorbidities (active malignant disease, renal insufficiency), treatment details (type of surgery, type of anesthesia, duration of surgery,), duration of prophylaxis, efficacy (death, pulmonary embolism, deep vein thrombosis), safety (major bleeding, cerebrovascular accident), cause of drug interruption.
Results: Of 195 patients, 129 patients received thromboprophylaxis with enoxaparin (group 1; our hospital standard since March 2009), 66 received rivaroxaban (group 2; our hospital standard since February 2011). Symptomatic venous thromboembolism was found in 0.7% of patients in the group 1 (1/129 patients) compared to 1.5% of group 2 (1/66 patients; P=0.627). No significant differences in the rates of symptomatic VTE were found. However, patients with received rivaroxaban had significantly more rates of major bleeding (0 in group 1 *vs* 3% (2/66 patients) in group 2; P=0.047). Although group 1 patients were planed receiving thromboprophylaxis with rivaroxaban from day one post operatively, mean time from the end of surgery to first rivaroxaban intatake was 4.2 days.
Summary and Conclusions: Despite lower compliance in rivaroxaban group, venous thromboembolism prophylaxis with rivaroxaban is not inferior to prophylaxis with enoxaparin with regard to the prevention of symptomatic venous thromboembolisms. But, more bleeding complications and wound problems revealed in rivaroxaban group. Further studies and experiences are needed to assess the efficacy and safety of rivaroxaban in clinical practice.

P1070

THROMBOSIS IN CHILDHOOD ACUTE LEUKEMIA

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Background: Thromboembolism can occur during acute leukemia, especially acute lymphoblastic leukemia (ALL) treated with L-asparaginase and steroids and acute promyelocytic leukemia (AML M3). The presence of central venous lines, usage of Escherichia coli asparaginase, sepsis and hereditary thrombophilic abnormalities are risk factors for thrombosis in children.
Aims: To evaluate the risk of thrombosis in children with ALL and AML.
Methods: One hundred and ninety-seven consecutive patients with acute leukemia were recruited for this study from April 2005 to October 2010 retrospectively. Ages of patients at time of diagnosis, symptomatic thromboembolic events, type of leukemia, chemotherapy protocol, time of thromboembolic events during chemotherapy, site of thromboembolic events, hereditary and acquired risk factors for thromboembolic events had been examined from the patients' records retrospectively.
Results: One hundred seventy-five patients with ALL, 10 patients with AML M3 and 12 patients with non-M3 AML were detected. All were in treatment according BFM protocols; ALL BFM 95 and AML BFM 2004 protocols for ALL and AML patients, respectively. Sixteen patients of the 197 patients had tromboembolic events (8,1%). Fourteen of sixteen were patients with ALL, one with AML M3, one with non-M3 AML. Thromboembolic events were more common in follow-up of patients with high risk ALL (23,8%) than patients with standart and intermediate risk. A half (50%) of thromboembolic events were seen in patients who were under treatment for standart and intermediate risk ALL during the induction phase and reinduction phase of treatment. Site of thrombus were iliac vein, catheter tip, finger tip, arteria dorsalis pedis, spleen, femoral vein, renal and central nervous system. The use of central vein increased risk of tromboembolic events to 20,3%. Hereditary thrombophilic factors were positive in nine of patients with thrombosis. Factor V Leiden heterozygosity, MTHFR heterozygosity, prothrombin 20210 heterozygosity, Factor V Leiden heterozygosity+MTHFR heterozygosity were found in3,4,1,1 of these patients respectively. None of these patients were elevated activated protein C resistance and homocystein level of these patients were not evaluated. Three of the 16 patients died due to sepsis during thromboembolic events.
Summary and Conclusions: The incidence of thromboembolic event in patients with leukemia is not negligible. Guidelines for leukemia specific risk factors, thrombosis prevention and treatment strategies in acute leukemia patients are required.

P1071

PROTHROMBIN TIME AND ANTIPHOSPHOLIPID ANTIBODIES: THE NUMBER OF POSITIVE TESTS PREDICTS PROTHROMBIN TIME PROLONGATION

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Background: The presence of antiphospholipid antibodies (aPLA) - lupus-like anticoagulant (LAC), anti-Beta2-glycoprotein I (B2G) or anti-cardiolipin (aCL) - is one of the necessary criteria for the diagnosis of antiphospholipid syndrome (APS), a prothrombotic state. LAC prolongs the activated partial thromboplastin time (aPTT), *in vitro*; in contrast, in the majority of patients, LAC does not prolong the prothrombin time (PT) - this finding is clinically relevant, underlying the rationale of using PT (through the INR) to monitor anticoagulation in patients with APS, in whom PT results are assumed to reflect the effect of warfarin, and not an interference of the underlying disease. Nevertheless, some authors have noted a prolonged PT in a selection of patients with LAC; in these patients, it can be postulated that there is a risk of undertreatment with warfarin, with PT prolongation being in part due to an *in vitro* artifact, and not solely to the effect of treatment. The identification of patients with a prolonged PT ab initio will help optimize patient management and reduce the risk of undertreatment. The characterization of the subset of patients in whom aPLA associate with a prolonged PT is the first step towards this goal.
Aims: To determine whether the nature or the number of positive aPLA are determinants of PT prolongation.
Methods: We reviewed all lab requests from 01-01-2000 to 12-31-2012, selecting patients with simultaneous diagnostic PT, aPTT, LAC, G2B and aCL determinations (IgM and IgG considered together). Patients were grouped according to the type (LAC, B2G and aCL) and number of positive results; the mean PT and aPTT (normalized to control) were compared across the groups.
Results: Inclusion criteria were fulfilled by 1669 results. The difference between PT and control was 1.3±3.3s when all tests were negative, and increased to 2.1±3.9s, 3.4±6.3s and 4.4±7.3s when one, two or all three tests were positive (P<0.001). When only one test was positive, the difference was 1.6±4.3s with B2G, 1.7±2.7 with aCL and 2.6±4.2 with LAC (P=NS); when two tests were positive, the difference was 2.7±6.0 for B2G and LAC, 3.5±6.4 for B2G and aCL, and 3.5±6.6 for LAC and aCL (P=NS). The ratio of aPTT to control was 1.0±0.2 when all three tests were negative, increasing to 1.3±0.5, 1.4±0.6 and 2.4±1.3 when one, two or all three tests were positive (P<0.001). When only one test was positive, the ratio was 1.0±0.2 with B2G, 1.1±0.3 with aCL (P=NS) and 1.5±0.6 with LAC (P<0.001); when two tests were positive, 1.2±0.4 for B2G and aCL, 1.6±0.6 for aCL and LAC, and 2.0±0.7 for B2G and LAC (P<0.001).
Summary and Conclusions: Considering the three APS-associated tests (B2G, aCL and LAC), we found a significant increase in PT and aPTT with an increase in the number of positive tests. For aPTT, not just the number, but also the type of test that was positive influenced the ratio: single positivity for B2G or aCL was comparable to negativity for all tests, but positivity for LAC had the strongest impact on aPTT; two-positive-test combinations including LAC were also associated with higher aPTT ratios than the B2G-aCL combination. These findings are in accordance with the known mechanism of LAC interference on lab testing. On the other hand, for PT, we found that only the number of positive tests was significant, with no differences between test combinations; positivity for LAC did not impact on PT results. These results can contribute to our understanding of PT-prolongation in LAC-positive patients: our data suggests that increased PT, in these patients, could be due to simultaneous positivity for B2G and/or aCL. We propose that in patients with positivity for two or, especially, all three of the aPLA, special care should be taken to identify an ab initio increased PT, and INR results obtained during anticoagulation should be interpreted in its light.

P1072

ULTRASTRUCTURAL ANALYSES OF THE NOVEL CHIMERIC HEMOSTATIC AGENT GENERATED VIA NANOTECHNOLOGY, ABS NANOHEMOSTAT, AT THE RENAL TISSUE LEVEL

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Background: Biomaterials used as tissue engineering scaffolds have specific physical properties and might form fibrous networks similar to collagenous extracellular matrix. They also can be programmed to carry chemical and physical cues to provide bioactivity for cell-materials interactions. In the search for more improved bioactive materials for tissue engineering purposes, peptide amphiphile (PA) molecules are good candidates to bring scaffold properties and bioactivity together.
Aims: We have generated a chimeric hemostatic agent, ABS Nanohemostat, via combining self-assembling peptide amphiphile (PA) molecules with the traditional ABS (Ankaferd Hemostat). The synthesis of the specific self-assembling peptide molecules capable of being a part of the combined ABS Nanohemostat compound and the assembly of the peptide nanofibers and ABS to generate the ABS Nanohemostat have already been published (Int J Biomaterials 2013, ID 949460, http://dx.doi.org/10.1155/2013/949460). The aim of this study is to assess renal tissue effects of the ABS Nanohemostat formed by the combination of self-assembling PA molecules and ABS.
Methods: Peptides were constructed on Rink Amide MBHA resin. Amino acid couplings were done with 2 equivalents of Fmoc protected amino acid, 1.95 equivalents HBTU and 3 equivalents of N,N-diisopropylethylamine (DIEA) for 2 hours. The PA was synthesized by Fmoc Solid Phase Peptide Synthesis (SPPS) method. It is composed of a lauryl (C12) group, hydrophobic region of the PA, and a peptide sequence. VVAG peptide sequence is used as β-sheet-inducer that causes nanofiber formation, while the lysine (K) residue is protonated at physiological pH and increase solvation of PA molecule in aqueous solution at pH 7. Renal artery and vein was revealed by hilar vascular dissection in 24 Wistar rats weighing 200 to 300 g. Subsequently renal artery and vein were clamped with Rommel vascular clamp. The lower third of the left kidney was resected in guillotine fashion with a single stroke of an amputating knife. Scanning Electron Microscopy (SEM) experiments were performed with FEI Nova NanoSEM 230, using the ETD detector at low vacuum mode with 30 keV beam energy to assess renal tissue alterations.
Results: SEM analyses revealed that significant erythroid aggregation are present inside the capillary bed of the renal tissue. However, neither the signs of necrosis nor any other sign of a tissue damage are not evident in the surrounding renal tissue supplied by those microcapillary vasculature. Furthermore, the appearance of the nucleus, cytoplasm of the vascular endothelial cells and their organelles are completely normal.
Summary and Conclusions: In previous investigations, histopathological examination of the damaged vascular structures revealed ABS-induced red blood cell aggregates supporting the hypothesis that ABS-induced formation of the protein network with vital erythroid aggregation covers the entire physiological hemostatic process (Critical Reviews in Oncology/Hematology 83 (2012) 21–34). We have observed the same structures in the kidney tissue in the present study via SEM analyses. In the current study, ABS Nanohemostat has lead to a more pronounced erythroid aggregation at the renal tissue level in comparison to the traditional ABS (Ankaferd hemostat).

P1073

MORTALITY OF PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND RELATION TO COAGULATION AND INFLAMMATORY BIOMARKERS

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Background: Patients with hematological malignancies often have a hypercoagulable state due to the production of substances with procoagulant activity. Also the role of inflammatory cells and pathways in the pathogenesis of cancer has become well established. Venous thromboembolism and inflammatory events are considered imortant causes of mortality in cancer patients. The impacts of vascular and inflammatory markers on the prognosis of hematological malignancies are still to be studied.
Aims: to study whether vascular and inflammatory biomarkers (as well as clinical variables) can be used as predictors of mortality in patients with hematological malignancies.
Methods: This study is a prospective observational cohort study; it was conducted on a group of 86 patients with malignant haematological conditions. They have been followed up for an average period of 314.45 days with an endpoint of mortality. **Exclusion criteria included** Overt bacterial or viral infection within the last 2 weeks, Venous or arterial thromboembolism within the last 3 months, Continuous anticoagulation with vitamin K antagonists or low molecular weight heparin (LMWH). Hypercoagulability and inflammation were assessed at the initiation of the study by measuring the circulating levels of the following parameters: Markers of coagulation and fibrinolysis activation (D-dimer, Fibrinogen, Thrombin, plasminogen activator inhibitor 1 [PAI-1]); Markers of endothelium and platelet activation (von Willebrand Factor [vWF], soluble P-selectin); and Markers of inflammation (Tumor necrosis factor alpha [TNF-α], Interleukin-6 [IL-6]).
Results: In our study, the mean age was 48.8 years. Our study included 38 (44.19%) female patients and 48 (55.81%) male patients. Out of 86 patients, 23 (26.74%) were diagnosed to have Lymphoproliferative disorders, 26 (30.23%) were diagnosed to have Myeloproliferative neoplasms, 19 (22.09%) were diagnosed to have AML (or MDS progressed to AML), 9 (10.47%) were diagnosed to have ALL, 9 (10.47%) were diagnosed to have Paraproteinaemias. Thirty two (37.21%) patients died during follow up. Twenty four (75%) patients died within 6 months after diagnosis. Eighteen patients (56.25%) died of disease progression, 7 patients (21.88%) died of infection, 4 patients (12.5%) died suddenly with suspected pulmonary embolism, 1 patient (3.13%) died of each of heart failure, bleeding, liver cell failure. There were statistically significant associations between mortality and ECOG performance status (P value: 0.001), duration of hospital stay (P value: 0.006), platelet count (P value: 0.033), transfused blood units (P value: 0.002), transfused platelet units (P value: 0.04), PTT (P value: 0.014), serum albumin (P value: 0.004), total bilirubin (P value: 0.007) Antithrombin (P value: 0.016), soluble P-selectin (P value 0.038), vWF (P value: 0.009), IL-6 (P value: 0.042). For prediction of mortality, ROC Curve of Albumin level showed that a level of 3.35 g/dl showed the highest likelihood ratio (LR) of 2.04 with sensitivity of 60% and specificity of 72.5%. For prediction of mortality, ROC Curve of total bilirubin level showed that a level of 0.58 mg/dl showed the highest likelihood ratio (LR) of 1.67 with sensitivity of 65.6% and specificity of 60.8%. ROC Curve of Antithrombin level showed that a level of 16.25 mg/dl showed the highest likelihood ratio (LR) of 1.8 with sensitivity of 61.3% and specificity of 66%. ROC Curve of soluble P-selectin level showed that a level of 28.28 ng/mL showed the highest likelihood ratio (LR) of 1.8 with sensitivity of 61.3% and specificity of 66%. ROC Curve of vWF level for showed that a level of 2.525mU/mL showed the highest likelihood ratio (LR) of 1.9 with sensitivity of 61.3% and specificity of 68%. ROC Curve of IL-6 level showed that a level of 3.35pg/mL showed the highest likelihood ratio (LR) of 1.62 with sensitivity of 61.3% and specificity of 62.3% (Figure 1).

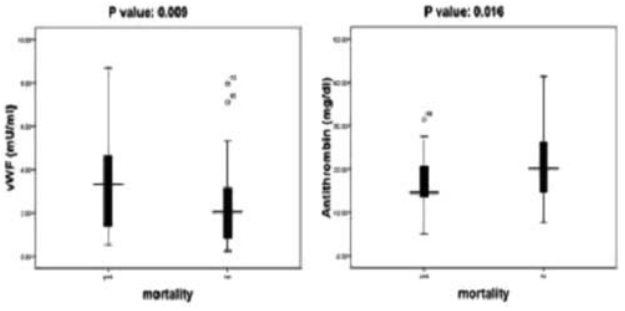

Figure 1. Relation between coagulation and inflammatory biomarkers and mortality in hematological malignancy.

Summary and Conclusions: our study concluded that initial levels of Albumin below 3.35g/dl, Total bilirubin above 0.58 mg/dl, Antithrombin below 16.25 mg/dl, soluble P-selectin below 28.28 ng/mL, vWF above 2.525mU/mL and IL-6 above of 3.35pg/mL are associated with poor outcome with increased mortality. these biomarkers can be included in a larger pridective model for mortality in patients with hematological malignancies

P1074

EPCR SER219GLY POLYMORPHISM AND SUSCEPTIBILITY TO VENOUS THROMBOEMBOLISM IN THE POPULATION OF NORTH-WESTERN RUSSIA

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Background: Venous thromboembolism (VT) is a multifactorial disorder. The genetic basis of predisposition to VT is not fully understood. Endothelial protein C receptor (EPCR) plays an important role in protein C anticoagulant pathway. An amino acid change Ser219Gly is associated with increased plasma levels of soluble EPCR and can affect thrombin degradation rate by activated protein C. The data on the role of EPCR Ser219Gly substitution in susceptibility to VT are still scarce.
Aims: To assess the role of EPCR Ser219Gly polymorphism in susceptibility to VT in the population of the North-Western region of Russian Federation.
Methods: We examined 300 patients with VT (147 men and 153 women, mean age 39.9±12.4 years) and 172 age- and sex-matched healthy controls (HC). In 200 patients, the first episode of VT was diagnosed at young age (before 45 years of old). All individuals originated from the North-Western region of Russia and gave informed consent for participation in the study. Allelic variants of the EPCR gene corresponding to Ser219Gly polymorphism were discriminated by PCR-RFLP method. The differences in genotype distributions between the groups were estimated by Fisher`s exact test. Odds ratios (OR) with their 95% confidence intervals (CI) as well as P-values were calculated by using GraphPad Prism software, version 4.0.
Results: The EPCR Gly219 variant was more frequently detected in the VT group (25.7% *vs.* 18.6% in controls, OR=1.5, 95% CI: 0.9-2.4, P=0.09). Notably, both hetero- and homozygosity for the EPCR Gly219 were more prevalent in patients than in HC, although not statistically significant (24.0% *vs.* 18.0%, and 1.7% *vs.* 0.6%, respectively). In the group of patients under 45 years of old, the frequency of individuals positive for the EPCR Gly219 variant was 2-times higher than in those with late-onset VT (31.0% *vs.* 15.0%, respectively, OR=2.5, 95% CI: 1.4-4.8, P=0.003). When comparing to HC, we found an increased risk of early-onset VT development in persons having EPCR Gly219 (OR=2.0, 95% CI: 1.2-3.2, P=0.008). Moreover, this variant of EPCR was present in 52 (33.5%) out of 155 young patients without factor V (G1691A) and factor II (G20120A) gene mutations (OR=2.2, 95% CI: 1.3-3.7, P=0.002, compared to control group).
Summary and Conclusions: Our data suggest that the EPCR Gly219 variant could independently increase the risk of VT development at young age in the population of North-Western Russia.

P1075

POLYMORPHISM OF VASCULAR TONE REGULATING GENES AND THE RISK OF VENOUS THROMBOEMBOLISM IN INDIVIDUALS WITH INHERITED THROMBOPHILIA

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Background: Venous thromboembolism (VT) is one of the most actual multifactorial diseases in the world. Genetic predisposition plays a significant role in pathogenesis of VT. Mutations in the factor II (FII G20210A) and factor V (G1691A, FV Leiden) genes are the most frequent inherited risk factors for VT and could be detected in about 9% and 20% of VT cases, respectively, in the population of North-Western Russia. Endothelial dysfunction is an important mechanism underlying thrombosis, and it frequently occurs as a result of imbalance between vasoconstriction and -dilatation processes. Variations in the genes coding for components of the renin-angiotensin system (RAS) and endothelial NO-synthase (eNOS) can lead to changes in their structure and/or functional activity and modulate the risk of VT.
Aims: To investigate the role of angiotensinogen (AGT), angiotensin II receptor type 1 (AGTR1), angiotensin-converting enzyme (ACE) and eNOS genes polymorphism in the development of VT at young age in individuals with inherited thrombophilia.
Methods: Retrospective study involved 181 patients with early-onset VT (mean group age 34,0±8,6 years) and 156 sex- and age-matched healthy controls (HC). All individuals originated from the North-Western region of Russia and gave informed consent for participation in the study. Variations in the FII

(G20210A), FV (G1691A, Leiden), ACE (Ins/Del), AGT (T704C, Met235Thr), AGTR1 (A1166C) and eNOS (T−786C) genes were discriminated by PCR-RFLP method. The differences in genotype distributions between groups were estimated by Fisher`s exact test.
Results: The distributions of alleles and genotypes of the vascular tone regulating genes in patients without known inherited risk factors, as well as in those having FII G20210A mutation were not significantly different from HC. At the same time, the positive association between the FV G1691A and eNOS −786CC genotypes was observed in the VT group (OR=3,2; 95% CI:1,3-7,5; P=0,01). Homozygosity for the eNOS −786C allele was more frequently seen among carriers of FV Leiden mutation than in patients with normal FII and FV genotypes (29,7% *vs.*13,1%, respectively, P=0,024). The "unfavorable" variants of the RAS genes were also over-represented in patients with FV Leiden. In particular, the simultaneous presence of the ACE Del/Del and AGT 704CC genotypes was almost 5-times more frequently seen in these individuals than in patients with normal FV and FII genotypes (15,2% *vs.*3,1%, respectively, P=0,018) Interestingly, neither the eNOS −786CC variant nor the "ACE Del/Del-AGT 704CC" combination was detected in VT patients having FII G20210A mutation and normal FV genotype.
Summary and Conclusions: We suggest that polymorphism of the vascular tone regulating genes can affect the imbalance between vasoconstriction and vasodilatation processes and play a provocative role in the development of early-onset VT among patients with FV Leiden variant.

P1076

PROPHYLAXIS FOR VENOUS THROMBOEMBOLISM IN PATIENTS TREATED FOR ACUTE LYMPHOBLASTIC LEUKEMIA–A SYSTEMATIC REVIEW

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Background: Venous thromboembolism (VTE) occurs frequently in patients with acute lymphoblastic leukemia (ALL). Reported incidences vary between 2 and 36%. Occurrence is often associated with treatment components, particularly L-asparaginase. Efficacy and optimal approach of VTE prevention during ALL treatment are unclear.
Aims: To investigate the efficacy and safety of systemic thromboprophylaxis, using blood-derived products, *i.e.* fresh frozen plasma, cryoprecipitate or antithrombin concentrate, or anticoagulant agents, *i.e.* (low-molecular-weight) heparin, fondaparinux or oral anticoagulants (vitamin K antagonists), on VTE incidence in pediatric and adult patients treated for primary ALL with L-asparaginase therapy. Also, impact of thromboprophylaxis on overall survival and treatment outcomes of ALL were investigated.
Methods: We systematically searched The Cochrane Central Register of Controlled Trials (CENTRAL, *The Cochrane Library*, Issue 7 2012), MEDLINE (January 1966 to August 2012; accessed via Pubmed) and EMBASE (January 1980 to August 2012; accessed via OVID). We handsearched conference proceedings and checked references of included studies. All randomized controlled trials (RCTs) that assessed the efficacy and safety of systemic thromboprophylaxis in patients treated for primary ALL with L-asparaginase therapy were eligible. Interventions included any dose of the above mentioned blood-derived products or anticoagulants, in comparison with no intervention or placebo, or a comparison of two different interventions. Three authors independently assessed eligible articles and systematically extracted the data from selected articles. Discrepancies were resolved by discussion or with the opinion of a fourth author. Risk of bias, quality of evidence, potential heterogeneity and reporting biases were explored.
Results: Of 304 identified citations, 44 articles were selected for full-text evaluation. Cross-referencing of articles yielded another 20 articles. Finally, one RCT enrolling 109 patients fulfilled our inclusion criteria and was analyzed for our review. This study assessed a randomization between antithrombin concentrate infusions (once weekly for 4 weeks) and no intervention in children treated for primary ALL. Outcomes were symptomatic and asymptomatic thrombosis (by radiographic screening following completion of the induction phase), and bleeding events. 7 of 25 analyzed children with antithrombin had thrombosis (28.0%) *versus* 22 of 60 patients without antithrombin (36.7%; OR 0.67; 95% CI 0.3-2.3, P=0.43). One major bleeding event (1.7%) occurred in the non-antithrombin arm, *versus* no major but two minor bleeds in the antithrombin arm. Impact of thromboprophylaxis on survival or ALL treatment outcomes was not assessed.
Summary and Conclusions: Only one RCT in children with ALL was identified, with a high risk of bias due to an open-label design and incomplete outcome data as a result of a per protocol analysis. In this study, no statistically significant effect of antithrombin infusions was seen on the outcomes of interest. However, the sample size was small with a skewed randomization ratio, and may have missed a clinically important effect. No RCTs were found addressing other blood-derived products or anticoagulants. Therefore, the efficacy and safety of thromboprophylaxis to prevent VTE during ALL treatment remain unclear. The use of thromboprophylaxis during ALL treatment, in particular during L-asparaginase therapy, needs to be assessed in randomized controlled trials.

P1077

THROMBOPHILIAS INCIDENCE IN PATIENTS WITH FETAL LOSS IN LAST TRIMESTER AND EVALUATION OF THE EFFECTIVENESS OF TREATMENT

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Background: Up to 5% of women of reproductive age, have >2 fetal losses, one of the most common causes of female infertility. Several studies identify thrombophilia as a major cause of these events.
Aims: We set as our primary objective to analyze the incidence of thrombophilia in a population of women who had pregnancies with fetal deaths (defined as a gestational age>20 weeks). Secondly, we evaluate both the presence of hereditary thrombophilia(HT), as the existence of acquired variations, to estimate the influence of both hypercoagulable states over the course of pregnancy. Finally correlate the use of low molecular weight heparin(LMWH) with gestational viability in these patients.
Methods: Retrospective January1999/December2011 taking as its starting point the existing number of stillbirths during this period in the Gynecology Service of the Hospital of Segovia. Mothers who were evaluated after the event came to the Hematology Service to perform hypercoagulability´s study. Collect the incidence of HT, acquired or absence of these alterations in coagulation studies. In parallel we analyzed the characteristics of obstetric patients for the presence of abortions, bleeding events during birth and number of live births. Finally the women were assessed in held LMWH prophylaxis and treatment, collecting potential medication side effects and the success of this therapy.
Results: During the study period, we collected a total of 97 patients with at least one pregnancy, in which the result was a dead fetus, performing hypercoagulability in 50 patients (51,5%). The average age of women at the time of the event was 33 years (range: 20-42). 38% of these patients (n=19) had suffered a previous abortion, distributed by episodes: 1 abortion (n=13;68%), 2 abortions (n=4;21%), 3 abortions (n=1;5%) and 5 (n=1;5%). Stresses that 36% (n=18) of women in the study had at least one living child prior to the event, not objectified in neither case malformations in children born. Regarding coagulation studies, 64% (n=32) were diagnosed with various syndromes of hypercoagulability (SH). The percentage distribution of final diagnoses are shown in Table 1. Since one of the most common diagnoses in the population was the mutation at the gene level of homocysteine(C-677-T), we performed a subanalysis in which we evaluated the mean serum levels of homocysteine in patients with a homozygous mutation *vs.* heterozygous, no statistically significant differences (8,61*vs.*7,96;P>0.5). 78% patients (n=25) diagnosed with SH, were treated with LMWH and folic acid as needed. In all cases we chose Bemiparin for easy administration (once daily).Close monitoring was performed during pregnancy and postpartum by measuring anti-Xa and platelet count to detect possible side effects. The mean platelet count at baseline was 270,000/mm³(range:135-402), and monitoring functions at3, 6 and 9 months (mean: 252, 243 and 207 respectively) demonstrated the absence of thrombocytopenia. Of all patients with SH 78%(n=25) had a pregnancy after the event, when assessed by subgroups was observed that 92%(n=23) of patients treated getting a viable fetus compared to 28%(n=2) of the untreated group.

Table 1.

DIAGNOSTIC	CASE NUMBER	% TOTAL
MUTATION HOMOCYSTEINE HOMOZYGOUS	13	41%
MUTATION HOMOCYSTEINE HETEROZYGOUS	3	10%
FACTOR V LEIDEN MUTATION	2	6%
ANTIPHOSPHOLIPID SYNDROME	2	6%
PLASMINOGEN DEFICIT	1	3%
MIXED PATTERNS	11	34%
—	N=32	100%

Summary and Conclusions: The incidence of fetal deaths in our population is similar to that reported in the literature. The most common types are the inherent SH and combined. Hypercoagulability studies identify patients with high-risk pregnancies, candidates for the implementation of therapeutic strategies. The use of LMWH in these patients achieved pregnancy with good fetal viability, with success rates similar to those reported.

P1078

IS THERE AN ASSOCIATION BETWEEN ABO BLOOD GROUP AND MICROANGIOPATHIC HEMOLYTIC ANEMIA?

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Background: Bachground: Several studies have reported the relationship between ABO blood groups and thrombosis and hemorrhagic disorders, most of them showing that non-O blood groups have a high risk for thrombosis and O blood groups have a high risk for hemorrhage. However, there are no studies about the relationship between the microangiopathic hemolytic anemia (MAHA) and the ABO blood groups.
Aims: Due to the different risks for hemorrhage and thrombosis in relation to ABO blood groups in different studies, in this study we evaluate the relationship between ABO blood groups and MAHA.
Methods: A prospective case-control study was conducted in the ICU centers of the Hasheminejhad and the Imam Reza Hospital of the Mashhad University of Medical Science in Mashhad, Iran between May 2011-Desember 2012. Patients admitted to the ICU with different etiology showed symptoms and signs of microangiopathic hemolytic anemia. There were 80 patients (age: 20-70 years) and 100 controls in this study. Controls were selected at random from a laboratory.
Results: In this study, we show that there is a significant difference in all blood groups between patients with microangipatic hemolytic anemia and the control group (P. value=0.009), with a significant difference between O and non-O blood groups (P. value=0.023).
Summary and Conclusions: previous studies confirm the historical linkage between some vascular disorders and non-O blood group status. In this study we show the significant relationship between microangiopathic hemolytic anemia and non-O blood groups; therefore, we recommended non-O blood group consider as a risk factor for this group of disease.

P1079

THE NOVEL NOX INHIBITOR 2-ACETYLPHENOTHIAZINE IMPAIRS COLLAGEN-DEPENDENT THROMBUS FORMATION IN A GPVI-DEPENDENT MANNER

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Background: Besides classical agonist-induced signal transduction pathways, platelet activation is also regulated by reactive oxygen species (ROS). In particular, superoxide ions from exogenous and endogenous sources increase collagen-dependent aggregation and thrombus formation. NADPH oxidases (NOXs) play a critical role in the generation of superoxide ions in platelets and contribute to platelet activation, although their mechanism of action remains largely unknown. Therefore, NADPH inhibitors may represent novel potential candidates for the development of anti-platelet agents.
Aims: In this project, we studied the effect of the novel NOX inhibitor 2-acetylphenotiazine (2-APT) on human platelet functional responses and intracellular signalling pathways.
Methods: The generation of superoxide ions was assessed by single cell imaging on adhering platelets using dihydroethidium (DHE), while cumulative ROS generation was detected with 5-(and-6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate (CM-H2-DCFDA). Whole blood thrombus formation, washed platelet aggregation, integrin αIIbβ3 inside-out signalling, Syk phosphorylation, and protein kinase C (PKC) activation were analysed to understand the functional consequences of NOX inhibition by 2-APT in platelets.
Results: Superoxide ion generation stimulated by platelet adhesion on collagen and fibrinogen was significantly inhibited by 2-APT in concentration-dependent manner within the submicromolar range, whereas this pharmacological agent did not affect cumulative ROS generation. 2-APT impaired washed platelet aggregation in response to collagen but not thrombin and abolished whole blood thrombus formation stimulated by collagen but not fibrinogen. The activation of integrin αIIbβ3 and protein kinase C in response to the GPVI-specific agonist collagen-related peptide (CRP) was significantly reduced, whereas the same responses to thrombin were not significantly affected by 2-APT. Finally, Syk activation in response to collagen but not thrombin was inhibited by 2-APT, which suggests a stimulatory role for NOX-generated superoxide ions in the early events of the signalling cascade of GPVI.
Summary and Conclusions: Taken together, our results suggest that 2-APT attenuates GPVI-specific signalling and is a novel inhibitor of collagen-induced platelet activation. Therefore, 2-APT can represent a novel candidate for the development of anti-thrombotic drugs and NOXs are promising new targets for anti-thrombotic drug discovery.

Quality of life

P1080

COMPARISON OF PATIENTS' AND PHYSICIANS' PERCEPTIONS OF PATIENTS' BASELINE HEALTH-RELATED QUALITY OF LIFE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: The use of novel therapeutic agents has significantly improved both progression-free survival and overall survival over recent years in multiple myeloma (MM) patients. With increasing extension of patients' life expectancy, elements related to health-related quality of life (HRQoL) gain in importance. One aspect not considered so far in the literature on HRQoL in MM is the level of concordance between patient- and physician- reported outcomes.
Aims: To compare MM patients' assessment and physicians' judgment of perceived patients' HRQoL.
Methods: A multicenter, observational study was conducted in 31 sites in Italy, Germany, France, UK, Ireland and Belgium in relapsed/refractory multiple myeloma (RRMM) patients starting 2nd or 3rd line treatment. Patients were asked to complete three EORTC questionnaires: 1) Quality-of-Life Core Questionnaire (QLQ-C30), including 15 domains (*Global Health Status/QOL, Physical Functioning, Role Functioning, Emotional Functioning, Cognitive Functioning, Social Functioning, Fatigue, Nausea and Vomiting, Pain, Dyspnea, Insomnia, Appetite Loss, Constipation, Diarrhea* and *Financial Difficulties*); 2) The EORTC QLQ-Multiple Myeloma (QLQ-MY20), including four domains (*Disease Symptoms, Side-Effects of Treatment, Body Image* and *Future Perspective*); and 3) The EORTC QLQ-Chemotherapy-Induced Peripheral Neuropathy (QLQ-CIPN20), including three domains (*Sensory scale, Motor scale* and *Autonomic scale*). All EORTC questionnaires were adjusted according to robust international standards to be completed by physicians. Paired t-tests and Intra-Class Correlation Coefficients (ICC) were calculated to compare patient- and physician-reported scores at baseline. Discordant scores were defined as those with an ICC<0.40.

Table 1. Results of analyses of comparison of patients' and physicians' perceptions of patients' HRQoL at baseline.

EORTC questionnaires	Domains	Mean score (patient – physician)	p-value*	ICC
QLQ-C30	Global Health Status/QOL	-0.2	0.9247	0.5016
	Physical Functioning	-0.5	0.7508	0.7713
	Role Functioning	-1.3	0.5627	0.6257
	Emotional Functioning	1.4	0.5161	0.4304
	Cognitive Functioning	-1.9	0.3547	0.4006
	Social Functioning	6.3	0.0153	0.4503
	Fatigue	0.8	0.6967	0.5100
	Nausea and Vomiting	2.2	0.1027	0.3430
	Pain	2.0	0.3699	0.6277
	Dyspnea	5.1	0.0257	0.3465
	Insomnia	6.5	0.0297	0.3548
	Appetite loss	5.3	0.0148	0.4590
	Constipation	12.7	**<.0001**	0.3735
	Diarrhea	6.3	**<.0001**	0.2482
	Financial Difficulties	2.7	0.2019	0.3585
QLQ-MY20	Disease Symptoms	3.6	0.0451	0.4773
	Side Effects of Treatment	9.7	**<.0001**	0.2769
	Body Image	-0.0	1.000	0.2574
	Future Perspective	4.1	0.0773	0.4754
QLQ-CIPN20	Sensory scale	5.3	**0.0002**	0.4760
	Motor scale	5.3	**<.0001**	0.4975
	Motor scale: using the pedals	-4.1	0.1291	0.3395
	Autonomic scale	6.5	**<.0001**	0.3753
	Autonomic scale: getting or maintain an erection	14.1	0.0232	0.3853

*From paired t-test;
In grey, ICC <0.40 considered as discordant scores

Results: As of Dec 2012, 155 patients (mean age=69; 52.0% male) were enrolled in the study and included in this interim analysis, with an average time since diagnosis of 3 years. At baseline, 14.2% of patients were ECOG ≥2 in terms of their performance status. A total of 87.7% of patients started 2nd line treatment and 12.3% of patients started 3rd line treatment. The majority of EORTC questionnaires were completed at baseline by physicians (n=152; 98.1%) and patients (n=151; 97.4%). The mean difference between patient- and physician-reported scores was highly statistically significant (P-value <.001) for *Constipation, Diarrhea, Autonomic scale, Motor scale, Sensory scale* and *Side-Effects of Treatment* (Table 1) and mostly also showed a low ICC (<0.40, showing high discordance). Additionally, low ICC (high discordance) was observed in the following scores: *Nausea and Vomiting, Dyspnea, Insomnia, Financial Difficulties, Body Image, "Motor scale: using the pedals"* and *"Autonomic scale: getting or maintaining an erection"*. In comparison, for *Global Health Status/QoL* and all five functioning scales, the mean differences between patients' and physicians' scores were not statistically significant and showed a high ICC (>0.40), reflecting lower discordance.
Summary and Conclusions: The level of concordance between patients' and physicians' ratings of functioning domains and QoL was high. However, when rating symptom and side effect domains, physicians underestimated the burden of MM disease and possible effects of prior treatment as compared to the individual patient's experience. A greater awareness especially of an MM patient's symptom level may help to better target improvements in symptom-related QoL.

P1081

PROFILING PATIENTS WITH HIGH-RISK MYELODYSPLASTIC SYNDROMES WHO PREFER TO RECEIVE SURVIVAL PROGNOSTIC INFORMATION. FINAL RESULTS FROM A LARGE INTERNATIONAL PROSPECTIVE COHORT OBSERVATIONAL STUDY

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Background: Physicians often face challenges in prognostic discussions with their patients at the time of diagnosis. This might be critical in patients with high-risk myelodysplastic syndromes (MDS), given the often poor prognoses of these patients.
Aims: The main objective was to investigate preferences for prognostic information on survival at the time of diagnosis and to identify clinical and socio-demographic factors associated with this preference. Also, we explored whether preference for information was associated with functional status and symptom burden so as reported by patients themselves.
Methods: This was an international prospective cohort observational study.

Newly diagnosed IPSS intermediate-2 and high risk patients were consecutively enrolled in 37 centers from nine countries in Europe, Asia and the USA. Patients were invited to participate during one of the first consultations, after confirmed diagnosis of MDS. Physicians completed a survey including a question on whether patients explicitly requested information on life expectancy (yes *vs.* no). Patients also completed the European Organization for Research and treatment of Cancer, Quality of Life Questionnaire-Core30 (EORTC QLQ-C30). The EORTC QLQ-C30 is a psychometrically robust quality of life (QoL) cancer measure assessing both symptoms and functional aspects. The following baseline socio-demographic and clinical variables were investigated for their association with desire for prognostic information: age, gender, education, living arrangements, ECOG performance status (0 *vs.* ≥1), IPSS risk category (int.2 *vs.* high-risk), comorbidity (0 *vs.* ≥1) and hemoglobin level. Univariate and multivariate logistic regression analyses were performed to investigate possible predictors for request of information, amongst socio-demographic and clinical variables (α=.05). Also, we explored possible associations between desire for information and EORTC QLQ-C30 scales by Chi-square and Wilcoxon-Mann-Whitney test as appropriate.

Results: Overall, 280 patients (37% female and 63% male) were enrolled between November 2008 and August 2012. Mean age of patients was 70 years (range: 32-89). Seventy-four percent were diagnosed with IPSS intermediate-2 while 26% with high-risk IPSS. Sixty-one percent of patients explicitly requested information about expected survival to their physicians and 39% did not. In univariate logistic regression analysis, with request for prognostic information (yes *vs.* no) as dependent variable, a younger age (P=.002) and having no comorbidity (P<.001) were the only variables significantly associated with desire for prognostic information. These two factors were also confirmed in multivariate logistic regression analysis. Mean age of patients requesting information was 68 (range 32-88 years) while mean age of patients who did not requested information was 73 (range 47-88 years). When we explored desire for prognostic information by patients' self reported functional status and symptom burden, we found that those who wished to receive information where generally doing better. This was mainly evident for fatigue (P=.007), physical functioning (P=.009) and role functioning (P<.001) so as measured by the EORTC QLQ-C30.

Summary and Conclusions: This data might be of help to physicians in clinical practice by suggesting that younger patients and those who are generally in better health conditions are more likely to be interested in receiving prognostic information on survival.

P1082

CROSS-CULTURAL DEVELOPMENT OF AN EORTC QUESTIONNAIRE FOR ASSESSING QUALITY OF LIFE IN CHRONIC MYELOID LEUKEMIA PATIENTS: THE EORTC QLQ-CML24

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Background: Quality of Life (QoL) is now an important treatment otucome for chronic myeloid leukemia (CML) patients. However, there is lack of internationally validated QoL measures for this population.

Aims: To present final results of the cross cultural development of an European Organization for Research and Treatment of Cancer (EORTC)-QoL questionnaire for patients with CML (*i.e.* EORTC QLQ-CML24).

Methods: The process followed a predefined and systematic stepwise iterative process as defined by the EORTC guidelines for Questionnaire development. The CML Advocates Network was involved providing logistic and administrative support and in key steps of the development process and participated in the Investigators' meetings. The process was divided into 3 phases: 1) generation of relevant QoL issues, 2) operationalization of the QoL issues into a set of items, 3) pre-testing the questionnaire for relevance and acceptability. Descriptive statistics and psychometric analyses were performed. Internal consistency was assessed using Cronbach's alpha coefficient. The construct validity was also examined by correlation with the core questionnaire (EORTC QLQ-C30) using Pearson's product moment correlation coefficients. Clinical validity was assessed with known-group comparisons to assess the extent to which questionnaire scores were able to discriminate between subgroups of patients known to differ in terms of clinical status (*i.e.*, Karnofsky performance status and complete cytogenetic response –CCyR- to therapy). Group differences were assessed for significance using the Wilcoxon rank sum test.

Results: A preliminary list of 74 issues was identified from the literature and was the basis to conduct semi-structured interviews with 137 patients recruited in seven hospitals from five countries. Forty-three percent of patients were in treatment with first line imatinib and 46% were in second line treatment with second generation Tyrosine Kinase Inhibitors (TKI). A preliminary version of the questionnaire with 30 items was then tested in a larger international sample. The provisional questionnaire was tested on 312 patients at 14 centres in 10 countries (including Europe, Asia and the USA) by also conducting cognitive debriefing interviews. The resulting questionnaire consisted of 24 items assessing the following aspects: *symptom burden, impact on daily life, impact on worry/mood, body image problems and satisfaction with care an information and satisfaction with social life.* Cronbach's alpha coefficients were 0.83, 0.74, 0.73 and 0.83, for symptom burden, impact on worry/mood, impact on daily life, and satisfaction with care and information scales, respectively. Clinical validity was confirmed as comparisons between responders and non-responders (*i.e.*, reaching at least a CCyR) showed statistically significant better QoL outcomes (P<0.01) for patients responding to therapy on four out of six scales. Construct validity analyses confirmed that all scales of the QLQ-CML24 correlated in the expected directions with scales of the EORTC QLQ-C30. To illustrate, the symptom burden and impact on daily life scales from the module and the fatigue scale from the core questionnaire were highly correlated (0.64 and 0.65, respectively).

Summary and Conclusions: The use of our questionnaire could help better inform physicians of a true intolerance to a given therapy by providing the unique patient's perception of the burden of treatment over time. Also, its implementation in comparative effectiveness studies will provide invaluable information to help guide clinical decision-making.

P1083

QUALITY OF LIFE AND RETURN TO NORMAL LIVING PATTERNS TWENTY YEARS AFTER TRANSPLANTATION FOR THALASSEMIA

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Background: More than 30 years have passed since the first successful hematopoietic stem cell transplantation (HSCT) was performed for thalassemia. Since that time, HSCT has become a widely used therapeutical approach for the treatment of this disorder.

Aims: For the first time, we investigated health related quality of life (HRQoL) and return to normal living patterns in a large cohort of thalassemia patients who underwent HSCT more than 20 years ago.

Methods: Socio-demographic and clinical data were collected from surviving Sardinian ex-thalassemic patients transplanted in the 1980s and 90s in the Centers of Pesaro and Cagliari. Patients were sent Short Form 36 (SF-36) and Functional Assessment of Cancer Therapy–Bone Marrow Transplant (FACT-BMT) health survey questionnaires. HRQoL outcomes were compared to those of matched peers from the Italian SF-36 normative database. Separate comparisons were performed to account for age at HSCT (≤15 and >15 years) and the presence or absence of graft-*versus*-host disease (GVHD).

Results: One hundred and nine out of 130 thalassemia-free surviving patients responded to the survey (83.8%; median age 34 years, range 21-48). Median age at HSCT was 12 years (range 1-36) with a median follow up of 22.8 years (range 11.7- 30.3). Acute and chronic GVHD were registered in 35.8% and 18.3% of the patients, respectively. About 75% reported at least one disorder at the time of the survey, with a higher incidence for liver, metabolic or endocrine alterations. Two patients underwent solid organ transplantation (kidney, liver) and 4 reported a secondary malignancy. Seven pregnancies were reported by 6 of the 44 female patients (13.6%); 16 pregnancies regarded 11 partners of the 65 male patients (16.9%). Mean scores as well as adjusted mean differences for SF-36 scores only showed clinically meaningful differences between patients and controls for the General Health scale (-8.9; 95% CI, -15.0 to -2.7, P=0.005); no differences were observed for the 4 physical and 5 mental domains of SF-36. Comparisons of patients, with or without acute/chronic GVHD, to the respective population norms showed significantly worse scores for GVHD patients (Figure 1). The HRQoL profile of patients without GVHD was similar to that of the control population with even better scores for Mental Health and Mental Component Summary (5.3; 95% CI, 1.6 to 9; P=0.003). Worse and clinically significant scores for general health (-12.8; 95% CI, -23.1 to -2.6; P=0.015) were also observed in the group of patients transplanted above 15 years (adults). Most patients (74.4%) had either resumed or completed their studies after HSCT, with results comparable to the healthy population. Surprisingly, employment status was considerably higher

among ex-thalassemia patients (77.1%) in comparison with a healthy Sardinian control population aged 20-45 years (59.9%).

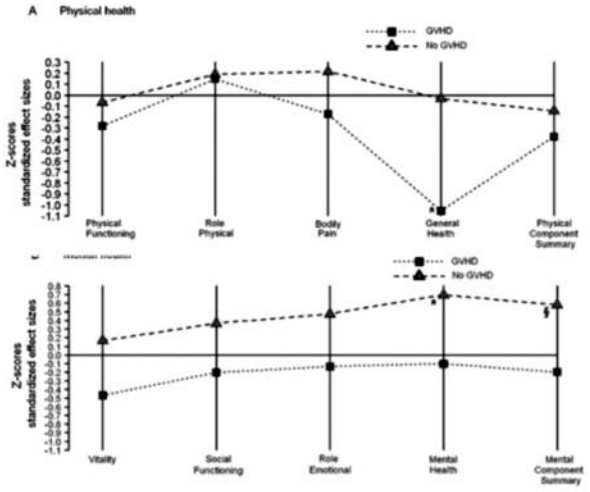

Figure 1.

Summary and Conclusions: Our data provide robust evidence that in the long term ex-thalassemia patients achieve good HRQoL after HSCT with return to normal life style. Nonetheless, GVHD remains an impairing factor and so, whenever possible, HSCT should be performed in pediatric age.

P1084

HOW ACCURATE ARE PHYSICIANS IN ESTIMATING SYMPTOM SEVERITY AND HEALTH STATUS OF THEIR CHRONIC MYELOID LEUKEMIA PATIENTS?

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Background: Several studies conducted in patients with solid tumors have shown that patients more frequently report worse symptom severity than physicians. On this ground, we hypothesized this would hold true in the chronic myeloid leukemia (CML) clinical setting.
Aims: The main objective of this study was to compare the reporting of health status and symptom severity, for a set of core symptoms related to first line imatinib therapy, between patients and their treating physicians. A secondary objective was to investigate whether either physician or patient-reported symptoms best reflected patient's overall health status.
Methods: Analysis was performed on 422 CML patients consecutively enrolled in a large survivorship project. All respective paired physicians (N=29) completed a paired questionnaire and the following analyses are thus based on 422 patient-physician paired questionnaires. The questionnaire administered to patients and physicians consisted of a nine core symptoms checklist developed for patients treated with imatinib. In addition, to these symptoms a question on health status from the well validated Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) was also included. Severity of the following symptoms was investigated: abdominal discomfort, diarrhea, edema, fatigue headache, muscle cramps, musculoskeletal pain, nausea and skin problems. Severity was rated on a four-Likert scale (*i.e.*, not at all, a little, quite a bit and very much). The self-rated question on health status was formulated as follow: "in general you would say your health is" with the following response options: excellent, very good, good, fair, poor. To avoid any risk of convergence in symptom ratings, the study was designed to avoid that patients and their treating physicians knew of each other's answer.
Results: Agreement on symptom ratings ranged between 34% to 66% respectively for muscular cramps and nausea. For all symptoms, patients graded higher severity more often than their physicians. The top three symptoms most frequently underestimated by physicians were respectively fatigue (51%), muscular cramps (48%) and musculoskeletal pain (42%). The majority of discrepancies were within one point, however, for edema, skin problems, musculoskeletal pain, muscular cramps and fatigue, differences of 2 points were also noted in at least 10% of evaluations. Agreement on overall health status rating was observed in 26% of pairs. However, health status was overestimated by physicians in 66% of paired evaluations. When physicians underestimated symptom severity, this was most frequently evident in the lower scales intensities. In the majority of cases, physicians rated the symptom as not being present while patients rated the same symptom as of mild intensity (*i.e.*, a little). Investigation on whether either patient-reported or physician-reported symptoms better reflected overall health status, revealed that patient reports had higher levels of concordance compared to physician reports. Fatigue was the most correlated symptom with overall health status for both patients and physicians. However, the level of concordance was higher for patient reports (Kendall=0.43; 95% C.I.=0.354 to 0.500), compared to physician reports (Kendall=0.26; 95% C.I.=0.186 to 0.348).
Summary and Conclusions: Physicians often underestimate symptom severity and overestimate health status of their patients. This miss-mach might have major implications in clinical management. Physicians' failure to note the presence of mild symptoms, for example, could lead them to underestimate the risk that their patients do not fully adhere to treatment schedule to minimize burden of side effects.

P1085

PATTERNS OF FATIGUE IN PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA: A MULTICENTER OBSERVATIONAL STUDY

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Background: Health-related quality of life (HRQoL) and fatigue are major concerns for patients with primary immune thrombocytopenia (pITP) due to the symptoms associated with the disease and its treatment. However, while these are critical issues, there is paucity of evidence-based data in this area.
Aims: The main objective of this study was to identify specific patterns of fatigue in pITP patients by disease phase (i.,e, chronic, persistent and newly diagnosed). A secondary objective was to investigate socio-demographic and clinical factors that could be associated with fatigue severity in this pITP population overall.
Methods: Data were gathered through an ongoing multicenter cross-sectional observational study. The Multidimensional Fatigue Inventory (MFI) was used to assess patient-self reported fatigue. This is a psychometrically robust measure consisting of 20-items. This questionnaire covers the following five dimensions (i.e, scales): General Fatigue (GF), Physical Fatigue (PF), Mental Fatigue (MF), Reduced Motivation (RM) and Reduced Activity (RA). Each scale includes four items with five-point Likert scales. GF was regarded as the primary outcome of our analysis as this was designed to encompass both physical and psychological aspects of fatigue. PF concerns physical sensations related to fatigue. MF pertains to cognitive functioning, including difficulty concentrating. RA refers to the influence of physical and psychological factors on the level of activity. RM relates to lack of motivation for starting any activity. Scores on each subscale range from 4 to 20, with higher scores indicating greater fatigue. Socio-demographic, clinical variables examined for their association with fatigue were: age, gender, education level, comorbidity, time since diagnosis, as well as hemoglobin and platelets counts at diagnosis. Descriptive statistics and univariate and multivariate linear regression analyses were used.
Results: To date 342 pITP patients are enrolled in this study and current analysis is based on 257 patients with full data available for analysis. Of these, there were 67%, 16% and 17%, respectively diagnosed with chronic, persistent and newly diagnosed pITP. At study participation, mean age of patients was *52 years* (*64% female and 36% male*). At least one comorbidity was present in *53%* of patients. The median time from initial diagnosis to study entry was *0.6, 8 and 64 months*, respectively for newly diagnosed, persistent and chronic patients. The three groups were well balanced (*i.e.*, no statistically significant differences) for the following variables: age, gender, education level and comorbidity. Newly diagnosed pITP patients reported lower fatigue severity compared to persistent and chronic patients. This finding was consistent across all scales

of the MFI (see Figure 1), however this was statistically significant only for GF (P=0.014). Further investigation revealed that this difference in fatigue levels between pITP groups was independent of time since diagnosis. In multivariate analysis, investigating factors independently associated with GF, gender was the only statistically significant factor. Female patients reported greater levels of fatigue (P=0.009).

Fatigue dimensions	Values	Newly diagnosed	Persistent	Chronic	Total	P
General Fatigue	Mean (SD)	10.20 (4.16)	12.38 (4.67)	12.20 (4.35)	11.88 (4.42)	0.014
	median	11	12	12.5	12	
	n	45	40	172	257	
Physical Fatigue	Mean (SD)	9.80 (4.16)	11.33 (5.03)	10.73 (4.30)	10.66 (4.40)	0.307
	median	9	11.5	11	11	
	n	45	40	172	257	
Reduced Activity	Mean (SD)	9.22 (3.60)	10.03 (4.67)	9.74 (4.05)	9.70 (4.07)	0.694
	median	9	10	10	10	
	n	45	40	172	257	
Reduced Motivation	Mean (SD)	8.11 (3.16)	9.48 (4.22)	8.80 (3.59)	8.78 (3.63)	0.267
	median	7	9	8.5	8	
	n	45	40	172	257	
Mental Fatigue	Mean (SD)	8.44 (3.37)	10.05 (4.84)	9.59 (4.27)	9.46 (4.24)	0.251
	median	8	9.5	9	9	
	n	45	40	172	257	

Legend: the higher the score the higher fatigue severity (score range between 4 to 20); SD. Standard deviation.

Figure 1. Fatigue severity by pITP groups.

Summary and Conclusions: Fatigue severity is higher in patients with persistent and chronic pITP patients compared to that reported by newly diagnosed patients. Also, female patients are at greater risk of experiencing higher fatigue levels. Current results begs for more research and can potentially help guide the development of tailor-based supportive care interventions.

P1086

PAIN AND EMOTIONAL DISTRESS IN HEMATOLOGIC PATIENTS THROUGHOUT ALL PHASES OF DISEASE: RESULTS FROM A MULTIDISCIPLINARY RESEARCH TEAM IN MODENA UNIVERSITY HOSPITAL

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Background: According to some outdated reports, physical pain has been considered for many years a rare feature in the majority of blood malignancies, especially in acute leukemias, with the exception of advanced and terminal phases of disease. Unlike myeloma and lymphoma, there are few published data regarding the frequency of pain in patients with leukemia. Based on the modern concept of total cancer pain and on the importance of patient-reported outcomes, routine symptom assessment for hematologic patients should include, together with pain, even emotional distress, expressed in terms of anxiety and depression.
Aims: In order to investigate prevalence and clinical relevance of pain and emotional distress in patients with acute myeloid (AML) and lymphoid (ALL) leukemia referred to our center, a multidisciplinary team consisting of nurses, physicians and psychologists has adopted two validated tools in the daily clinical practice: NRS (Numeral Rating Scale) and HADS (Hospital Anxiety and Depression Scale). ESAS (Edmonton Symptom Assessment System) has been compared with HADS with the aim to evaluate its diagnostic accuracy.
Methods: NRS, HADS and ESAS scales were administered to newly diagnosed AML and ALL patients at diagnosis (T0), during the neutropenic phase (T15) and at discharge (T30), throughout hospital admissions and different phases of treatment. According to NRS scale pain intensity was classified as absent (0), mild (1-3), moderate (4-6) or severe (7-10). HADS is 14-item scale given by 7 questions related to anxiety and 7 to depression, determining a score from 0 to 21. ESAS is a multiple-item visual analogue scale from 0 to 10. Anxiety and depression were considered positive with HADS 8 and ESAS 2 or more. Sensitivity and specificity tests were also performed. Results were mainly focused on induction phase, bone marrow transplation (BMT) and home care.
Results: From June 2007 to December 2011 137 patients with AML and ALL were enrolled in the study (AML=109, ALL=28, M=85, F=52, median age=60). Another cohort of 31 patients referred to the home care program and affected by several blood disorders (NHL=8, MM=6, AML=5, MF=3, ITP=2, MDS=2, AA=1, ALL=1, CLL=1, CML=1, ET=1) was evaluated in parallel (M=18, F=13, median age=79) on monthly basis. 842 questionnaires were collected in the AML-ALL group. At diagnosis pain was reported in 46.2% of cases (mild=30.3%, moderate-severe=15.9%). The highest prevalence and intensity of pain was observed in post-BMT neutropenic phase associated to mucositis (overall pain=61.5%, severe=30.8%). At diagnosis anxiety scores were positive in 33.6% for HADS and 51.1% for ESAS, while depression was present in 22.4% and 42.4% of cases, respectively. A higher prevalence of anxiety and depression was documented at T15 both in induction and post-BMT phases. Considering all HADS questionnaires anxiety and depression were positive in 26.7% and 25.2% of cases, respectively (10% with HADS from 11 upward, accounting for more severe symptoms), while an ESAS score of 2 or more was reported in 36.5% (anxiety) and 31.9% (depression) of cases. In the home care group pain was reported in 78 of all 157 questionnaires (overall pain=49.7%, mild=26.1%, moderate-severe=23.6%). Anxiety and depression were positive in 31.2% and 45.2% of HADS and in 45.9% and 49% of ESAS questionnaires, respectively. Overall test accuracy of ESAS (score of 2 or more) was 77.5% for anxiety and 76.4% for depression.
Summary and Conclusions: Pain, anxiety and depression are common symptoms in a significant proportion of acute leukemia patients, impacting quality of life, clinical decisions and outcomes. Compared with HADS, ESAS showed adequate diagnostic accuracy in screening for anxiety and depression, and could be an excellent candidate for large-scale and routine assessment of physical pain, emotional distress and other symptoms, at diagnosis and during the active phase of disease. Some issues remain open, such as the identification of appropriate cut-offs for ESAS and the development of strategies to implement a sustainable integration of early interventions of palliative care in hematology units and to prevent/reduce the burden of physical symptoms and emotional distress.

P1087

EFFICACY OF FENTANYL IN COMPARISON TO KETAMINE IN ANALGESIC EFFECT FOR HEMATOLOGY /ONCOLOGY PROCEDURES IN CHILDREN. A RANDOMIZED, DOUBLE-BLINDED, CROSSOVER, PLACEBO-CONTROLLED TRIAL

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Background: Children often require relief of pain and anxiety when undergoing painful procedures.
Aims: The purpose of this study was to determine the differences in the comparison between Fentanyl and Ketamine used in cancer-diagnosed children undergoing painful procedures. These include the degree in satisfaction, painfulness and nausea and vomiting.
Methods: The study was conducted with fifty-five children undergoing painful procedures (intrathecal chemotherapy and/or bone marrow aspiration/biopsy) at department of pediatrics, Phramongkutklao Hospital. The informed consents and assents were obtained. Patients were randomly assigned in a double-blinded fashion to receive either intravenous Fentanyl or Ketamine at 1 mcg/kg/dose and 1 mg/kg/dose, respectively. Each patient acted as his or her own control, and each patient was studied at two time points. The result in effectiveness of the drug was measured using 3 parameters. The first parameter was satisfaction score ranging from 0 to 10. Secondly perception of procedural pain using FLACC scale, Wong-Baker FACES Pain Rating Scale and Visual Analog Scale in patient age 3 month to 4 years, 4 to 8 years and more than 8 years old, respectively. Finally, the last studied parameter was nausea score and the frequency of vomiting.
Results: Period effect, sequence effect and carry over effect were not demonstrated. The satisfaction in patient receiving Fentanyl was significantly greater than Ketamine (P=0.007) in the age group more than 8 years old (P=0.005). In addition, both painful and nausea/ vomiting were significantly less in the patient receiving Fentanyl (P=0.002 and P<0.001, respectively**).** No child required admission to hospital and there were no serious complications.
Summary and Conclusions: This study demonstrated that intravenous Fentanyl had a superior efficacy in satisfaction, decreased painful and nausea/ vomiting with no significance side-effects over Ketamine. Fentanyl may also be recommended as a reasonable option before undergoing oncology procedures (intrathecal chemotherapy and/or bone marrow aspiration/biopsy) in children with cancer.

P1088

IS SCREENING FOR MALNUTRITION USEFUL IN OLDER PATIENTS WITH AGGRESSIVE HAEMATOLOGICAL MALIGNANCIES?

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Background: Treating older patients with haematological malignancies is a challenging task. The age- and gender-specific incidence rates increase dramatically over the age of 70 for Acute Myeloid Leukaemia (AML), Myelodysplastic Syndromes (MDS), Non-Hodgkin's Lymphoma (NHL) and Multiple Myeloma (MM). Malnutrition is a common feature in many types of cancer and is responsible not only for a poor quality of life and poor response to chemotherapy, but also for a

shorter survival time. Patients with haematological malignancies have specific challenges associated with eating and nutrition because of the intensive and aggressive treatments they endure. However, studies focusing on this aspect in older haematological patients are very scarce. Comprehensive geriatric assessment (CGA) is a multidisciplinary systematic approach using validated instruments and aiming at assessing nutritional status, physical functioning, co-morbidity, cognition, mood and polypharmacy in older patients. CGA is strongly recommended as part of the evaluation of older patients with cancer.
Aims: To establish the usefulness of screening for malnutrition in older patients considered for intensive chemotherapy against an aggressive haematological malignancy.
Methods: The patients, 70 years or older, with a new diagnosis of AML, intermediate or high grade MDS, MM or high grade NHL, referred to the haematology department of a tertiary hospital, were enrolled in the current study after providing a written consent. Before the start of therapy, a geriatric assessment measuring nutritional status, physical functioning, hand grip strength, co-morbidity, cognition, mood, quality of life, polypharmacy and falls was completed for each patient. CGA was repeated 2 months after the start of therapy. We used the Mini Nutritional Assessment Short Form, as part of the CGA, to screen for malnutrition. The study was approved by the local Ethical Committee.
Results: Fifty patients were included. Median age was 76 years (range 70-87). Patient characteristics are summarized in Table 1. Eighty four percent of the patients (n=42) had a BMI ≥23. Remarkably, 16% of the patients (n=8), assessed by MNA SF, were malnourished and 66% of the patients (n=33) were at risk for malnutrition. Of the malnourished patients, 50% (n=4) had a BMI>23. Apart from their haematological diagnosis, recent weight loss and declined food intake were the most important MNA SF parameters predicting (risk of) malnutrition. For 12 patients (29%), (risk of) malnutrition was the only impairment detected by CGA on initial evaluation. On the second evaluation, 2 months later, 6 more patients scored positive on MNA SF (1 malnourished, 5 at risk).

Table 1.

		No. of patients	%
Multiple myeloma		5	10
Non-Hodgkin lymphoma		19	38
Myelodysplastic syndrome		9	18
Acute myeloid leukemia		17	34
Female		25	50
G8 ≤ 14		39	78
Dependent in activities of daily living (ADL)		12	24
Dependent in instrumental ADL (iADL)		19	38
ECOG performance score	0 – 1	38	76
	≥ 2	12	24
4-item Geriatric Depression Scale ≥ 2		15	30
MMSE	< 24	2	4
	missing	5	10
At least 1 fall in the previous year		16	32
> 3 medications		33	66

Summary and Conclusions: The prevalence of malnutrition or risk for malnutrition in older patients with severe (aggressive) haematological malignancies is high, even before the start of treatment. Intensive treatment will further increase this risk. Therefore, instead of screening for malnutrition, we recommend nutritional assessment by a dietician with individualised dietary advice and follow up during treatment to become an integral part of the treatment plan in this group of older patients.

P1089

IMPAIRED HEALTH-RELATED QUALITY OF LIFE IN ACUTE MYELOID LEUKEMIA SURVIVORS

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Background: Prognosis of acute myeloid leukemia (AML) has progressively improved in the last decades. As a consequence of the increasing number of AML survivors it is becoming more important to assess the impact of AML and its treatments on health-related quality of life (HRQOL).
Aims: The aim of this study was to assess the impact of AML on HRQOL by comparing the HRQOL of AML survivors with the HRQOL in the general population. We also examined which patient characteristics were associated with HRQOL in AML survivors.
Methods: HRQOL was measured with the EQ-5D-5L and the EORTC QLQ-C30. Questionnaires were sent to 103 patients diagnosed with AML between 1999 and 2011 at a single academic hospital and still alive in 2012. The HRQOL in the general population was derived from previously published studies. T-tests were used to test for differences in mean scores on the QLQ-C30 scales and the EQ-VAS between AML survivors and the general population. Multivariate linear regression was used to identify factors associated with HRQOL. The study was approved by a local medical ethics committee.
Results: Questionnaires were returned by 92 of the 103 patients (89%). AML survivors had significantly lower scores on the functioning scales of the QLQ-C30, reported significantly more fatigue, pain, dyspnea and appetite loss and had significantly lower EQ-VAS scores than the general population. Impaired HRQOL in AML survivors was mainly found in survivors aged <65 years without a paid job. Other factors associated with a poor HRQOL were allogeneic hematopoietic stem cell transplantation and absence of social support.
Summary and Conclusions: The HRQOL in AML survivors can return to levels comparable with the HRQOL in the general population. However, a subgroup of AML survivors continues to experience symptoms and functioning problems which complicate the return to work. HRQOL in these patients can be improved by adequately treating and preventing fatigue, pain, dyspnea and appetite loss.

P1090

VALIDATION OF THE G8 IN OLDER HAEMATOLOGICAL PATIENTS

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Background: Given the increased age- and gender-specific incidence rates for Acute Myelogenous Leukaemia (AML), Myelodysplastic Syndromes (MDS), Non-Hodgkin's Lymphoma (NHL) and Multiple Myeloma (MM) over the age of 70, haematologists are more and more confronted with necessity of decision making in older patients. However, the group of older patients is very heterogeneous with more comorbidities, more often a poor performance status at diagnosis and less tolerance to intensive therapy.
Comprehensive geriatric assessment (CGA) is a multidisciplinary systematic approach using validated instruments and aiming at assessing nutritional status, physical functioning, co-morbidity, cognition, mood and polypharmacy in older patients. CGA is strongly recommended as part of the evaluation of older patients with cancer to identify frail patients for whom a tailored treatment might be imperative. As CGA can be very time-consuming, a screening tool is necessary to select those patients in need of a CGA. In the literature the G8 was validated as a screening tool for older patients in oncology.
Aims: In this study, we tested the performance of the G8 as a screening tool in identifying older haematological patients who would benefit from CGA.
Methods: Patients, 70 years or older, with a new diagnosis of AML, intermediate or high grade MDS, MM or high grade NHL, referred to the haematology department of a tertiary hospital, were enrolled in the current study after providing a written consent. For each patient both the G8 and the geriatric assessment were completed before therapy was started. For the CGA validated scales were used, measuring nutritional status, physical functioning, hand grip strength, co-morbidity, cognition, mood, quality of life, polypharmacy and falls. Patients were considered frail when scoring abnormal for at least one CGA domain. ROC-curve analysis was used to determine inherent validity of G8. The study was approved by the local Ethical Committee.
Results: Fifty patients were included. Median age was 76 years (range 70-87). The AUC-value of 0.949 (95% CI 0.889–1.00) indicates that the G8 tool has a high diagnostic accuracy to identify disabilities. At the cut-off score proposed in the literature (G8 ≤14), a sensitivity of 88.6% and a specificity of 100% was obtained. Overall, one or more impairments were detected by CGA in 88% of patients with a majority of patients scoring positive on (risk for) malnutrition. A comparison of our results with the literature is summarized in Table 1.

Table 1.

	Screening tool	CGA	Sensitivity (%)	Specificity (%)	Prevalence (%)
Velghe A. et al, 2013	G8 ≤ 14	At least 1 abnormal score	88.6	100	88
Soubeyran P. et al, 2011	G8 ≤ 14	At least 1 abnormal score	76.6	64.4	80.1
Pottel L. et al, 2011	G8 ≤ 14	At least 1 abnormal score	85.7	75	68.6
Bellera C. et al, 2012	G8 ≤ 14	At least 1 abnormal score	85	65	94

Summary and Conclusions: Our results show that the G8, at the proposed cut-off of ≤14, can be used as a valid screening tool for older patients with aggressive haematological malignancies who would benefit from CGA.

P1091

PSYCHOLOGICAL ASSESSMENT OF SIBLINGS OF PATIENTS WITH CHILDHOOD CANCER: A FORGOTTEN GROUP

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Background: The disruption created by diagnosis of cancer in a child within a family reach beyond the diagnosed child to impact the entire family. It is no surprise, that siblings within these families are at risk for emotional, behavioral and academic problems, and hence it is important to understand their consequences and develop feasible interventions to reduce their distress and promote their adjustment.
Aims: Our aim was to assess of psychological state of siblings of children with cancer.
Methods: A cross-sectional study included 100 children with age range (6-18 years). Group 1 included 70 siblings of 39 patients with cancer attending the oncology unit at Ain Shams University- Pediatrics Hospital. Any child receiving prior treatment for depression or anxiety, having chronic illness, mentally handicapped and siblingsof patients on end of life care program were excluded from participation. Group 2(control) included 30 healthy children from 13 families without any childhood chronic illness. Children receiving treatment for depression or anxiety and mentally handicapped were excluded from participation. Participants were subjected tohistory taking with special emphasis on the onset of the oncological disease in their sick sibling, duration and frequency of hospitalization, socioeconomic standards (SES) and educational level as well as the presenting symptoms, diagnosis and treatment stage of the ill child was collected from patient records.Psychological assessment for depression using children depression inventory (CDI),for anxiety using revised children's manifest anxiety scale (RCMAS) and for personal compatibility using Child Behavior Checklist (CBCL)
Results: Mean age for group1 was 11.36±3.48 years, and group 2 11.77±3.28 years with no significant difference as regard the distribution of sex, SES, educational leveland parental divorce (P>0.05). 65.7% of siblings had depression compared to 40% in group 2 (P=0.046) yet no significant difference was found in mean depression and anxiety scores (P>0.05), and a significantly lower mean score of personal compatibility in group 1 (375.03±33.56) compared to control group(411.6±43.79) (P<0.05). No statistical significant difference was found as regardlow self-esteem, anorexia and sleep disorder (P>0.05).
As regard the effect of gender, there was significantly higher scores and grades of depression in females when compared to males (P<0.05); a significantly higher rate of loneliness, insecurity and low self-esteem in females when compared to males (P<0.05). There was no significant difference between different SES or levels of education in psychological tests (P>0.05). There was no significant association between duration of cancer therapy and psychological tests. We further divided group1 participants into 2 groups (hospital admission more and less than twice per month), significantly lower score and grades of anxiety, lower rate of nervousness and insecurity, lower score and grades of depression, lower rate of low self esteem and sleep disorder in siblings of patients admitted <twice per month when compared to those admitted ≥twice per month (P<0.05); however no statistical significant difference was found in the total personal compatibility score.
Summary and Conclusions: Depression and impaired personal compatibility represent an appreciable problem in siblings of patients with childhood cancer which become more evident in the settings of frequent hospitalization; readjustment of these problem may have a positive impact on the whole family.

P1092

HEALTH-RELATED QUALITY OF LIFE AMONG LONG-TERM SURVIVORS OF ADOLESCENT HODGKIN'S LYMPHOMA TREATED WITH PEDIATRIC APPROACH

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Background: Reduced health-related quality of life (QoL) and late complications after curative treatment of Hodgkin's lymphoma (HL) are of special relevance because most of the cured are young adults.
Aims: The purposes of the present study were to compare QoL of HL survivors with that of health young adults and to evaluate the relationships between disease/treatment features and QoL in the HL survivors.
Methods: Fifty sex (male - 22, female - 34) survivors of HL with a median ages 27.5 years (range, 22-41) were evaluated. For comparison 94 (male - 44, female - 50) health subjects with a median ages 28.0 years (range, 22-46) was drawn to the study of QoL. All HL survivors were treated with modified pediatric protocol DAL-HD-90 in 1997-2007 in our clinic. Patients are allocated to three treatment groups (TGs). The original protocol is modified in the following positions: (1) procarbazin was replaced by dacarbazin; (2) young adults received vinblastin instead vincristin; (3) all patients with advanced stages received 2 cycles of ODPA independently from gender; (4) doses of involved field radiotherapy were increased from 20-25 Gy to 30 Gy. QOL was assessed by the Short Form 36 (SF-36), which generate 8 separate scales and 2 general scores (0=worst health state, 100=best health state). All survivors have a time of complete remission (CR) of Hodgkin's lymphoma ≥5 years.
Results: The HL survivors had lower scores than the normal controls on all scales and scores (Figure 1). Statistically significant differences were found in general health - GH (53±3 *vs.* 72 ±2, P<0.001), vitality - VT (55±2 *vs.* 72 ±2, P<0.001) and mental health - MH (57±2 *vs.* 72 ±2, P<0.001). Patients with the international prognostic scores for advanced stages (IPS; Hasenclever, 1998) ≥4 (n=7) had the lowest scores in role physical - RF (29±15 *vs.* 81 ±5, P<0.001) and role emotional limitations - RE (33±15 *vs.* 77 ±5, P=0.006). The adverse events including relapsed disease (n=7) and second malignancies (n=2) correlated with IPS ≥4 and reduced QoL. The patients with ages on moment of HL diagnosis ≥18.5 years (methods of ROC-curves, P=0.047) have reduced QoL when compared to younger patients in GH (48±3 *vs.* 61 ±4, P=0.027), VT (50±3 *vs.* 61 ±4, P=0.013) and physical component scale - PCS (48±1 *vs.* 53 ±2, P=0.046). Time since diagnosis, ages on QoL evaluation, gender, treatment groups (2, 4 or 6 cycles of primary chemotherapy plus radiotherapy), Ann-Arbor stages, bulky disease, current married status and education levels were not associated with statistically significant differences in QoL.

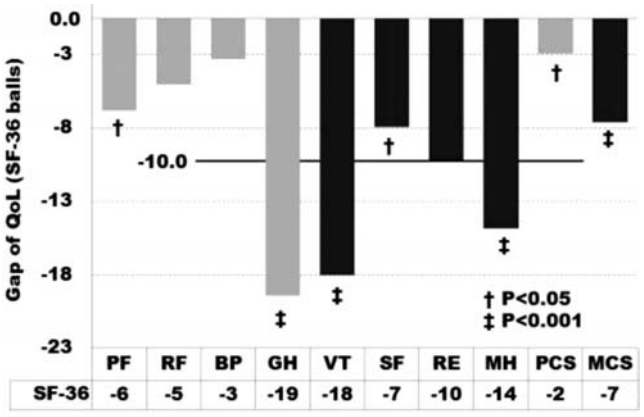

Figure 1. Gap of SF-36 scale-scores for the HL survivors.

Summary and Conclusions: Long-term HL survivors have poorer physical and mental QoL than the general population of young adults. The age on moment of LH diagnosis ≥18.5 years was associated with significant reduced predominantly physical QoL. IPS ≥4, relapsed HL and secondary malignancies were associated mostly with the deterioration of role physical and emotional functioning, which may indicate a lack of confidence in the future health.

P1093

THE PATIENT-REPORTED BURDEN OF ADVERSE EVENTS RELATED TO DASATINIB VERSUS OTHER BCR-ABL INHIBITORS, FROM A PATIENT PERSPECTIVE

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Background: The tyrosine-kinase inhibitors (TKIs) imatinib,dasatinib and nilotinib have improved clinical outcomes in patients with chronic myeloid leukemia (CML). While the prevalence of adverse events (AEs) for each TKI has been widely reported, the impact of these AEs from the patient perspective is less widely documented.
Aims: The study assessed the patient-reported prevalence, bothersomeness and severity of AEs associated with TKIs. Based upon these data, the study aimed to identify differences in patient-reported prevalence, bothersomeness and severity of AEs between the different TKIs.
Methods: Patients with chronic-phase CML were identified from the National Health and Wellness Survey and the Lightspeed Research Chronic Illness panel in the US and EU (United Kingdom, Spain, France, Italy, Germany). Respondents were aged ≥18 years, receiving a TKI or on a drug holiday with an intention to return to treatment. An Internet-based self-administered survey assessed prevalence of AEs, bothersomeness of AEs (1=not present, 2=present, 3=most bothersome) and severity of AEs in the past 24 hours using the MDASI-CML (0=AE not present to 10=bad as you could imagine). Prevalence,

bothersomeness and severity of AEs were compared between dasatinib and the other TKIs in a series of bivariate analyses, using Chi-square tests and independent sample t-tests for categorical and continuous variables, respectively.

Results: Among 303 patients in the study, 208 were on imatinib (3.4% on a drug holiday), 38 on dasatinib (7.9% on holiday) and 49 on nilotinib (2.0% on holiday). Compared with imatinib, dasatinib (imatinib reported first) was associated with lower diarrhoea (43.8% *vs.* 23.7%; P=0.021), muscle cramps (62.0% *vs.* 26.3%; P<0.001), stomach pain (28.4% *vs.* 13.2%; P=0.049) and tear production (19.2% *vs.* 5.3%; P=0.035), but higher constipation (9.6% *vs.* 23.7%; P=0.013) and lost sexual appetite rates (25.5% *vs.* 44.7%; P=0.016). Dasatinib patients experienced significantly lower bothersomeness associated with abnormal kidney function (P=0.003), abnormal liver function (P<0.001) and muscle cramps (P<0.001) than imatinib patients; severity of numbness/tingling (P=0.014), diarrhea (P=0.007), muscle cramps (P=0.002) and swelling (P=0.028) was lower with dasatinib. When compared with nilotinib (nilotinib reported first), dasatinib was associated with fewer abnormal kidney tests (10.2% *vs.* 0.0%; P=0.043) and liver tests (16.3% *vs.* 0.0%; P=0.009) but was associated with more patients showing weight gain (18.4% *vs.* 39.5%; P=0.029). Dasatinib patients showed significantly lower bothersomeness for abnormal kidney function (P=0.031), abnormal liver function (P=0.006) and skin irritation (P=0.039) than nilotinib patients. No differences were reported in AE severity between dasatinib and nilotinib.

Summary and Conclusions: The analysis suggests that patients regard individual AEs associated with dasatinib as equally or less bothersome, and equally or less severe, than the same AEs for other TKIs. Muscle cramps and diarrhea appear to affect dasatinib less than imatinib. Further research is ongoing to assess the impact of these AEs on quality of life and resource use in larger, better-powered samples of dasatanib and nilotinib users.

P1094

EARLY SUPPORTIVE/PALLIATIVE CARE INTEGRATED WITH STANDARD HEMATOLOGICAL CARE (SIMULTANEOUS CARE) IN MULTIPLE MYELOMA PATIENTS

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Background: Patients with multiple myeloma (MM) have a substantial symptom burden at the onset of the disease. We have recently set up a multi-disciplinary team aimed at introducing early supportive/palliative care integrated with standard hematological care (simultaneous care).

Aims: The goal of the current study was to examine the effect of early simultaneous care on health-related quality of life and symptom assessment in MM patients. The primary endpoint was the change in quality of life.

Methods: Quality of life and symptom assessment were evaluated using the M.D. Anderson Symptom Inventory (MDASI: where scores range from 0 to10, with higher scores indicating worse symptoms) at baseline, on days 7 and 28, and every month. The simultaneous care ambulatory team consists of a palliative care physician, a hematologist, a counselor, a psychologist and a social worker. Specific attention was paid to pain, by assessing both physical and psychosocial symptoms, establishing goals of care, assisting in the decision making process regarding treatment, and coordinating care on the basis of the individual needs of the patient.

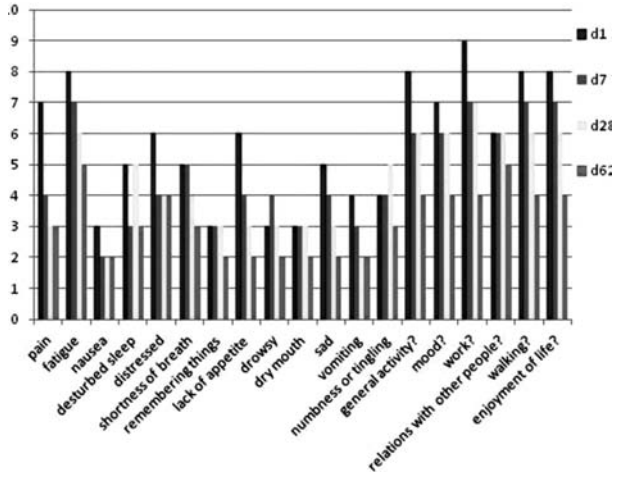

Figure 1.

Results: Between November 2011 and January 2013, 65 MM patients were followed by the simultaneous care team. The clinical characteristics were: median age 67 years (range 40-78); 21 men and 44 women; 40 and 25 patients were, respectively, in Durie and Salmon stages I-II and III. Forty patients were undergoing first line treatment, 13 were in relapse and 17 in advanced stage. The symptoms most frequently reported at baseline were: pain in 83% of patients, fatigue in 92%, anxiety in 67%. The actions taken included pain management in 56 patients; among these, 37% were treated with opioids and NSAIDs, and 63% with strong opioids at the third level of the WHO pain ladder. Oral transmucosal fentanyl delivery technologies were used in 35% of patients for the management of breakthrough cancer pain. Psychosocial interventions were carried out in 18 patients. Among the 17 patients with advanced phase, 12 were assigned to early palliative home care. The MDASI questionnaire scale showed a significant reduction in the median value of all symptoms reported by patients after 2 month (Figure 1).

Summary and Conclusions: Our study demonstrates the feasibility of early palliative care integrated with the standard hematologic care through a multiprofessional team dedicated to the management of symptoms in patients with MM. Based on the reduction in the intensity of symptoms and improvement of the quality of life score, the effectiveness of this type of organization should be emphasized.

P1095

WHAT DO PRELIMINARY ANALYSES HIGHLIGHT AS THE KEY FEARS AND WORRIES OF CHILDREN AND YOUNG PEOPLE WITH SICKLE CELL DISEASE AND WHAT CAN WE DO TO SUPPORT THEM?

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Background: SCD children are now living longer and over 95% survive into adulthood (Telfer *et al.*, 2007; Quinn *et al.*, 2004). As a result, improved survival issues that require psychological input are becoming apparent. Access to a psychology service is not universal for families living with a chronic illness and SCD children may be adversely affected by the inability to address some unique issues they encounter. From audit data and clinical observations it is apparent that despite providing evidenced-based, protocol-driven care to these families, there are gaps in our understanding as clinicians as to what families are most concerned about and opportunities for us to learn from them. In a single centre setting we set out to highlight what these priorities may be and suggest implications and lessons to be learned from them, alongside objectives for future research.

Aims: We qualitatively explore the fears and worries reported by adolescents with SCD and discuss these in relation to team clinical observations of younger children, with the aim of understanding what most affects families living with SCD and how clinicians can best support them.

Methods: The data presented is from a retrospective study using a convenience sample of adolescents attending a London Children's Hospital. A semi-structured questionnaire jointly developed by doctors, psychologists, nurses and teenagers was administered as part of a service evaluation to 41 adolescent sickle cell patients. The questionnaire consisted of 4 sub-sections. For the purpose of this analysis, we focus on the 'impact on life' sub-section, specifically the free-text question, 'What are your fears about having sickle cell disease?' Participants were 24 males and 17 females aged 12 to 19 years (mean 15yr5m). 31 patients had HbSS, 9 had HbSC, and 1 had $HbSb^0$.

Results: 36 adolescents (87.8%) responded to the question in the survey. Of these, 11 adolescents (30.5%) reported not having any fears about SCD. Free-text answers provided were analysed qualitatively using thematic analysis (Braun & Clarke, 2006). Data provided produced 10 codes which led to the emergence of 3 overarching themes (see Figure 1). Themes were interrelated and highlighted a range of fears reported by the adolescents. Most prevalent were fears of death and dying (26.2%), having a crisis (14.63%), stroke (7.31%) and illness (7.31%).

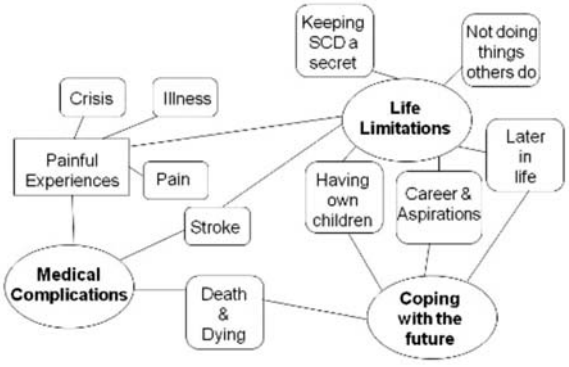

Figure 1. Thematic Map showing 3 main themes to describe adolescents' reported fears of SCD.

Summary and Conclusions: This survey shows that adolescents with SCD have wide ranging fears in relation to medical and psychosocial issues. The adolescents are clearly thinking ahead to their futures and are acutely aware of illness, stroke and death. The results demonstrate a need to incorporate these reported fears into transition planning and provide coping strategies as our adolescents move into adult services. Furthermore, our clinical observations of a younger cohort of patients aged 10-14 attending psycho-educational groups run by the team indicate that younger children also have similar fears and worries to the adolescent cohort, including death and dying, pain, limitations to activities and are also thinking about the future. Clinical observations also highlight parents as sharing similar fears. We propose two objectives for future studies: to examine the direction of the relationship between the fears of parents and children; and to explore the developmental trajectory of reported fears and worries throughout the lifespan. This information would enable us to address such fears both during transition and when determining the support provided to younger children.

P1096

COMPREHENSIVE ANALYSIS OF CLINICALLY RELEVANT IMPROVEMENT IN PROGRESSION-FREE SURVIVAL IN HEMATOLOGIC MALIGNANCY FROM THE VIEWPOINT OF REVISED EMA'S GUIDELINE ON ANTICANCER MEDICINAL PRODUCTS

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Background: Whether prolongation of progression-free survival (PFS) for a short term means clinically significant benefit has been a major concern regarding marketing authorization of some anticancer drugs for solid tumors. The European Medicines Agency's (EMA's) revised guideline on the evaluation of anticancer medicinal products was released this January. Concerning this revised guideline, the Committee for Human Medicinal Products (CHMP) scientific advisory group (SAG) for oncology suggested that improvement in median PFS in the order three to four months or larger is generally considered adequate if the prognosis is in the order of two to three years overall survival (OS) or less.
Aims: Each of the Pharmaceuticals and Medical Devices Agency (PMDA) in Japan and the EMA reviews drug applications for market approval. In order to examine relevance of the EMA's guideline to hematologic malignancy and what clinically relevant improvement in PFS is, the effect of hematologic anticancer drugs approved in the European Union (EU) or Japan on improvement in PFS as the primary endpoint and OS as the secondary endpoint in pivotal trials was analyzed in this research.
Methods: 38 indications of 28 hematologic anticancer drugs approved in Japan after the year of 2000 and 36 indications of 24 hematologic anticancer drugs which were granted central marketing authorization by the European Commission listed in the EMA's website were examined. Information was obtained from the PMDA's review reports and the European public assessment reports.
Results: All the pivotal trials in which the primary endpoint was PFS were randomized comparative trials, as both the EMA's guideline and the FDA's guidance concerning endpoints requested. Of these indications, the pivotal trials in which OS as the secondary endpoint was significantly improved are as follows. Rituximab as maintenance therapy for relapsed or refractory follicular lymphoma (FL) prolonged median PFS for 27.9 months. Rituximab for newly diagnosed chronic lymphocytic leukemia (CLL) extended median PFS for 7.6 months. On the other hand, the pivotal trials in which significantly improved OS as the secondary endpoint was not observed are as follows. Ibritumomab tiuxetan for newly diagnosed FL extended median PFS for 23.5 months. Rituximab for relapsed or refractory CLL prolonged median PFS for 10.0 months. Rituximab as maintenance therapy for newly diagnosed FL extended median PFS for 191 days. Temsirolimus for relapsed or refractory mantle cell lymphoma prolonged median PFS for 2.9 months. In addition, all these indications were approved in the EU but not Japan while bortezomib and lenalidomide for multiple myeloma (MM) approved in Japan as well as the EU prolonged both time to progression (TTP) as the primary endpoint and OS as the secondary endpoint, suggesting that TTP may be worthy to examine in MM.
Summary and Conclusions: There are few trials of hematologic anticancer drugs in which the primary endpoint was PFS and drugs for lymphoma or CLL were examined in these trials. All the drugs examined in this analysis prolonged PFS for more than 80 days, which was considered to be nearly adequate improvement by the CHMP SAG. Therefore, this research suggests that the revised EMA's guideline is relevant to hematologic anticancer drugs and that more efforts to conduct comparative trials are globally necessary. Because this analysis also shows that it is difficult to define a minimum relevant difference in PFS which leads to improved OS, however, it is important to at least rule out a detriment in terms of OS.

P1097

PREGNANCY ROLE IN THE OCCURRENCE OF MALIGNANT HEMATOLOGICAL DISEASES REMISSION

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Background: In recent years treatment of several malignant hematological diseases (MHD) has improved considerably thanks to more profound understanding of the pathogenesis, implementation of new medicines and optimization of accompanying therapies. For this reason the issues pertaining to the realization of reproductive function in this category of patients are becoming an ever more topical subject.
Aims: Pregnancy planning, evaluation of the disease prognosis and creation of protocols for management of pregnancy and childbirth, all this serves to improve the quality of life of such patients. Research Center for Obstetrics, Gynecology and Perinatology has been developing the optimal strategy for management of pregnant patients with MHD.
Methods: From 1986 to 2012, we have analyzed 158 pregnancies in 149 women remaining in remission after MHD, including 117 patients with Hodgkin lymphoma (HL), 19 patients with non-Hodgkin lymphomas (NHL) and 13 patients with acute leukemia (AL). All of them underwent the treatment of hematological disease to achieve remission. The median time from the moment of achieving remission to the onset of pregnancy was 6 years in patients with HL, 3.2 years in patients with NHL and 7.4 years in patients with AL.
Results: In pregnant women with full MHD remission the progression of the disease was registered in 3 (23%) patients with AL and 2 (1.7%) patients with HL. All patients with diagnosed disease relapse needed the resumption of special chemotherapy. 152 (96.2%) of pregnancies ended up with a birth of full-term healthy infants without birth defects. Premature birth occurred in 5 (3.2%) cases. Neonatal mortality occurred in 1 (0.6%) newborn with unfavorable nervous system defect (anencephaly). In 1 (0.6%) patient pregnancy was terminated for medical reasons in the 2nd trimester. The mode of delivery was determined by the obstetric conditions in all cases. No thrombotic or hemorrhagic complications during labor or postpartum period have been observed.
Summary and Conclusions: Therefore, pregnancy does not influence on the progression of lymphoproliferative diseases in patients with long-lasting remission. Patients with lymphomas could be recommended to plan a pregnancy in 3 years after achieving the remission. Pregnant women in remission after AL are at a considerably higher risk for relapse, hence, the pregnancy planning in this category of patients is feasible but no sooner than in 5 years since the moment of achieving full remission, and only after detailed examination including the molecular and cytogenetic methods, in order to rule out the minimal residual disease. There is no difference between the health of infants born by mothers in MHD remission and the newborns from general population.

P1098

VALIDATION OF A COMPREHENSIVE HEALTH STATUS ASSESSMENT SCALE IN OLDER PATIENTS (≥65 YEARS) WITH HEMATOLOGICAL MALIGNANCES. GAH STUDY

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Background: Cancer affects mainly older people. The US National Comprehensive Cancer Network and the International Society of Geriatric Oncology have recommended that some form of geriatric assessment should be conducted to help oncologists to determine the best treatment for older patients, in order to identify current health problems and to guide interventions to reduce adverse outcomes and optimize the functional status. Currently, the main tool for assessing older patients is a comprehensive geriatric assessment, although its complexity and duration may hinder its regular use in daily practice as a tool for clinical decision making. Several attempts have been made to assess comorbidities in the specific field of mielodysplasia, but they focused mainly on organic damage rather than global assessment.
Aims: To develop and validate an assessment scale (called Geriatric Assessment in Haematology, GAH) for use in older patients with hematological malignancies (myelodysplasic syndrome (MDS), acute myeloblastic leukemia (AML), multiple myeloma (MM) and chronic lymphocytic leukemia (CLL), that, while integrating the essential dimensions of geriatric assessment, is easier to apply,

accessible, does not interfere with daily clinical practice and could be useful in clinical decision making.
Methods: After item-pool generation, stakeholder consultation and content validation, a brief scale with selected items of several domains has been created. Feasibilty was confirmed in 83 hematological patients in a previous exploratory experience. After that, a multicenter, observational, prospective study has been started in 18 hospitals in Spain, expecting to enroll 360 treatment-naïve, newly diagnosed patients belonging to one of the three groups of study (MDS or AML, MM and CLL). The scale validation process integrates the analysis of criterion validity, concept validity internal reliability, test-retest reliability, as well as the evaluation of intraclass correlation coefficient (ICC) and factor analysis. After psychometric validation phase, further studies will be carried out in order to evaluate its clinical use for prognosis and clinical decision making.
Results: Currently, 75 patients fulfilling inclusion criteria have been enrolled in the study, 51.5% men, median age at diagnosis 76 years (70-80). According to diagnosis, 44% of patients had MDS or AML, 36% had MM and 20% had CLL. Median time for filling in the questionnaire was 12 (10-15) min. In the initial testing, GAH showed satisfactory test-retest reliability. ICC was statistically significant for each dimension, being greater than 0.66 for 6 of the 8 dimensions ($P<0.05$). Factor analysis will be conducted when recruitment is completed. Some differences have been found in subjects with different diseases, although no statistical analysis has been made yet. Based on rough proportions, GAH shows a great potential in terms of sensitivity, specificity and reproducibility between trained observers.
Summary and Conclusions: To our knowledge, this is the first study performed to develop and validate a comprehensive health status assessment in older patients with hematological malignances, which could be used in clinical practice to help haematologists to improve decision making processes in older patients.

P1099

PSYCHOLOGICAL MORBIDITY FOLLOWING THE DIAGNOSIS OF A HAEMATOLOGICAL MALIGNANCY

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Background: It has been shown that psychological morbidity occurs frequently following the diagnosis of a haematological malignancy. A number of factors may contribute to the development of mental health issues in this patient group including exposure to new medications and prolonged periods of isolation as well as multiple other physical & emotional factors. Cork university hospital (CUH) is one of the largest teaching hospitals in the Republic of Korea Ireland, serving a population of over 1 million people. The Haematology department within CUH acts as a regional tertiary referral centre for the treatment of acute leukaemia and other complex haematological disorders. A specialist psycho-oncology/liaison psychiatry service has been in operation in CUH since 2006. In cases where there is a clinical suspicion of a mental health issue, the clinical haematology team submits a referral directly to the psycho-oncology service, following which the patient is assessed and recommendations for further specific interventions made if appropriate.
Aims: The purpose of this study was to examine the nature of mental health issues among patients that were diagnosed with a haematological malignancy within our institution.
Methods: Data with regard to mental health assessment, diagnosis and therapy were obtained from a database that was compiled prospectively on all referrals to the liaison Psycho-oncology service from August 2006 to August 2011 in CUH.

Table 1.

Psychological assessment/Mental Health diagnosis	% of Patients (N=254)
Declined assessment/ Referral cancelled	8% (20)
Reaction to severe stress/Adjustment disorder	24% (61)
No mental health diagnosis	19% (49)
Stable mental state (history of psychiatric disorder)	9% (23)
Organic mood/anxiety disorders a) Secondary to steroids b) Not secondary to steroids	15% (38) a) 9% (23) b) 6% (15)
Mood and anxiety disorders	14% (36)
Delirium/Dementia	7% (18)
Mental retardation or Autistic Spectrum Disorder	0.8% (2)
Mental disorders due to psychoactive substance use	1% (3)
Not specified	1% (3)
Disorders of adult personality and behaviour	0.4% (1)

Results: During the study period, 254 patients (146 male, 108 female) with a haematological malignancy were referred for assessment to the liaison psycho-oncology service. The mean age at time of referral was 52 (SD+/-15.4, range 19-85) years. The most frequent haematological diagnosis within the cohort was myeloma (27.6%) followed by non-Hodgkin lymphoma (27.1%). Patients with acute leukaemia accounted for 26.4% of the cohort, with the remainder comprising of patients with chronic leukaemias, myelodysplastic syndromes and myeloproliferative neoplasms. The majority of referrals were prompted for assessment of "low mood" (32%), "anxiety/insomnia" (25%), "poor coping" (14%) and for review with regard to a "past psychiatric history" (14%). No referrals were initiated specifically for assessment of suicidal ideation or behaviour, however, one patient was re-referred following an episode of suicidal behaviour on the haematology ward. Each patient was requested to complete a modified Roth distress thermometer questionnaire. The median distress score was 5 (range 0-10). 75% of patients recorded a score of 4 or greater, which has been validated as being highly sensitive and specific for detecting high levels of psychological distress. Table 1 illustrates the outcome of the initial mental health assessments. Following assessment and diagnosis, a recommendation for either further active observation or specific interventions was made in 84% of cases, including medication change (52%), anxiety management/psychotherapy (38%) and psychoeducation (16%).
Summary and Conclusions: Psychological wellbeing is often adversely affected following the diagnosis of a haematological malignancy. Organic mental disorders, including steroid induced mental disorders, represent a significant component of the morbidity observed. Access to specialized psychiatric assessment is vital in order to make an accurate diagnosis and to identify patients who may benefit from specific therapy.

P1100

QUALITY OF LIFE OF THE PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background: The evaluation of quality of life (QOL) is widely used in medical practice, as it allows to assess QOL of patient to get the most complete picture of his health, before starting and during treatment.
Aims: *The Aim* of this study was to determine quality of life of the patients with hematological malignancies (HM) at the time of diagnosis, at the time of chemotherapy (PCT) and during complete clinical remission (CCR).
Methods: Surveyed group were 170 patients (77 patients with non-Hodgkin's lymphoma, 57 - Hodgkin's lymphoma and 36 patients with acute leukemia. Mean age is 44.5±3.75 years. Gender distribution was: women–89 (52.4%) and men–81 (47.6%). The control group consisted of 170 persons, representing the "healthy" population. The average age of respondents was 46±4.6 years: women of them–93 (54.7%) and men–77 persons (45.3%). QOL was estimated by the SF-36.
Results: We noticed a significant reduction of QOL for all investigated scales of the SF-36 in HM group before treatment compared with the control group. During the chemotherapy program QOL of patients with HM were significantly lower compared with the control group on the such scales as: physical functioning (PF)–63.1±2.26 points in the group of patients at the time of chemotherapy and 87,2±1,13 points in the control group, role physical functioning (RFF)–39.4±3.51 points in the patients with HM during treatment and 59.5±2.82 points in the control, general health (GH)–45±1.68 points compared with 56.5±1.35 points respectively, vitality (V)–43±2.09 points in the group of patients at the time of chemotherapy and 58.4±1.54 points in the control group, social functioning (SF)–54.7±2.51 points in HM during treatment and 69.3±2.02 points in the control, role social functioning (RSF)–49.8±3.54 points and 54.5±3,12 points respectively, mental health (MH)–54.5±1.57 points in the group of patients at the time of chemotherapy and 59.7±1.45 points in the control group. At the same time, the analysis of QOL of patients with HM during the course of induction and consolidation of remission showed a significant increase QOL compared with patients before treatment for the pain scale (P) (due to reduction of pain), GH and V: V–66.1±2.36 points in the group of treated patients in comparison with 53.2±2.38 points in the group of patients before treatment, GH–45±1.68 points in the patients during treatment and 39±1, 68 points in the patients at the time of diagnosis, V–43±2.09 37±1.77 points respectively. Comparative analysis of QOL during the therapy and CCR showed the significant increase QOL for all investigated scales of the SF-36, but in comparison with the control group, QOL of the patients with HM were significantly lower for majority of the studied scales: FF–76.2±1.45 points in the patients with CCR and 87.2±1.13 points in the control group, RFF–50±3.25 points in patients in remission and 59.5±2.82 points in the control, GH–44.8±1.77 points and 56.5±1.35 points respectively, V–49.8±1.67 points in patients during CCR and 58.4±1.54 points in the control group, PH–53.5±1,32 points in the patients in remission and 59.7±1.45 points in the control group.
Summary and Conclusions: The study showed the reduction of quality of life for patients with HM before therapy. Chemotherapy and radiation therapy in patients with HM can improve quality of life. During the period of clinical remission QOL was increasing on many studied scales of the SF-36, but the results of QOL on the GH, PH and V scales was significantly lower in comparison with the control group.

SIMULTANEOUS SESSION III

Chronic myeloid leukemia - Clinical

S1101

ULTRA-DEEP SEQUENCING WITH COMPUTED THRESHOLDS FOR SENSITIVE MUTATION ANALYSIS IN THE KINASE DOMAIN OF BCR-ABL: FOCUSED ON DEEP MUTATION DETECTION IN CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE

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Background: Sanger sequencing (SS) is widely used for mutation detection in the BCR-ABL kinase domain (KD) with a sensitivity of 15-20%. Due to its clonal nature, next generation sequencing (NGS) improves resolution through ultra-deep sequencing (UDS) of targeted genes. It is necessary to have tools separating errors produced in NGS pipeline from true mutational events.
Aims: 1) We develop a model for error correction and estimating thresholds for detection of mutations in the KD of BCR-ABL by UDS of an amplicon library that was prepared using BCR-ABL plates developed within the IRON-II study (Roche Applied Science). 2) We applied the thresholds in a retrospective UDS analysis of BCR-ABL mutations associated with resistance under TKI treatment in CP-CML patients to assess applicability to future clinical practice.
Methods: *BCR-ABL*mutations were analyzed in 129 samples from 15 CP-CML patients by UDS on a 454 platform (Roche Applied Science). Each patient received 2 or 3 lines of TKIs. BCR-ABL oligonucleotide primer plates containing fusion primers were used to create 4 partially overlapping amplicons covering the KD coding region. For estimation of SNS (Single Nucleotide Substitution) error rates, we used 8 samples from healthy donors. The SNS caller and software framework for estimation of errors was written in Java. We estimated error rates using the Lea Coulson distribution. The distribution parameter was estimated by the maximum likelihood method (Foster, PL in DNA Repair, 2006, Part B, Vol409). If the probability, that the same or higher count of SNS reads from single base occur in error distribution, is less than 1% then we call this SNS significant mutation. On the level of 1% for each nucleotide substitution we also set a threshold.
Results: 1) The SNS error frequency was much higher for nucleotide transitions *vs.* transversions (*e.g.*, mean 2.2 SNS events for T/C *vs.* 0.1 for T/A per 3000 reads). Therefore, computations for each type of nucleotide substitution were performed separately and resulted in higher thresholds for transitions, resulting in *e.g.* T315I and Y253H with 77 (2.57%) and 96 (3.20%) mutations per 3000 reads, respectively, in contrast to transversions, resulting in *e.g.* V299L with 10 (0.3%) per 3000 reads. 2) Using the computed thresholds UDS revealed insignificant mutations at the time of diagnosis that subsequently developed under TKIs in 8/15 patients. We examined, if UDS can outperform SS in detecting mutations earlier considering significant mutations only. UDS detected 5 mutations 3 months earlier, 4 mutations 4 months earlier and 2 mutations 17 months earlier during imatinib treatment. UDS detected other minor mutant populations (G250R, L387F, L364I, Y253H) in 4 patients. In 3 patients, UDS, but not SS, revealed minor populations with F317L, Y253H and T315I before therapy switch that emerged under selected 2nd TKI. Finally, we compared the level of baseline mutations detected by UDS and SS after TKI switch. Baseline mutations were still detectable by UDS in 2 patients who achieved MMR at the time of analysis.
Summary and Conclusions: Since enzymes create errors during library preparation, the threshold computation is essential for relevant interpretation of BCR-ABL KD mutations detected with the highly sensitive UDS technology. Higher SNS error rate was found for nucleotide transitions. UDS outperformed SS in detection sensitivity and thus provided more information. For future reference, we suggest using UDS even for critical clinical applications provided that the precise methods for error management presented here are utilized.
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S1102

IKAROS DELETION AND LEVELS OF FULL-LENGTH TRANSCRIPT ARE CRITICAL FOR CML BLAST CRISIS TRANSFORMATION

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Background: The *IKZF1* gene encodes Ikaros, a zinc finger protein which acts as a master regulator of lymphoid and myeloid development as well as a tumour suppressor. Deletions of *IKZF1* coding exons 3 to 6 can result in the expression of dominant-negative short forms of *IKZF1* (*Ik6*, *Ik9* and *Ik10*). *IKZF1* deletions have been reported in blast crisis (BC) of chronic myeloid leukaemia (CML).
Aims: To investigate the role of Ikaros as a predictor of BC in CML, we investigated the expression of *IKZF1* variants and the expression levels of the full length *IKZF1* transcript in chronic phase (CP), accelerated phase (AP) and BC.
Methods: The following cases were studied: 53 cases of BCR-ABL1+ acute leukaemia (25 myeloid, 22 lymphoid, 3 biophenotypic and 3 of unknown lineage), 27 cases of AP CML, 52 cases of CP CML who failed imatinib, nilotinib or dasatinib and 25 cases of newly diagnosed CP CML. RNA was extracted and cDNA synthesised from whole blood white cells. PCR was performed using various combinations of primers to detect *IKZF1* variants. Real-time quantitative RT-PCR was applied to quantify the gene expression levels of the full-length *IKZF1* on a LightCycler.
Results: *Ik6/Ik10* were detected in 9/53 (17%) of BCR-ABL1+ leukaemia including 8 cases with *Ik6* and one case with *Ik10*. These were found at a higher incidence in BCR-ABL1$^+$ ALL (7/22; 32%) and biphenotypic BC (1/3; 33%) than in BCR-ABL1$^+$ AML (1/25; 4%). Strikingly, all 9 cases with *Ik6/Ik10* variants had major *BCR-ABL1* transcripts; while none of the 10 cases of *de novo* BCR-ABL1+ ALL with minor or other *BCR-ABL1* variant had *Ik6/Ik9/Ik10*. Analysis of *Ik6/Ik10* in serial CP CML samples prior to BC transformation showed that no *Ik6/Ik10* was detected when patients were responding to treatment. *Ik6/Ik10* was only detected when the patients progressed from CP to BC. No dominant-negative form of *IKZF1* was detected in any CP or AP case. *IKZF1* isoforms *Ik5*, *Ik7* and *Ik8* with no crucial zinc finger motifs were also screened in all samples, but no consistent expression patterns were seen for particular disease subgroups. Surprisingly, expression levels of full length *IKZF1* were greater in BCR-ABL1$^+$ acute leukaemia patients (n=39) than those in CP CML (n=20) (P=0.001; M-W test). For BC cases without shorter dominant-negative *IKZF1* isoforms, the full length *IKZF1* levels were also higher than those in CP CML (P=0.001, M-W test). Expression levels of full length *IKZF1* levels were not significantly different between lymphoid (n=11) and myeloid BC (n=18) of CML, nor between CML BC with and without *Ik6/Ik10* or between imatinib responders and non-responders (n=8).
Summary and Conclusions: This study shows that 1) dominant negative isoforms of *IKZF1* are more prevalent in lymphoid BC of CML than in *de novo* BCR-ABL1$^+$ ALL; 2) BCR-ABL1$^+$ ALL without dominant-negative *IKZF1* isoforms have greater levels of full length *IKZF1* transcript. However, we do not find that IKZF1 dominant negative isoforms are useful in CP CML as a biomarker of BC. Further study is warrantied to investigate the role of full length Ikaros in BC transformation.

S1103

DEEP MOLECULAR RESPONSE (MR4.5) IS REACHED BY THE MAJORITY OF CML PATIENTS, PREDICTS BETTER SURVIVAL, AND IS ACHIEVED FASTER BY OPTIMIZED HIGH DOSE IMATINIB - RESULTS FROM THE RANDOMIZED CML-STUDY IV

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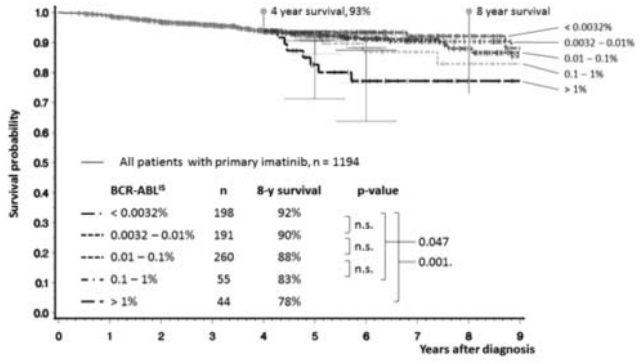
Background: Deep molecular response (MR$^{4.5}$) defines a subgroup of CML patients who may stay in unmaintained remission after treatment discontinua-

tion. It is unclear, how many patients achieve $MR^{4.5}$ and whether $MR^{4.5}$ predicts survival.
Aims: Determine proportion of CML patients who achieve deep molecular responses, analyse predictive power of $MR^{4.5}$for survival, determine whether $MR^{4.5}$is achieved faster by optimized high-dose imatinib.
Methods: Patients from CML-Study IV (Hehlmann *et al.*, JCO 2011, 29:1634-42), a five arm randomized comparison of imatinib in three combinations and two dosages, were analyzed for confirmed $MR^{4.5}$ which was defined as ≥4.5 log reduction of BCR-ABL on the international scale (IS) and determined by RT-PCR in two consecutive analyses. Landmark analyses were performed to evaluate the impact of $MR^{4.5}$on survival at various time points.
Results:1,524 of1,551 randomized patients were evaluable. After a median observation time of 67.5 months 6-year overall survival was 88.2%. The cumulative incidence of $MR^{4.5}$ after 9 years was 70%, of confirmed $MR^{4.5}$ 54%. $MR^{4.5}$ was reached significantly faster with optimized high-dose imatinib than with imatinib 400 mg. Independent of treatment approach confirmed $MR^{4.5}$ at 4 years predicted significant higher survival probabilities than >1% IS (8 years survival 92% *vs.* 78%, P=0.001) and than 0.1–1% IS which corresponds to complete cytogenetic remission (8 years survival 83%, P=0.047, Figure 1). The interim levels of 0.01–0.1% IS defined as major molecular remission (MMR, 8 years survival 88%) and of 0.0032–0.01% IS defined as MR^4 (8 years survival 90%) were not significant compared to $MR^{4.5}$or 0.1-1% IS. Early MMR predicted $MR^{4.5}$. No patient with confirmed $MR^{4.5}$ has progressed.

Figure 1.



Summary and Conclusions: $MR^{4.5}$ is a new molecular predictor of long-term outcome, is reached by a majority of imatinib-treated patients and is achieved faster with optimized high-dose imatinib. Optimized high-dose imatinib may provide an improved therapeutic basis for treatment discontinuation in CML.

S1104

NON-ADHERENCE IN CHRONIC MYELOID LEUKEMIA: RESULTS OF A GLOBAL SURVEY OF 2546 CML PATIENTS IN 79 COUNTRIES

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Background: Optimal adherence to oral chronic myeloid leukemia (CML) therapy is of key importance to maximise treatment effectiveness. Non-adherence as well as its biological effect on CML has been observed in clinical research, but data on potential driving factors of non-adherence are lacking in the scientific literature.
Aims: The aim of this study is to investigate motivations and behavioural patterns of adherence in CML and subsequently support hematologists and patients to improve adherence and develop suitable adherence tools.
Methods: A European workgroup of the CML Advocates Network, a network of leukemia patient groups in 55 countries, has conducted a large international study enrolling patients from 09/2012 to 01/2013 in 12 languages. The study was also supported by CML investigator groups in Germany, Italy and France. An *ad hoc* questionnaire was developed for the purpose of this study and this included questions on potential factors associated with non-adherence and other aspects related to patients' perception of disease and treatment burden. Also, medication taking behavior was assessed with the 8-item Morisky Medication Adherence scale (MMAS-8). This scale allows classification of patients into their level of adherence to treatment (*i.e.* low, medium and high adherence). Patients completed this questionnaire online. An additional sample of patients were given this questionnaire, along with a pre-stamped envelope, by their own treating physicians in hospital with the request to completing it at home. These questionnaire were then returned to an independent Data Center for analyses. Differences in adherence groups were determined by χ^2 tests, differences of mean adherence were determined with Mann Whitney U tests and Kruskal Wallis tests.
Results: Overall 2546 questionnaires filled in by CML patients from 79 countries were analyzed. 2151 were completed online, 395 were returned by patients invited to participate in hospital, all others (N=2151) were filled in online. 52.4% of participants were male. Mean age of participants was 50.4 years (range 18-96) and 52.4% of these were male. According to the MMAS-8, 33% of participants could be classified as highly adherent, while 47% and 21% were respectively in the medium and low adherence group. Several factors influenced adherence: Men were more often highly adherent than women (57.3% *vs.* 42.7%, P<0.001). Mean age was higher in the more adherent groups (low 44.8 years, medium 50.6, high 58.8; P<0.001). Patients who live with a partner or family member (85.7%, P=0.006) are more adherent, suggesting that adherence tools should support not only patients but also their relatives. 27.1% of patients on Nilotinib treatment categorized into the low adherence group, compared to only 18.8% of patients on other CML therapies (P<0.001). This might also be due to the fact that most patients (91.2%) have to take Nilotinib twice a day while Imatinib and Dasatinib are mostly administered only once per day (86.8% and 92.5% respectively). Frequency of medication is a major factor of influence on adherence (P<0.001). 51.6% of participants accidentally missed at least one dose of medication within the last year. Main reasons were forgetting (41%) and interruption in the daily routine (27%). As reminder tools, 43% are reminded by family members, 40% use pill dispensers and 24% use cellphones. The majority of patients (85.7%) are aware of the importance of CML therapy to their health. However 19.5% of patients consciously decided to miss a dose during the last year. Main reasons were not feeling well (35%) and the wish to reduce side effects (26%), of which 79% state gastrointestinal events as the reason. Patients state that CML medication impacts their work life (26%) and their social life (23.5%), which both influence adherence to treatment schedules (P<0.001). Patients managed by an approachable doctor providing sufficient information on disease and medication were more adherent than patients with lower satisfaction with their doctor (P<0.001). Doctor's support in managing side effects was also driving adherence (P<0.001).
Summary and Conclusions: This is the most comprehensive study conducted to date to gain knowledge about motivations behind non-adherence in CML. Better information on disease, medication and management of side effects, supported by hematologists, is key to improve adherence.

S1105

INDIVIDUAL EARLY DYNAMICS OF BCR-ABL TRANSCRIPTS, BUT NOT BCR-ABL TRANSCRIPT LEVELS AT BASELINE PREDICT SURVIVAL IN PATIENTS WITH CHRONIC PHASE CML

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Background: Early assessment of molecular and cytogenetic response at 3 months of imatinib treatment has been shown to predict survival and might trigger treatment intensification in slow responders at risk of treatment failure. The use of Beta-Glucuronidase (GUS) as a reference gene allows a linear measurement of high BCR-ABL transcript levels at diagnosis and after 3 months of treatment. A ratio of 3 month and baseline levels might best reflect individual

response kinetics. The linear transcript scale is a prerequisite for the optimization of cut-off levels as predictive landmarks.
Aims: Evaluation of the prognostic significance of 1) BCR-ABL/GUS at diagnosis, 2) the individual reduction of transcripts given by (BCR-ABL/GUS at 3 months)/(BCR-ABL/GUS at diagnosis), 3) BCR-ABL/GUS at 3 months and 4) the established 10% BCR-ABL/ABL^{IS}landmark.
Methods: A total of 408 patients (pts) were investigated. 58 pts with imatinib onset before initial blood sampling within the study were excluded from the analysis. A total of 350 evaluable patients (median age 52 years, range 17-85, 42% female) were treated with an imatinib-based therapy consisting of imatinib 400 mg/d (n=84), imatinib 800 mg/d (n=146) and combinations of standard dose imatinib with interferon alpha (n=101) and low-dose cytarabine (n=19). Median follow-up was 4.8 years (range 1-10). Transcript levels of BCR-ABL, ABL and GUS were determined by quantitative RT-PCR from initial samples, taken before imatinib onset ("at diagnosis") and 3 month samples. Only patients expressing typical BCR-ABL transcripts (b2a2 and/or b3a2) were considered. Disease progression was defined by the incidence of accelerated phase, blastic phase or death from any reason. A landmark analysis was performed for progression-free survival (PFS) and overall survival (OS) after dichotomizing patients by a cut-off with optimized hazard ratio and sensitivity.
Results: Disease progression was observed in 26 patients (7.4%), 18 of them died (5.1%). The median BCR-ABL/GUS ratio was 15% at diagnosis (0.07-107) and 0.70% at 3 months (0-84) reflecting a decline to the 0.05 fold (1.3 log decline). With regard to the above described parameters the following findings were observed: 1) At diagnosis no prognostic cut-off level could be identified. 2) A reduction to the 0.35-fold of the initial BCR-ABL transcript level at diagnosis (0.46 log reduction) was identified as single best cut-off according to a maximal hazard ratio (HR) of 6.4 for OS and separated a high-risk group of 64 pts (18% of pts, 8-year PFS 77%, 8-year OS 80%) from a good-risk group of 286 pts (82% of pts, 8-year PFS 93%, 8-year OS 94%, P<0.001, respectively). 3) At 3 months, four BCR-ABL/GUS cut-offs with comparably high hazard ratios were identified: 6%, HR 5.6; 10%, HR 6.0; 14%, HR 6.5 and 21%, HR 7.0. The 6% cut-off was used for further analysis due to its superior sensitivity including the largest proportion of high-risk patients. This optimized cut-off separated a high-risk group of 86 pts (25% of pts, 8-year PFS 79%, 8-year OS 83%) from a good-risk group of 264 pts (75% of pts, 8-year PFS 93%, 8-year OS 94%, P<0.001, respectively). 4) When the established 10% BCR-ABL^{IS} landmark at 3 months was investigated, 88 pts were high-risk (25% of pts, 8-year PFS 83%, 8-year OS 86%) and 262 good-risk (75% of pts, 8-year PFS 92%, 8-year OS 93%, P=0.007 for PFS, P=0.008 and HR=3.4 for OS).
Summary and Conclusions: The individual reduction of BCR-ABL transcripts to the 0.35-fold of baseline levels and a 6% BCR-ABL/GUS cut-off at 3 months identify patients at risk more precisely than the 10% BCR-ABL^{IS} landmark.

Myelodysplastic syndromes - Clinical

S1106

IS IT POSSIBLE TO CURE SEVERE APLASTIC ANEMIA REFRACTORY TO IMMUNOSUPPRESSIVE THERAPY WITHOUT TRANSPLANT? A LONG TERM FOLLOW UP ANALYSIS OF A PHASE II STUDY OF ELTROMBOPAG

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Background: About one quarter of patients with severe aplastic anemia are refractory to immunosuppressive therapy. Management of this population is challenging with no standard therapies available. Our group has shown that eltrombopag has good efficacy with minimal toxicity in this setting (Olnes MJ NEJM 2012; 367:11-19). In this pilot, phase II, non-randomized study patients received eltrombopag at a dose of 50 mg with dose escalation every 2 weeks to a maximum of 150 mg. The primary endpoint was improvement in blood counts with defined responses for each lineage. Forty-four per cent (11/25) of patients had a hematological response after 12-16 weeks, with responses in all three lineages seen. The majority of responders became transfusion-independent for platelets (9/11), 6 had erythroid responses with 3 patients previously dependent on red cell transfusion becoming transfusion -independent. Nine of 11 responders had increased neutrophil counts.
Aims: The aim of this analysis is to report updated safety and efficacy data in a larger patient cohort, and ask whether eltrombopag can be discontinued in patients with severe aplastic anemia achieving robust count recovery.
Methods: This current analysis includes 37 patients with refractory severe aplastic anemia, including 25 from the initial cohort and an additional 12 patients, enrolled in a second cohort of 18 patients, who have reached time to response assessment. Patients reaching response criteria at 12-16 weeks were offered continued therapy in an extended access study. Patients with robust blood count recovery (platelets >50,000/ul, Hb >10 gr/dL in the absence of RBC transfusion, and neutrophils >1,000/ul for more than 8 weeks) had eltrombopag tapered and discontinued.
Results: Median age was 44 years (range 18-77). Median number of prior courses of immunosuppression was 2 (range 2-4) and median time since last immunosuppression was 10 months (range 6-55). Thirty-eight per cent (14/37 patients) had a response in at least one lineage: 10 patients had platelet responses; 8 had erythroid responses, and 9 neutropenic patients had increased neutrophil counts. One non-responder who had drug stopped prematurely because of hepatitis B infection was retreated off protocol and attained red cell- and platelet-transfusion independence. Of the 11 patients who entered the extension study, 7 have and continue to maintain trilineage responses. We discontinued study drug because of sustained and robust counts (platelets >50,000uL, Hb >10 gr/dL in the absence of transfusion and neutrophils >1,000 for more than 8 weeks) in 4/7 patients and all have maintained stable counts with a median follow up of 7.5 months (range 7-9) off drug. Five non-responders had cytogenetic evolution, all found at response assessment with 3 developing monosomy7, 1 trisomy 21, and 1 trisomy 8. A 77 year old male who was enrolled in the extended access study with a non-robust response developed monosomy 13 after 14 months on eltrombopag. Baseline reticulocytes counts continued to be predictive of response (45.22 per cubic millimeter in responders *vs.* 24.45 per cubic millimeter in non-responders, P=0.015). There was no increased reticulin noted in bone marrow biopsies performed at response assessment and thereafter every 6 months on drug.
Summary and Conclusions: Eltrombopag is an effective treatment for refractory severe aplastic anemia. Continued exposure to drug may not be required for a sustained remission in those with robust count recovery. As in all patients with BM failure syndromes, serial BM biopsies are recommended to monitor for cytogenetic or morphologic evolution. Patients treated with eltrombopag for marrow failure states should be enrolled in clinical trials to allow data collection regarding risk of progression to clonal hematopoiesis. We are currently investigating patients who evolved with comparative genomic hybridization to assess whether unbalanced chromosomal abnormalities existed prior to treatment.

S1107

A RANDOMISED STUDY OF LENALIDOMIDE (LEN) +/- EPO IN RBC TRANSFUSION DEPENDENT (TD) IPSS LOW AND INT-1 (LOWER RISK) MYELODYSPLASTIC SYNDROMES (MDS) WITHOUT DEL 5Q RESISTANT TO EPO

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Background: ESAs, the first line treatments of anemia in non del 5q lower risk MDS, yield only 40-50% responses. LEN gives RBC transfusion independence (TI) in about 25% of ESA resistant (or relapsing) TD lower risk MDS without del 5q (Raza, Blood, 2008), and a gene expression signature can predict response (Ebert, Plos Med 2008).
Aims: We randomized in this patient population LEN alone and LEN+EPO.
Methods: In this prospective multicenter open-label phase II study (NCT01718379), lower risk MDS patients without del 5q, with TD (≥4 RBC units during the previous 8 weeks (w)) and with ESA resistance or relapse after a response were randomized between LEN alone, 10mg/d × 21 d/4 w (L arm) or LEN (same schedule)+EPO beta, 60 000 U/w (LE arm). The primary endpoint was erythroid response (HI-E, IWG 2006 criteria) after 4 treatment cycles. Secondary objectives included identification of biomarkers of response.
Results: Between July 2010 and June 2012, 132 patients (pts, 66/arm), median age 73 (range 46-88), M/F: 88/44 were enrolled. Median TD was 6 RBC units/8w (range 2-18). IPSS was Low in 45% and Int-1 in 55% pts. Pretreatment characteristics did not differ between the 2 groups. All but 3 pts, who withdrew consent (2L+1LE), were evaluable for response. In this ITT population, HI-E was obtained in 15 pts (23.4%) in L arm and 26 (40.0%) in LE arm (RR=1.7, P=0.043, chi2 test), and TI in 9 (14.1%) *versus* 16 (24.6%) pts (RR=1.7, P=0.13). In the 99 pts who completed 4 treatment cycles, 41 achieved HI-E, including 15/49 (30.6%) in L arm *versus* 26/50 (52.0%) in LE arm (P=0.03), and TI in 9 (18.4%) *versus* 16 (32.0%) pts (RR=1.7, P=0.12). Side effects (cytopenias and 1 DVT/arm) were similar in the 2 arms. A 29-gene expression profile signature predicting HI-E to L or LE, different from that previously published, was identified and a polymorphism in the CRBN gene (Kosmider, submitted) was significantly associated with HI-E in the entire cohort (P=0.034)
Summary and Conclusions: LEN+EPO yielded a significantly better erythroid response than LEN alone in lower risk MDS patients with anemia resistant to ESA alone. A gene expression signature and a CRBN gene polymorphism correlated with the erythroid response.

S1108

PLACEBO-CONTROLLED, RANDOMIZED, PHASE I/II TRIAL OF THE THROMBOPOIETIN RECEPTOR AGONIST ELTROMBOPAG IN THROMBOCYTOPENIC PATIENTS WITH ADVANCED MYELODYSPLASTIC SYNDROMES OR ACUTE MYELOID LEUKEMIA

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Background: Patients (pts) with advanced myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) often develop platelet (plt) transfusion-dependent thrombocytopenia. Eltrombopag (EPAG), an oral thrombopoietin receptor agonist, increases plts in chronic immune thrombocytopenia, hepatitis C virus-associated thrombocytopenia, and severe aplastic anemia.
Aims: To evaluate the safety and tolerability of EPAG in thrombocytopenic pts with advanced MDS and AML (primary end point). Secondary end points include plt transfusions, plt response, and overall survival (OS).
Methods: Pts with relapsed/refractory MDS or AML ineligible for antileukemic therapies, with 10%>50% bone marrow (BM) blasts and plts <30 Gi/L were randomized 2:1 to EPAG 50 mg qd (increases q2 weeks in pts without a plt response, up to 300 mg [150 mg for Asian pts]) or placebo (PLB) for 6 months. Standard supportive care and disease-modifying treatments were permitted at the investigator's discretion.
Results: Overall, 98 pts were enrolled (EPAG: n=64; PLB: n=34). Most pts had AML (Table 1) and received ≥1 prior antileukemic treatments, including hypomethylating agents (EPAG: 24 [38%]; PLB: 11 [32%]) and chemotherapy (EPAG: 10 [16%]; PLB: 3 [9%]). Most pts received the maximum dose (EPAG: 36 [56%]; PLB: 20 [59%]). Mean treatment duration was 102 days for EPAG and 78 days for PLB; 9 (14%) pts on EPAG continued treatment >6 mo *versus* 1 (3%) pt on PLB. Twenty-one (33%) EPAG and 17 (50%) PLB pts died on therapy or <30 days from the last dose; primary cause of death in both arms was underlying disease. The most common (≥20% in the EPAG arm) adverse events (AEs) on therapy +30 days were pyrexia, nausea, diarrhea, fatigue, decreased appetite, and pneumonia. Serious AEs in ≥5% of pts in either arm included sepsis, pyrexia, febrile neutropenia, and pneumonia. Hepatobiliary events were reported in 11 (17%) EPAG and 5 (15%) PLB pts; 3% (EPAG: 2; PLB: 1) reported thromboembolic events. Of 26 pts with MDS (WHO criteria) at baseline, 14 (EPAG: 9; PLB: 5) had postbaseline BM examination results available; 8 (EPAG: 5 [56%]; PLB: 3 [60%]) of 14 pts developed BM blasts ≥20% during treatment. Plt transfusion independence for ≥8 weeks was reported for 24 (38%) EPAG and 7 (21%) PLB pts (*P*=0.0979). Ten (16%) EPAG and 9 (26%) PLB pts had ≥Grade 3 hemorrhages (*P*=0.1472). More EPAG *versus* PLB pts started antileukemic/palliative treatment during the study (26 [41%] *versus* 11 [32%], respectively), including hypomethylating agents and salvage chemotherapy. Median OS was 27 weeks for EPAG *versus* 15.7 weeks for PLB (hazard ratio=0.71, *P*=0.1931). During treatment weeks 5-12, fewer pts receiving EPAG experienced clinically relevant thrombocytopenic events (plt counts <10 Gi/L, plt transfusions, or ≥Grade 3 hemorrhagic events) per week than PLB (weighted average [range]: EPAG: 38% [30%>48%]; PLB: 66% [56%>88%]).

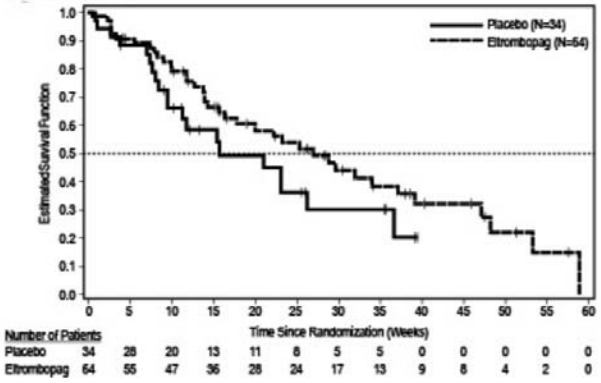
Table 1.

Baseline Disease Characteristics

	Placebo (N=34)	Eltrombopag (N=64)
WHO criteria, n (%)[a]		
MDS	11 (32)	15 (23)
AML	22 (65)	48 (75)
French-American-British criteria n, (%)[b]		
MDS	15 (44)	23 (36)
AML	19 (56)	41 (64)
Poor prognosis karyotype, n (%)	14 (41)	19 (30)
Received prior treatments, n (%)[c]	21 (62)	46 (72)
	Median (range)	
Absolute neutrophil count, Gi/L	0.55 (0-9.8)	0.85 (0-17.6)
Hemoglobin, g/dL	8.5 (6.0-11.2)	8.8 (4.3-13.2)
Platelets, Gi/L[d]	12 (2-38)	17 (2-71)
% BM blasts	20 (10-50)	26 (10-50)

[a]Two pts (1 PLB, 1 EPAG) had missing information. [b]FAB criteria assessed by local morphology review. [c]Excludes palliative treatments (eg, hydroxyurea). [d]Baseline platelet count was derived using an average of platelet counts during screening, excluding within 3 days of a transfusion.

Overall Survival



Summary and Conclusions: EPAG ≤300mg was well tolerated in pts with advanced MDS or AML. Pts treated with EPAG showed a trend toward fewer plt transfusions, fewer ≥Grade 3 hemorrhages, and improved OS compared with PLB. Additional studies with EPAG to evaluate potential antileukemic activity in advanced MDS or AML are warranted.

S1109

PROGNOSTIC VALUE OF DISTINCT ABERRANCIES IN ANTIGEN EXPRESSION AS ASSESSED BY MULTIPARAMETER FLOW CYTOMETRY IN PATIENTS WITH SUSPECTED MDS

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Background: Multiparameter flow cytometry (MFC) is increasingly used to diagnose myelodysplastic syndromes (MDS). The prognostic impact of distinct aberrancies in antigen expression remains to be clarified yet.
Aims: Evaluate the respective impact of distinct aberrancies in antigen expression on overall survival (OS) in pts with suspected MDS in relation to cytomorphology (CM) and cytogenetics (CG).
Methods: 804 pts with suspected MDS were analyzed in parallel by CM, CG and MFC (median age 70 yrs, range 2-89; median OS 6.2 yrs, median follow-up 3.2 yrs). Results of CM indicated MDS in 493 (61.3%) pts; in 170 (21.1%) pts CM revealed evidence of dysplasia but which was not sufficient to diagnose MDS; CM excluded MDS in 141 (17.5%) pts. Karyotypes were good/intermediate/poor according to IPSS in 684 (85.1%)/89 (11.1%)/31 (3.9%) pts. MFC was performed following ELN recommendations (Westers, Leukemia 2012) in myeloid progenitor cells (MPC), granulocytes, monocytes and erythroid cells. MFC parameters included increased or decreased antigen expression, expression of normally not expressed antigens, aberrant expression pattern of antigen pairs and expression of lymphatic antigens.
Results: The first set of analyses is based on the total of 804 pts, *i.e.* regardless of confirmation of MDS by CM. 11 MFC parameters were significantly associated with OS: MPC >5% (P<0.001, hazard ratio (HR) 2.5), expression of CD5 (P<0.001, HR 4.1), CD56 (P=0.043, HR 2.0), CD7 (P=0.015, HR 2.2) in MPC; reduced side-scatter signal (P<0.001, HR 1.9), aberrant CD13/CD16 expression pattern (P=0.007, HR 1.4), aberrant CD11b/CD16 expression pattern (P=0.003, HR 1.6), CD56 expression (P<0.001, HR 2.1), reduced CD33 expression (P=0.018, HR 1.6) in granulocytes; CD56 expression (P<0.001, HR 1.6) in monocytes; reduced CD71 expression (P<0.001, HR 2.1) in erythroid cells. A flow score was devised calculating for each pt the sum of all HRs for the respective parameters found positive. Pts were separated into 4 groups: group 1 (n=263 pts), score of 0; group 2 (n=259), score >0 and below the median (2.1); group 3 (n=149), score above the median and below the 75th percentile (5.0); group 4 (n=133), score above the 75th percentile. 4-yr-OS in groups 1/2/3/4 was 82.4%/67.1%/54.7%/36.2%, respectively (P=0.001 1 *vs*2, P=0.022 2 *vs*3, P=0.003 3 *vs*4, P<0.001 all other comparisons). Cox analysis of MFC score revealed a significant association with OS (P<0.001, HR 1.4 per group). Other parameters univariately related to OS were: diagnosis of MDS by CM (P<0.001, HR 2.2),% bone marrow blasts by CM (P<0.001, HR 1.9 per 10% increment), cytogenetic IPSS-group (P<0.001, HR 5.1 per group), WBC count (P<0.001, HR 1.2 per 10 G/L increment), hemoglobin level (P<0.001, HR 0.8 per g/L), platelet count (P<0.001, HR 1.4 per 100 G/L increment), and age (P<0.001, HR 1.5 per decade). Multivariate Cox analysis including flow score and established diagnostic markers as covariates revealed an independent impact on OS for all of them: flow score (P<0.001, HR 1.3), diagnosis of MDS by CM (P=0.003, HR 1.4), bone marrow blasts by CM (P=0.015, HR 1.4), and cytogenetics (P<0.001, HR 2.9). The flow score was still indenpendently related to OS when age and peripheral blood counts were included as covariates. The second set of analyses was performed in subgroups of patients according to results of CM. In cases with MDS proven by CM flow score was confirmed independently associated with OS (P=0.001, HR 1.2). Interestingly, even in cases with signs of dysplasia by CM, which are not sufficient to diagnose MDS, the 4 groups derived from the flow score differed significantly in OS (2-year-OS 85.9%/76.4%/73.0%/62.9%, P=0.024), Furthermore, multivariate analysis confirmed the independent impact of the flow score on OS (P=0.016, HR 1.3) within this cohort.
Summary and Conclusions: MDS-related findings by MFC in MDS provide prognostic information not just in pts with MDS proven by CM but also in a comprehensive cohort of pts being diagnosed for suspected MDS. Furthermore, even in pts with evidence of dysplasia not sufficient to diagnose MDS by CM a prognostic impact of MFC was demonstrated. This data thus suggests to integrate MFC into the diagnostic work-up of pts with suspected MDS.

S1110

ELTROMBOPAG FOR THE TREATMENT OF THROMBOCYTOPENIA OF LOW AND INTERMEDIATE-1 IPSS RISK MYELODYSPLASTIC SYNDROMES: RESULTS OF A PROSPECTIVE, RANDOMIZED, TRIAL

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Background: Low and Int-1 International Prognostic Scoring System (IPSS) risk MDS patients may experience severe thrombocytopenia associated with risk of hemorrhage. Eltrombopag is an oral agonist of the thrombopoetin-receptor (TPO-R) indicated for treating chronic immune thrombocytopenic purpura. Eltrombopag's potential in increasing PLT counts in lower risk MDS has not been evaluated. We present interim results of a Phase II, multicentre, prospective, placebo-controlled, single-blind study (EQoL-MDS).
Aims: Primary endpoints are safety and efficacy of eltrombopag in low and intermediate-1 IPSS risk. Secondary endpoints include changes in quality of life (QoL), PLT transfusion requirement, incidence and severity of bleeding, and survival.
Methods: Adult patients (N=171) are being included if: PLT<30 Gi/L; ECOG performance status <4; ineligible for, relapsed or refractory to other treatments; and naive to TPO-R agonists. Eltrombopag/placebo (2:1) will be administered at a 50 mg daily starting dose with 50 mg increases every 2 weeks to maximum 300 mg to target PLT 100 Gi/L. Dose interruptions or reductions are required for PLT >200 Gi/L or adverse events. PLT response is defined as Response (R) if: 1) baseline PLT>20 Gi/L: absence of bleeding and PLT≥50Gi/L; 2) baseline PLT<20 Gi/L: PLT>20 Gi/L and increase by at least 100%, not due to PLT transfusions; and Complete Response (CR) if PLT≥100 Gi/L and absence of bleeding. Peripheral and bone marrow blood morphology (centralized and blind) and cytogenetics are performed throughout the study. QoL scores are evaluated by EORTC QLQ-C30 and QOL-E instruments.
Results: Thirty-one patients (21 on eltrombopag–Arm A), have been randomized and 5 are in screening at the time of this report. Mean age is 66 (SD 12) years, male/female 18/13. Baseline mean PLT count was 16 (SD 8) Gi/L. Three cases in Arm A and 1 in Arm B had significant bleeding requiring PLT transfusions. Fifteen patients have reached a 12-week follow-up: 12 out of 15 cases in Arm A have obtained PLT responses at median 100 mg dosing associated with disappearance of bleeding and PLT transfusion independence. There were no responses in Arm B. At 12 weeks, PLT count increased from baseline by mean 64 (SD 77) Gi/L (P=0.006) in Arm A *versus* no significative changes in Arm B. Fatigue and general QoL improved significantly from baseline in Arm A. Grade III-IV unrelated adverse events occurred in 4 patients in Arm A (infectious complications, arthritis, heart diseae). Bone marrow blasts reduced in 3 cases during treatment in Arm A, *versus* 2 progressions in Arm B.
Summary and Conclusions: Preliminary results in low and Intermediate-1 risk IPSS MDS patients with thrombocytopenia suggest safety and efficacy of eltrombopag in terms of response rates and improvements in quality of life.

S1111

LONG-TERM OUTCOMES FROM A PHASE 3 STUDY COMPARING RUXOLITINIB WITH BEST AVAILABLE THERAPY (BAT) FOR THE TREATMENT OF MYELOFIBROSIS (MF): A 3-YEAR UPDATE OF COMFORT-II

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Background: Ruxolitinib is a potent JAK1/JAK2 inhibitor that has demonstrated rapid and durable improvements in splenomegaly, MF-related symptoms and quality of life in the 2 phase 3 COMFORT studies. COMFORT-II is a randomized, open-label study comparing ruxolitinib with BAT in patients (pts) with MF. Two-year follow-up of COMFORT-II confirmed that spleen volume reductions were sustained and ruxolitinib treatment remained tolerable with long-term use. This 3-year follow-up presents longer-term survival data and updates the long-term efficacy and safety findings of COMFORT-II.
Aims: To update the efficacy and safety findings of COMFORT-II with 3 years of follow-up (cutoff date: 01 Dec 2012).
Methods: Patients (n=219) with intermediate-2 or high-risk MF were randomized (2:1) to receive ruxolitinib (15 or 20 mg bid, based on baseline platelet count [100-200 or >200×10^9/L, respectively]), or BAT. Efficacy results are based on an intention-to-treat analysis. A spleen response was defined as a reduction ≥35% from baseline in spleen volume; a loss of response was defined as a spleen volume increase >25% over on-study nadir that was no longer a ≥35% reduction from baseline. Overall survival was estimated using the Kaplan-Meier (KM) method.

Results: After 3 years (median follow-up, 151 wk), 45.2% of patients in the ruxolitinib arm remain on treatment. All patients randomized to BAT discontinued treatment: 45 pts (61.6%) crossed over to receive ruxolitinib with 22 (48.9%) of these pts ongoing in the extension phase. Primary reasons for study discontinuation were AEs (16.4%) and disease progression (15.1%) in the ruxolitinib arm and withdrawal of consent and other (12.3% each) with BAT. The median duration of exposure was 136 wk to ruxolitinib (randomized and extension phases) and 45 wk to BAT (randomized treatment only). Overall, 75 pts (51.4%) treated with ruxolitinib achieved a ≥35% reduction from baseline in spleen volume, and 6 of these pts achieved a ≥35% reduction after the primary analysis at 48 wk. Reductions in spleen volumes were sustained with continued ruxolitinib therapy (median duration not yet reached; Figure 1). Overall, 51 patients died: 29 (19.9%) in ruxolitinib arm and 22 (30.1%) in BAT arm; there was a 52% reduction in risk of death in the ruxolitinib arm compared with BAT (HR=0.48; 95% CI, 0.28-0.85; log-rank *P*=.009 [unadjusted for multiple comparisons]; Figure 2). The estimated probability of being alive at 144 wk was 81% in ruxolitinib arm and 61% in BAT arm. With longer treatment duration (median exposure: ruxolitinib, 136 wk; BAT, 45 wk), there were no newly reported or unexpected AEs among those that were most common. Consistent with ruxolitinib's mechanism of action and previous reports, the most common AEs were anemia (ruxolitinib, 50.0%; BAT, 16.4%) and thrombocytopenia (50.7%; 13.7%). The most common non-hematologic AEs were peripheral edema (ruxolitinib, 36.3%; BAT, 28.8%), diarrhea (32.2%; 17.8%), asthenia (24.0%; 12.3%), dyspnea (23.3%; 20.5%), pyrexia (24.0%; 9.5%) and fatigue (23.3%; 11.0%). Five (3.5%) pts and 4 (5.5%) pts developed leukemia in the ruxolitinib and BAT arms, respectively.

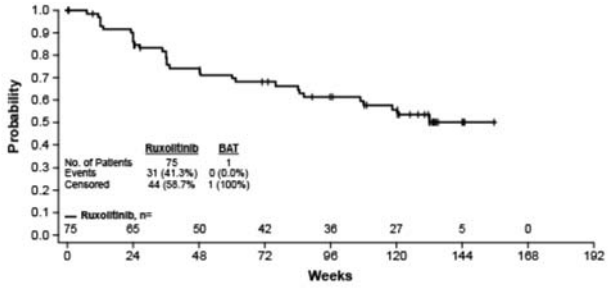

Figure 1. Kaplan-Meier analysis of maintenance of spleen response with Ruxolitinib treatment.

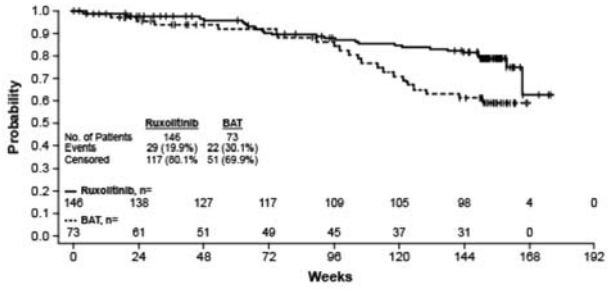

Figure 2. Kaplan-Meier analysis of overall survival.

Summary and Conclusions: This long-term analysis shows that ruxolitinib continues to be well tolerated and provides rapid and durable reductions in splenomegaly that are sustained for ≥3 years of treatment. Ruxolitinib-treated patients showed a longer survival over those receiving BAT, consistent with the survival advantage observed in previous analyses of COMFORT-II (Cervantes *et al. Blood.* 2013*)* and COMFORT-I (Verstovsek *et al. NEJM.* 2012).

Myeloproliferative neoplasms - Clinical

S1112

IMETELSTAT: A NOVEL APPROACH WITH ROBUST HEMATOLOGIC AND MOLECULAR RESPONSES IN A PHASE 2 STUDY IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET) WHO ARE REFRACTORY OR INTOLERANT TO PRIOR THERAPY

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Background: Myeloproliferative neoplasms (MPNs), such as essential thrombocythemia (ET), are driven by neoplastic progenitor cells. Telomerase is upregulated in neoplastic progenitor cells and sustains indefinite replication. Imetelstat is a first in class, potent, specific inhibitor of telomerase. *In vitro* studies suggest that imetelstat selectively inhibits spontaneous CFU-megakaryocyte growth from the blood of pts with ET but not from healthy individuals. In addition, imetelstat inhibited the proliferation of neoplastic clonogenic cells in ET pts. An update of the ongoing phase 2 study is presented here.
Aims: Primary endpoint was best overall hematologic response (HR) with complete response (CR) and partial response (PR) defined as platelet (plt) counts ≤400E3/µl and ≤600E3/µl (or >50% reduction from baseline), respectively, for ≥4 consecutive weeks (wks) in the absence of new thromboembolic events. A key secondary endpoint was molecular response (MR), which includes measurement of the JAK2V617F allele burden by allele-specific quantitative real-time PCR.
Methods: This study enrolled pts with ET who had failed or were intolerant to ≥1 prior therapy, or refused standard therapy. During the induction phase, pts were treated with imetelstat 7.5 mg/kg or 9.4 mg/kg IV weekly until attainment of plt count approximately 250-300E3/µl. Maintenance phase was then commenced with dosing titrated to plt count.
Results: As of 11 Jan 2013, 18 pts with ET were enrolled of which 16 (7 JAK2V617F-positive pts) were treated. Median age and yrs since initial diagnosis were 60 yrs and 7.2 yrs, respectively. Median initial plt count was 832E3/µl (range 521-1359E3/µl). Best overall HR was 87.5%, with 14 of 16 pts achieving confirmed HR (13 CR, 1 PR) after a median of 6.1 wks (range 5.1-14.1 wks). Both pts without HR received ≤3 cycles of imetelstat. Hematologic disease progression (PD) is defined as having plt count >600E3/µl for 2 consecutive cycles on the maximum 11.7 mg/kg/wk dose. Despite some fluctuations in plt count, 15 of 16 pts remain on imetelstat therapy (median time 33.1 wks) without PD, including five pts with >1 yr of treatment. Dosing frequency on maintenance therapy was generally reduced with time. A decrease in JAK2V617F allele burden was observed in 6 of 7 JAK2-positive pts (see Table 1). Five pts had a partial MR (≥50% reduction of JAK2V617F allele burden from baseline) with a 2-22 fold decrease within 3 months. One pt reached a partial MR after 12 months and no pt lost partial MR (up to 1.5 yrs treatment) despite less frequent imetelstat dosing. Long-term administration of imetelstat was generally well tolerated. Common adverse events reported were mild to moderate gastrointestinal toxicities, cytopenias, and fatigue. Early onset self-limiting grade 1 increases in alanine aminotransferase were often observed with concurrent grade 1 increases in aspartate aminotransferase, though the latter often persisted with treatment. With longer dosing new onset grade 1 increases in alkaline phosphatase were observed in 7 of 16 pts, associated with unconjugated hyperbilirubinemia (up to grade 2) in 4 pts.

Table 1.

Pt #	Baseline	3 mo	6 mo	9 mo	12 mo	15 mo	18 mo
1	26.4%	***7.7%***	6.1%	1.8%	8.3%	1.0%	2.6%
2	53.3%	52.2%	43.7%	44.5%	44.6%	43.4%	40.7%
3	10.8%	11.2%	14.3%	6.5%	***4.4%***	3.0%	
4	31.1%	***14.8%***	5.6%	n/a	6.5%		
5	10.8%	***1.2%***	3.0%	0.9%			
6	19.7%	***5.7%***	6.2%	3.5%			
7	26.7%	***1.2%***					

n/a: not available
Italic highlight: first tested time point with a partial molecular response

Summary and Conclusions: Imetelstat induced rapid and complete HR in 13 of 16 pts with ET who failed or were intolerant to conventional therapies while having acceptable safety and tolerability. All pts who achieved HR continue on imetelstat without PD. Six of 7 pts with JAK2V617F achieved and maintained a partial MR throughout treatment, confirming durable inhibition of the neoplastic clonogenic cells. Therefore, telomerase inhibition by imetelstat could have the potential to modify the underlying biology of MPNs. Additional and new data on pts with ET and polycythemia vera, respectively, will be presented from this ongoing study.

S1113

UPDATED RESULTS FROM A RANDOMIZED PHASE 2 DOSE-RANGING STUDY OF THE JAK2-SELECTIVE INHIBITOR SAR302503 IN PATIENTS WITH INTERMEDIATE-2 OR HIGH-RISK MYELOFIBROSIS (MF)

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Background: We previously reported that treatment with 3 cycles of the JAK2-selective inhibitor SAR302503 at doses of 300, 400, or 500 mg reduced splenomegaly and disease-related symptoms with acceptable toxicity in patients with MF enrolled in a phase 2 study (NCT01420770; *Blood* 2012;120:21 Abs 2837).
Aims: We assessed efficacy and safety in this study after 6 treatment cycles (24 weeks).
Methods: Patients ≥18 years old with intermediate-2 or high-risk primary, post-polycythemia vera, or post-essential thrombocythemia MF and splenomegaly (length ≥5 cm by palpation) were eligible. SAR302503 was administered orally, once daily as consecutive 4-week cycles until disease progression or unacceptable toxicity. Spleen response (≥35% reduction in spleen volume *vs* baseline) was assessed by MRI/CT with blinded independent central review. Symptom response was defined as either a 2-point improvement or resolution of the symptom. All patients provided written informed consent.
Results: A total of 31 patients were enrolled (300 mg n=10; 400 mg n=10; 500 mg n=11). Risk status was balanced in all but the 300 mg group (70% high risk). Most patients were *JAK2*V617F positive (90%) and blood transfusion independent (81%). Spleen response rates at the end of 6 cycles of treatment (secondary endpoint) were 30% (3/10) in the 300 mg group, 60% (6/10) with 400 mg, and 55% (6/11) with 500 mg compared with end of cycle (EOC) 3 rates of 30%, 50%, and 64%, respectively. One patient in the 500 mg group who had a spleen response at EOC 3 (39% reduction) but not at EOC 6 (25% reduction) had dose reductions to 200 mg due to anemia. The median number of cycles was 13 (range 2–17) and 24 patients have been on treatment for >12 months. SAR302503 reduced baseline constitutional symptoms at EOC 3 in all dose groups, with the greatest symptom responses seen for night sweats in 14/15 patients (93%), itching 10/14 (71%), early satiety 10/18 (56%), and abdominal pain 10/18 (56%). Most common adverse events across all doses were anemia and diarrhea, with grade 3/4 rates of 58% and 13%, respectively. The incidence of diarrhea tended to decrease after the first 2 treatment cycles. The rate of grade 3/4 thrombocytopenia was 16%. There was no grade 3/4 neutropenia. There have been no reports of withdrawal syndrome after stopping SAR302503. Median *JAK2*V617F allele burden was 93% at baseline, 87% at EOC3, and 78% at EOC 6 in 19/26 patients with all available samples. The expression of 22 of 97 cytokines was significantly regulated (≥1.5-fold difference; P<0.05) after cycle 1.
Summary and Conclusions: In this phase 2 trial, continued treatment with SAR302503 was associated with clinically meaningful reductions in splenomegaly. SAR302503 was generally well tolerated in this trial and adverse events were consistent with the known safety profile. Updated symptom and long-term outcome data will be reported at the meeting.

S1114

LONG-TERM OUTCOMES IN 62 PATIENTS TREATED WITH PEGYLATED INTERFERON ALPHA2A: EXPERIENCE OF THE FIM.

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Background: To date, the only curative treatment for patients with myelofibrosis is still allogeneic stem cell transplantation. Few of palliative therapies could act on both proliferative and cytopenic phases of the disease. Pegylated Interferon α2a (Peg-Ifn α2a) seemed able to do so *(Ianotto et al, 2009 and Ianotto et al, 2013 under submission)*. In a recent study, the French Intergroup of Myeloproliferative Diseases (FIM) proved the real efficiency of Peg-Ifn α2a by proceeding of an analysis of FIM (France Intergroupe des Syndromes Myéloprolifératifs) cases.
Aims: We reported here the long-term analysis of 62 identified patients treated for primary or secondary myelofibrosis and treated with Peg-Ifn α2a.
Methods: From December 2006 to April 2011, we identified 62 patients treated with Peg-Interferon-α2a in 19 different French and Belgian centres affiliated to the FIM. Characteristics of the patients were collected at diagnosis, clinical and biological parameters were collected every 3 months: doses of Ifn, palpable spleen size, constitutional symptoms, transfusion needs, leukocytes, myelemia, haemoglobin, platelets, circulating CD34+ cells, erythroblasts and quantification of $JAK2^{V617F}$ allele burden. Responses were assessed by the International Working Group for Myelofibrosis Research and Treatment criteria (IWG-MRT).
Results: The patients were aged from 33 to 81 years at the beginning of the Peg-Ifn α2a and were 36 males and 26 females. Twenty-nine of them were primary myelofibrosis, 19 were post-PV and 14 post-ET myelofibrosis. Forty patients were $JAK2^{V617F}$ positive. Forty-two patients received previous cytoreductive treatment (almost hydroxyurea). Concerning the best global results obtained, the reduction of the spleen size was observed in 46% (with 50% of complete response: CR), the debiliting symptoms disappeared in 82%, leukocytes abnormalities were reduced from 73% (100% of CR), anaemia was reduced in 64% (91% of CR), platelets abnormalities were reduced from 78% (90% of CR). The median duration of the study was 34.5 months for the global population and 26.5 months for patients still on therapy. At the time of the study-point, 25 patients were still under Peg-Ifn α2a among the 42 patients still alive. Twenty patients died during the study, almost from GVHd (5), evolution of myelofibrosis (4) or acute leukaemia (4). Among the 25 patients still under Peg-Ifn α2a, we did not seen any global CR because of the absence of bone marrow biopsy (at this time), we observed 40% of PR, 30% of CI and 15% of SD. Thirty-seven patients stopped the drug due to evolution of myelofibrosis (43%), cytopenia (16%) or psychiatric side effects (16%). The initial median dose of Peg-Interferon-α2a was 135µg/wk and decreased to 45µg/wk at the time of the evaluation.
Summary and Conclusions: We report here the results of the largest study of patients with primary and secondary myelofibrosis treated with Peg-Ifn α2a. We could observe that this drug is effective and safety in this indication, even for old patients. The positive effects could be maintained over years.

S1115

SYNERGISTIC EFFECT OF RUXOLITINIB AND PANOBINOSTAT TO OVERCOME DRUG RESISTANCE RELATED TO BM STROMA MICROENVIRONMENT IN PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background: The $JAK2^{V617F}$ constitutively deregulate signaling of the JAK/STAT pathway conferring proliferative and survival advantages in the chronic $Ph^{neg.ve}$Myeloproliferative Neoplasms (MPNs). MPNs do not evolve merely from hematopoietic progenitor cells intrinsic defects; rather they are heavily influenced by genetic and epigenetic events affecting the Bone Marrow (BM) niche. Many drugs target different pathways critical for MPN development, like JAK inhibitorruxolitinib, known to decrease spleen size and alleviate constitutional symptoms in myelofibrosis (MF). Other drugs work through remodeling of chromatin with a key role in epigenetics, like the pan-histone deacetylase inhibitor panobinostat. This drug in phase I study for patients with MF, showed to be clinically active, regardless of JAK2 V617F status.However several lines of evidence suggest that in MF, stromal cells are primed by the malignant hematopoietic clone, which, in turn, influences the stroma to create a "favorable" pathologic microenvironment that nurtures and protects the malignant cells. Then, humoral factors secreted by BM niche, may protect MPNs clones from JAK2 or HDAC inhibitortherapy.

Aims: In this study, we evaluate whether combination therapy with HDAC and JAK2 inhibitors has a potential value to overcomethe "protective effects" of the stroma BM on MPN cell lines or primary CD34+ progenitor cells derived from MPN patients.
Methods: $JAK2^{V617F}$ tumor cell lines HEL and SET2 were maintained in culture with RPMI medium, defined as regular media (RM). The human stroma cell line HS-5 serum-free supernatant, named HS5/Stroma Conditioned Media(HS5/SCM), was used to mimic BM microenvironment. Cell lines were incubated for 72hrs in the presence of panobinostat or/and ruxolitinib before the specified analysis. Apoptotic rate was evaluated by Annexin-V staining. Cell cyclewas assessed by BRdU Assay. IC50 was calculated based on the level of viable cells residual after treatment with increasing doses of drugs in the presence or absence of HS-5/SCM.
Results: Treatment with panobinostat or ruxolitinib induced a significant apoptosis in SET2 and HEL cells in a dose-dependent manner. Indeed, when SET2 cells are treated with 30nM panobinostat or 300nM ruxolitinib in the presence of HS-5/SCM, the drug-related apoptosis is significantly reduced (40% ±18% and 30%±8%, respectively) respect to cell line treated in RM (79% ±15% and 58%±12%, respectively; $P<0.05$). Similar results have been achieved for HEL cell line. Serial dilutions of HS-5/SCM, ranging from 50% to 0.1%, showed that the resistance was dose dependent. The IC50 of SET2 cells treated with panobinostat or ruxolitinib is significantly increased in the presence of HS-5/SCM (32nM and 1261nM, respectively) *versus* the IC50 in RM (15nM and 281nM, respectively). Co-treatment of panobinostat and ruxolitinib strongly synergize, increasing SET2 (96%±1%) and HEL (73%±5%) apoptosis, regardless HS5/SCM exposition. Finally co-treatment of MPN-CD34+ cells with panobinostat and ruxolitinib strongly decrease CFU counting in a stroma-independent manner.
Summary and Conclusions: Disrupting the cross-talk between the malignant clone and its BM milieu is an attractive therapeutic strategy in MPNs. Here, we present evidence that, combination therapy with HDAC and JAKinhibitors has a potential value to overcomethe "protective effect" of the stroma BM on the $JAK2^{V617F}$ cells.

Non-Hodgkin lymphoma - Biology

S1116

GENETIC ANALYSIS OF SPLENIC MARGINAL ZONE LYMPHOMAS REVEALS NOVEL CHANGES IN PATHWAYS IMPLICATED IN CARCINOGENESIS

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Background: The pathogenesis of Splenic Marginal Zone Lymphoma (SMZL) remains largely unknown. Deletions and mutations in *TP53*, as well as deletions of 7q and gains of 3q, are recurrent in SMZL but their role in disease pathogenesis and their clinical significance remains unclear. Recent whole exome sequencing (WES) studies have identified recurrent mutations in key pathways, and most notably *NOTCH2* mutations in >25% of patients. These studies are based on small, heterogeneous discovery cohorts, and therefore only captured a fraction of the genomic lesions present in SMZL. Considerable research therefore remains necessary to accurately describe the genetic landscape of SMZL.
Aims: To identify novel somatic mutations in a biologically-homogeneous (7q abnormalities and/or *IGHV*1-2*04 usage) cohort of eight SMZL patients.
Methods: High molecular weight genomic tumour DNA was extracted from purified peripheral blood lymphocytes or spleens, and matched with germ-line DNA from saliva. PCR for each patient's clonal *IGHV*-sequence was used to ensure germ-line DNA purity. WES (Agilent SureSelect enrichment and Illumina HiSeq2000 sequencing) and copy number profiling (Affymetrix SNP6.0) were performed. Sequence reads were aligned to the human reference genome (Hg19, Novoalign), then merged and compiled (SAMtools), prior to removal of duplicate reads (Picard). Finally, non-synonymous somatic variants were identified (Varscan 2), prioritised (somatic P-value), and annotated against the COSMIC, dbSNP and 1000 genomes databases using Annovar. The presence of somatic variants was confirmed using Sanger sequencing.
Results: Our analysis identified 74 somatic variants, including 66 non-synonymous or frame-shift mutations and eight splice-site mutations, with an average of 9/patient (2-17). Copy number analysis revealed 28 somatic copy number alterations, with an average of 3.5/tumour (0-9/tumour). Two gains of 3q (28.5Mb; 170.8-199.3) and a trisomy 12 were also found. A 12.2Mb MDR was identified at 7q32.3-34 (130.6-142.8) in seven patients. We identified mutations in several genes previously reported in SMZL, such as *MAP3K14* [n=2], *NOTCH2* [n=1] and *TNFAIP3* [n=1]. Importantly, our study identified two novel recurrently mutated genes, *CREBBP* and *CBFA2T3*, both in two cases. The CREB binding-protein, *CREBBP*, is involved in chromatin remodelling and transcription factor recognition, and the same mutation (Y1450C) occurs in other lymphoma subtypes. *CBFA2T3* is a core-binding factor that facilitates transcriptional repression and is targeted by the t(16;21) translocation in AML. We also identified mutations in *BRPF1*, *FAT4*, *SDK1*, *TRPM6* and *AMOTL1* in single cases that can now be considered recurrent, as they have also been observed in other studies [*JEM (2012); 209(9): 1553-63/1537-51*]. Pathway analysis identified involvement of MAPK signalling (*MAP3K14, FLNC, CACNA1E*), cell cycle control (*CREBBP, CUL1*) and Notch signalling (*NOTCH2, NOTCH4*).
Summary and Conclusions: This current study expands our understanding of the molecular pathogenesis of SMZL. We are currently extending this work by re-sequencing approximately 600 candidate genes, known or predicted to be involved in SMZL pathogenesis, in 300 patients (Haloplex, Agilent Technologies). This is an international collaborative project that will ultimately establish the frequency and clinical importance of gene mutations in SMZL, pushing forward our understanding of the disease and validating the clinical utility of these lesions to establish a stratified approach to care.

S1117

EPIGENETIC DEREGULATION OF MIRNA IN MALIGNANT LYMPHOMAS

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Background: One of the most significant recent advances in biomedical research has been the discovery of the 22-nt-long class of non-coding RNA designated miRNA. The miRNAs are pleiotropic molecules that can regulate multiple genes expression and consequently control cell fate. miRNA imbalance is observed in the pathogenesis of a range of diseases including lymphomas, in

which survival and proliferation signaling cascades are abnormally sustained. It is now unclear how miRNAs regulate lymphoma-specific signaling activation.
Aims: Elucidation of miRNA signature and its functional involvement in malignant lymphomas.
Methods: By integrated analyses of primary adult T-cell leukemia (ATL) cells, we uncovered unique molecular characteristics that include profiles of mRNA expression, DNA copy number, and miRNA signature. In addition, we also addressed miRNA abnormality and its functional roles in diffuse large B-cell lymphoma (DLBCL).
Results: Although crucial roles of miRNA in cancers have begun to emerge, detailed studies with ATL patients have not been achieved. Using 40 primary ATL samples and 22 samples of normal CD4+ T cells, we determined the ATL miRNA signature, *i.e.*, all ATL samples showed abnormal downregulated miRNA expression (Yamagishi *et al.*, Cancer Cell, 2012). Especially, microarray and qRT-PCR revealed drastic loss of miR-31, which has been reported as a metastasis-associated miRNA in breast cancer. Given that all ATL cases invariably showed undetectable or very low level of miR-31, the loss of miR-31 appears to be involved in ATL development. As a novel miR-31 target gene, we identified NF-κB inducing kinase (NIK) that plays central roles in noncanonical signaling and constitutive activation of NF-κB pathway in various cancers, including malignant lymphomas. Restoration of miR-31 decreased the levels of NIK expression and NF-κB activity, resulting in reduction of malignant phenotypes, including proliferative index, anti-apoptosis, and chemotaxis in ATL cells. Furthermore, lentivirus-introduced miR-31 could induce strong apoptosis in primary tumors freshly isolated from ATL patients, clearly indicating pivotal functions of miR-31 as a tumor suppressor. We also found that genetic loss and polycomb-mediated epigenetic deregulation directly involve in the silencing of miR-31. Knockdown of polycomb repressive complex restored the miR-31 expression and consequently inhibited NIK-dependent NF-κB cascade, leading to strong apoptosis in ATL cell lines as well as in primary patient samples. These findings illustrated that genetic and epigenetic abnormalities link to NF-κB activation through the loss of miR-31. Of note, we and other research groups have demonstrated that this novel molecular interconnection is well conserved in other cancers such as breast and prostate cancers, melanoma, and B cell lymphomas, in which polycomb and NF-κB are both activated. Thus, miR-31 downregulation appears to be one of the crucial molecular hallmarks of cancers. By comprehensive analysis of primary DLBCL samples and *in vitro* experiments, we also discovered specific miRNAs downregulation in DLBCL. We show that epigenetic silencing of some miRNAs contributes to chronic signaling activation and cell proliferation in B cell lymphoma cells.
Summary and Conclusions: Considering aberrant epigenomics associated with cancers, the emerging relationship provides us a conceptual advance in understanding the broad-acting oncogenic signaling and molecular hallmarks of malignant lymphomas.

S1118

REDUCED TET2 FUNCTION CONTRIBUTES TO DEVELOPMENT OF PERIPHERAL T-CELL LYMPHOMA THROUGH IMPAIRMENT OF EPIGENETIC REGULATION

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Background: TET family proteins convert methylcytosine (mC) to hydroxymethylcytosine (hmC). Loss-of-function mutations in *TET2* were initially reported in various types of myeloid malignancies, and later also in specific subtypes of lymphoid malignancies. *TET2* mutations are found more frequently in angioimmunoblastic T cell lymphoma (AITL) and peripheral T cell lymphoma, not otherwise specified (PTCL-NOS) with AITL-like features than PTCL-NOS without those or other types of lymphomas. The tumor origin of AITL is thought to be follicular helper T cells (Tfh). *Tet2* knockout mice demonstrated premalignant status of myeloid lineages, implicating that loss-of-function mutations of *TET2* play an important role in myeloid malignancies; however, there are no clues how impaired *Tet2* function provokes T-cell lymphomas.
Aims: We analyzed Tet2 gene trap mice to analyze the role of TET2 in T-cell lymphomagenesis.
Methods: The $Tet2^{gt}$ locus has a trapping vector inserted into the second intron of *Tet2* locus. Bone marrow, spleen, and tumors when developed were analyzed in adult $Tet2^{gt/gt}$ mice by flow cytometry and immunohistochemical staining. The comprehensive gene-expression profiles of tumors were assayed by microarray and the data was analyzed by Gene Set Enrichment Analysis (GSEA). The patterns of hmC were analyzed by hmDIP, followed by high throughput sequencing.
Results: Adult mice were obtained after crossing $Tet2^{+/gt}$ mice under the following ratio; $Tet2^{+/+}$:$Tet2^{+/gt}$:$Tet2^{gt/gt}$=48:52:25. The TET2 mRNA expression level in $Tet2^{gt/gt}$ Lin- FL cells was reduced to 20% of that in WT Lin- FL cells, while the hmC level to around a half of that in WT cells. In the spleen of 40 week-old mice, the proportion of CD4+/PD1+/CXCR5+ cells, immunophenotypically similar to Tfh, was significantly increased in $Tet2^{gt/gt}$ mice compared to $Tet2^{+/+}$ mice ($P=0.022$). Five out of the 7 $Tet2^{gt/gt}$ mice aged 60 weeks or older (median, 67 weeks) developed marked splenomegaly with swollen lymph nodes and multi-nodular tumors in liver and lungs. Histological examination of the enlarged spleen and swollen lymph nodes in these 5 mice demonstrated completely destroyed follicular structures and infiltration of morphologically abnormal lymphocytes, which showed the CD4+/PD1+/CXCR5^{dull} phenotype. CD4+ cells purified from the tumors revealed restricted rearrangement patterns of T-cell receptor gamma gene, implying a signature of T-cell lymphoma. CD4+ cells purified from the lymphomas represented similar gene-expression profiles with Tfh cells, *i.e.*, high expression of Bcl6 and c-Maf, two key transcription factors of Tfh. The patterns of hydroxymethylation were distinct between CD4+ cells purified from $Tet2^{gt/gt}$ mice and those from control mice.
Summary and Conclusions: Our observations indicate that reduced expression of TET2 leads to proliferation of Tfh cells and eventually to development of lymphomas that pathologically recapitulate PTCL-NOS with characteristics of Tfh in humans. These data suggest that TET2 serves as a gatekeeper of lymphoid malignancies in addition to myeloid malignancies.

S1119

A KEY ROLE FOR EZH2 IN EPIGENETIC SILENCING OF HOX GENES IN MANTLE CELL LYMPHOMA

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Background: HOX genes play a central role in body formation during early embryonic development, while deregulation of these genes adversely affects development and may also lead to malignancy. Using 27K methylation microarrays, we recently reported differential methylation of a number of HOX genes (n=13) in one of the most the aggressive lymphomas *i.e.* mantle cell lymphoma (MCL) *versus* the more indolent disease chronic lymphocytic leukemia (CLL). Interestingly, we observed that though differentially methylated, *i.e.* hypermethylated in MCL and hypomethylated in CLL, HOX genes remained unexpressed in both diseases, indicating that both DNA methylation-dependent and independent mechanisms operate to silence HOX genes.
Aims: We here characterized the differential mechanisms underlying the epigenetic silencing of HOX genes (specifically, HOXA cluster genes) in MCL *versus* CLL using representative cell lines and primary samples.
Methods: In order to investigate if HOXA genes could carry other epigenetic modifications in their upstream regulatory regions, we performed chromatin immune purification (ChIP) assays in MCL and CLL cell lines (Granta 519 and HG3) using antibodies for the repressive histone mark H3 lysine 27 trimethylation (H3K27me3) and its methyl transferase, EZH2. The DNA methylation status was analyzed using pyrosequencing and bisulfite sequencing assays, while the mRNA and protein expression levels were measured using RQ-PCR and western blot analysis respectively. Furthermore, siRNA transfection and EZH2 inhibitor (3-Deazaneplanocin A (DZNep)) treatment experiments were performed to investigate the direct role of EZH2 in DNA methylation.
Results: Overall, our results for the first time highlights the epigenetic silencing mechanisms underlying differential HOXA genes (*i.e.* HOXA2, HOXA7, HOXA 9 and HOXA13) regulation in MCL *versus* CLL. More specifically, we demonstrate that the chromatin modifier, EZH2, catalyzed repressive H3K27me3, which was sufficient to silence HOX genes in CLL, whereas in MCL both H3K27me3 and DNA methylation were required for efficient silencing of the HOXA genes. More importantly, HOXA gene promoters were more enriched with EZH2 in MCL compared to CLL, which in turn correlated with increased DNMT1 recruitment and CpG methylation, indicating that EZH2 levels indeed determine the levels of DNA methylation through DNMTs. Finally, the crucial role of EZH2 in orchestrating HOX gene silencing in MCL was underscored by our siRNA and EZH2 inhibitor experiments, whereby DNA methylation occurs as a secondary event following EZH2 recruitment to the HOX promoter.
Summary and Conclusions: These novel observations implicate EZH2 in long-term silencing of HOX genes in MCL, and this could form the basis for epigenetic therapy targeting EZH2 with potential clinical implications.

S1120

DEEP SEQUENCING REVEALS SMALL SUBCLONES OF STAT3 MUTATIONS IN LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

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Background: Large granular lymphocytic (LGL) leukemia is a clonal disorder of either cytotoxic CD8+ T-cells or NK-cells. We recently discovered that 40% of LGL leukemia patients with monoclonal Vbeta expansions harbor somatic mutations in the SH2 domain of *STAT3* gene. The found mutations cause constitutive activation of STAT3.
Aims: The purpose of the current project was to study the LGL leukemia samples with ultra-deep amplicon sequencing to discover the clonal architecture and mutation spectrum of expanded lymphocytes.
Methods: DNA samples from 190 T-LGL and 38 NK-LGL leukemia patients (both with the mono-and oligoclonal expansions) who met the diagnostic criteria of WHO2008 were included in the study. Locus-specific primers for *STAT3* exon 21 were used in the amplicon sequencing and up to 10 000× coverage was achieved with Illumina Miseq platform. The data was analyzed with Illumina MiSeq Reporter.
Results: The frequency of *STAT3* mutations in the whole study cohort was 21% (48/228) by capillary sequencing, whereas the amplicon sequencing revealed 27 additional patients with mutations (total freq. 33%, Figure 1). The most common mutations found were Y640F (38%) and D661Y (27%). In addition to previously described mutations, 4 novel *STAT3* mutations were discovered by amplicon sequencing (Q643H, I659L, K658H, and K658N). In total, 12 patients had multiple *STAT3* mutations: in 8 cases the mutations were in different clones and in 4 cases in the same clone. As extreme examples, two patients had 3 different *STAT3* mutations in different clones (Y640F, D661Y and I659L; D661Y, N647I and Y640F). Patients with single *STAT3* mutation had typically one large clone, whereas in patients with multiple *STAT3* mutations, small subclones were discovered in addition to main clone. As in some LGL leukemia patients a clonal drift (ie. change of the major Vbeta clone) has been described, we analyzed one T-LGL leukemia patient who had multiple lymphocyte expansions during the disease course in detail. At diagnosis, 40% of CD3+ cells expressed TCR-γδ, and *STAT3* amplicon sequencing of mononuclear cell (MNC) fraction revealed mutations Y640F (5%) and D661Y (20%) in different clones. After one year of treatment with cyclosporine and danazol, the patient had two new Vbeta expansions (Vβ14 18% and Vβ16 17%), whereas only 4% of CD3+ cells were γδ+. We sorted MNCs into 4 different CD3+ cell subsets: CD3+αβ+, CD3+γδ+, CD3+Vβ14+, and CD3+Vb16+ cells and sequenced the fractions with amplicon sequencing method. Both *STAT3* mutant clones had survived the therapy and were found in the small CD3+γδ+ fraction (Y640F 23% and D661Y 9%). However, no mutations were discovered in the expanded Vbeta fractions suggesting that they were only reactive clones and no real clonal drift had occurred.

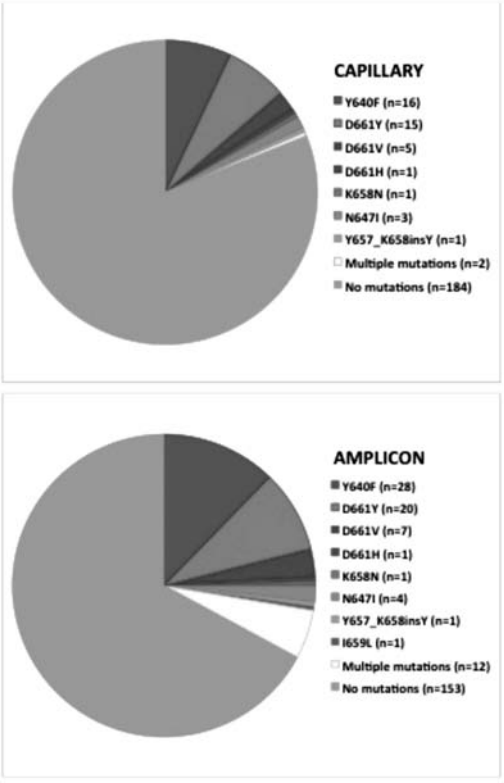

Figure 1.

Summary and Conclusions: With the ultra-deep amplicon sequencing method we were able to discover small lymphocyte clones with STAT3 mutations in LGL leukemia patients and reveal clonal heterogeneity in some patients. The total frequency of mutations found (33%) was lower than in the original LGL leukemia cohort with monoclonal expansions suggesting that some patients included may have reactive LGL proliferation. The multiple STAT3 mutations in individual patients suggest that STAT3 pathway plays a central role in the LGL leukemia pathogenesis and further studies are needed to understand the initial transforming events.

Acute lymphoblastic leukemia - Therapeutics

S1121

PROGNOSTIC VALUE OF COMPLEX KARYOTYPE AND MONOSOMAL KARYOTYPE IN PATIENTS WITH ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The karyotype is an important predictor of outcome in adults with acute lymphoblastic leukemia (ALL). Some groups have reported a negative prognostic value of complex karyotype (CK, defined as ≥5 unrelated chromosomal abnormalities) in adult ALL (Moorman *et al.*, Blood. 2007; 109: 3189-97). On the other hand, monosomal karyotype (MK, defined as ≥2 distinct autosomal chromosome monosomies or 1 single monosomy in the presence of structural abnormalities; Löwenberg *et al.*, JCO. 2008; 26: 4791-7) has been associated with a worse outcome in patients with acute myeloid leukemia.
Aims: We aimed to assess the prognostic value of CK and MK in adults with ALL treated with risk-adapted protocols of the Spanish PETHEMA Group.
Methods: The karyotypes of 881 adult ALL patients from 65 Spanish centers treated according to the protocols of the PETHEMA Group between 1993 and 2012 were reviewed. Central review of karyotype reports was performed for this study. Patients with t(9;22), t(v;11q23), t(1;19), t(8;14), and t(14q32) rearrangements were not considered for the analysis of CK according to the Moorman's criteria. All karyotypes were included for the analysis of the prognostic value of MK. A specific analysis for patients with t(9;22) was performed. Outcome measures were complete remission (CR) rate, CR duration, overall survival (OS) and event-free survival (EFS).
Results: The median age of the series was 33 years (range 15-82) and 498 patients (56.5%) were male. The karyotypes of 636 out of 881 patients were evaluable after review: 163 patients (19.5%) presented normal karyotype, 33 out of 364 evaluable patients without t(9;22), t(v;11q23), t(1;19), t(8;14), and t(14q32) rearrangements (9.1%) had CK, and 68 out of 535 evaluable patients (12.7%) had MK. The CR rate and the probabilities of CR duration, OS and EFS are shown in the Table 1.

Table 1.

Group	N (%)	CR rate	CR duration (6 years)	OS (6 years)	EFS (6 years)
CK	33 (9.1)	79%	55±13%	36±18%	33±17%
No CK	170 (46.7)	89%	52±10%	37±8%	35±8%
Normal	161 (44.2)	89%	52±10%	39±9%	39±9%
MK	68 (12.7)	82%	66±15%	40±14%	43±12%
No MK	467 (87.3)	87%	48±6%	33±5%	33±5%
Ph+ MK	15 (15)	79%	86±26%[1]	22±34%	40±28%
Ph+ no MK	85 (85)	84%	35±17%[1]	30±11%	20±12%
Ph+ (imatinib) MK	10 (19.6)	89%	100%	62±35%	55±34%
Ph+ (imatinib) no MK	41 (80.4)	93%	56±24%	45±18%	39±18%

No significant differences on comparison between groups except [1]p=0.08

Summary and Conclusions: Our study shows that CK and MK were not associated with a worse prognosis in adult patients with ALL treated with risk-adapted or subtype-oriented protocols from the PETHEMA group. In patients with Ph+ ALL MK did not have impact on prognosis irrespective to imatinib treatment.
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S1122

IDENTIFICATION OF NEW PROGNOSTIC FACTORS PREDICTIVE OF EXTRAMEDULLARY RELAPSE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia (ALL) is the most common cancer diagnosed in children. With the discovery and implementation of new drugs and a better risk stratification, survival rates in pediatric ALL patients increased over the last decades, and currently at least 80% of childhood ALL are cured, indicating that a relatively large proportion of the children suffer from relapse. Extramedullary (EM) involvement is defined as leukemic infiltration in EM sites like the central nervous system (CNS), testis, gut or skin. EM involvement at diagnosis is rare (1-4%) in childhood ALL patients, but is more frequent at relapse (~40%) suggesting that ALL cells at EM sites more easily escape from treatment. Because of the still developing and maturing of the CNS in children, the applied treatment modalities have a high change to induce late sequelae.To reduce the side effects of CNS-directed therapy and to prevent CNS relapses, it is important to predict which patients are at high risk of a CNS relapse. These patients will receive intensified treatment, while patients at low risk may receive less treatment, thereby improving quality of life at later age.
Aims: To find cellular and molecular characteristics of EM-derived cells and hypothesize that at diagnosis a subpopulation of ALL cells with such "EM signature" might already be present in bone marrow (BM), allowing early identification of ALL patients at risk of developing an EM relapse.
Methods: Microarray study was performed using Affymetrix GeneChip Human Genome U133 Plus 2.0 Array. Differential expression of genes as identified by microarray analysis was confirmed by realtime quantitative PCR (RQ-PCR). Differential gene expression between ALL cells derived from BM *versus* extramedullary locations was confirmed at protein level by flow cytometric analysis. Bio-plex pro human cytokine 20-plex assay was performed according to the manufacturer's protocol.
Results: Gene expression profiling was performed on B-cell precursor (BCP) ALL samples isolated from cerebrospinal fluid (CSF) or BM. Principal Component Analysis (PCA) and hierarchical clustering analysis showed that CSF-derived ALL samples perfectly cluster into one separate branche, indicating that ALL cells from the CNS show distinct gene expression profiles as compared to BM-derived ALL cells. Selection of potentially interesting genes was performed based on the following criteria: highest differential expression (FC) between CSF and BM samples in both the paired and the unrelated analysis; highest statistical significance; expression at the cell membrane; presence of (monoclonal) antibodies and biological relevance and confirmed by RQ-PCR. Genes involved in cell growth, survival, proliferation (ARHGAP18, RASA1), differentiation (RASA1), migration and cell adhesion (OPN, ARHGAP18), showed a significant increase in CNS localization compared to BM localization. Diagnostic BM and/or PB cells from four BCP-ALL patients with an isolated CNS relapse were analyzed by flow-cytometry for (sub) populations of ALL cells. Preliminary data show that minor ALL populations expressing the proteins encoded by these genes can be identified in diagnostic BM samples from patients with EM relapse.
Summary and Conclusions: We conclude that EM-derived and BM-derived BCP-ALL cells show distinct gene expression profiles. The presence of a small ALL population with an EM signature at diagnosis may be associated with a higher chance of EM relapse. In future, the identification of proteins and involvement of specific pathways in EM involvement may contribute to targeted therapies.

S1123

IKZF1 DELETION STATUS DISCRIMINATES FOR OUTCOME IN IMATINIB-TREATED BCR-ABL1-POSITIVE CHILDHOOD ALL

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Background: The *BCR-ABL1*-translocation (Philadelphia-chromosome) predicts for an unfavorable outcome in childhood acute lymphoblastic leukemia

(ALL). *BCR-ABL1*-positive ALL is characterized by a high frequency (70%) of *IKZF1* deletions. *IKZF1* deletions are correlated to an unfavorable outcome in *BCR-ABL1*-negative ALL.
Aims: We studied the prognostic value of *IKZF1* deletions in children with *BCR-ABL1*-positive ALL who were treated with and without Imatinib.
Methods: The *IKZF1*-status was evaluated by multiplex ligation-dependent probe amplification in 191 newly diagnosed patients; 84 cases were diagnosed before the introduction of Imatinib and 107 cases were enrolled in the European Philadelphia-chromosome positive ALL trial. 63 EsPhALL cases were stratified in the good-risk arm and were randomized for Imatinib, 44 EsPhALL cases were stratified in the poor-risk arm and all received Imatinib.
Results: 66% (126/191) of the *BCR-ABL1*-positive patients had an *IKZF1* deletion; 36.5% of these deletions resulted in haplo-insufficiency (covering monoallelic loss of at least exon 2), 52.4% had an exon 4-7 deletion that resulted in a dominant-negative variant. The frequency of hematopoietic stem cell transplantation (HSCT) did not differ between the *IKZF1*-deleted and wild-type group (both ~70%). In patients that were treated before the introduction of Imatinib, *IKZF1*-deleted patients had a poor 4-yr disease free survival (DFS) (30.0±6.8%) compared to wild-type patients (57.5±9.4%; P=0.01). *IKZF1*-deleted patients treated according to the EsPhALL good-risk protocol had a poor 4-yr DFS (51.9±8.8%) compared to wild-type patients (78.6±13.9%; P=0.03), and this was also evident for those who received Imatinib (4-yr DFS 55.5±9.5% for *IKZF1*-deleted *versus* 75· 0±21.7% for *IKZF1* wild-type good-risk patients, P=0.05).
Summary and Conclusions: This is the first study demonstrating that *IKZF1* deletions are correlated to a poor outcome in a relative large cohort of childhood *BCR-ABL1*-positive ALL. We advocate that *IKZF1*-deleted patients need a more intensified or alternative therapy. In addition, since *IKZF1* wild-type *BCR-ABL1*-positive patients treated with Imatinib have a good outcome comparable to *BCR-ABL1*-negative ALL, the absence of an *IKZF1* deletion may be used as marker to exclude HSCT.

S1124

FRENCH RESULTS WITH THE EWALL CHEMOTHERAPY BACKBONE IN OLDER PATIENTS WITH PHILADELPHIA CHROMOSOME-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA. A GRAALL REPORT.

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Background: Philadelphia chromosome (Ph)-negative acute lymphoblastic leukemia (Ph-neg ALL) occurring after 60 years of age is associated with a poor prognosis.
Aims: In order to test novel therapeutic interventions, the European Working Group for Adult ALL (EWALL) has developed a common chemotherapy backbone adapted to older patients.
Methods: After a dexamethasone (DEX) prephase, patients received two induction cycles, comprising DEX, vincristine and idarubicin for cycle1, followed by cyclophosphamide and cytarabine for cycle2, starting after hematological recovery or at d29. Consolidation included 6 cycles alternating intermediate-dose methotrexate and L-asparaginase (odd cycles) and intermediate-dose cytarabine (even cycles). Patients aged more than 70 years received half-dose of chemotherapy during consolidation. Maintenance therapy comprised then 6 cycles. Treatments are detailed in the Table 1.
Results: We report here the results of 59 patients with Ph-neg ALL treated with this backbone between 2007 and 2013 in 12 GRAALL centers. Median age was 65 years (60.9-82.5) and median follow-up was 13.9 months (2.3-64). After induction, 45 patients (76%) achieved CR, 10 (17%) were alive with resistant disease (RD). Four patients (7%) died during induction. Five patients who reached CR after the first induction cycle did not received the second one. Among the 45 CR patients, 29 died (25 after ALL recurrence). Among the 10 RD patients, 8 died. Median overall survival (OS) was 11.3 months. The probability to be alive at 1 year and 3 years were 49.9% and 24%, respectively. Median disease free survival (DFS) was 9.6 months with 1-year and the 3-years DFS estimation at 43% and 19%, respectively. We used the EWALL backbone to test two treatment intensification approaches. First, 9 of these 59 patients (median age, 64 years) received 6 additional infusions of6,000UI/m^2 L-asparaginase during each induction course. In these patients, we observed 6 CR (67%), two toxic-deaths due to encephalopathy and one RD. Their median DFS and OS were 15 and 16.7 months, respectively. Comparatively 39 CR (78%), two toxic-deaths, and 9 RD were observed in the 50 patients who received recommended induction, with 8.6 months median DFS and 11.3 months median OS. These differences were not statistically significant. Secondly, we tested consolidation intensification in 6 CR eligible patients (median age, 64 years). These patients received consolidation, late intensification (with 6 infusions of 6.000UI/m^2 L-asparaginase) and maintenance according to the GRAALL2005 protocol designed for younger adults. With median follow-up of 9.9 months, none of them relapsed, nor died in CR. Comparatively median DFS was 8.6 months in the 39 patients who received recommended consolidations, but had a longer median follow-up of 37.9 months. The difference was not statistically significant.

Prephase		
DEX	D-5 à D1	10 mg/m²/d
METHOTREXATE IT	D-5	15 mg
First induction		
DEX	D-2 and D11	10 mg/m²/d
VINCRISTINE	D1 and D8	1 mg flat dose
IDARUBICINE	D1-D2 and D8-9	10 mg flat dose
METHOTREXATE IT	D2-D9	15 mg
CYTARABINE IT	D2-D9	40mg
METHYLPREDNISOLONE IT	D2-D9	40mg
Second induction After hematologic recovery or at D29		
CYCLOPHOSPHAMIDE	D1-3	300 mg/m²/d
CYTARABINE	D2-5 ; D9-12	60 mg/m²/d
METHOTREXATE IT	D2-D9	15 mg
CYTARABINE IT	D2-D9	40mg
METHYLPREDNISOLONE IT	D2-D9	40mg
Consolidation course n°1, n°3 and n°5 (D1=D21)		
METHOTREXATE	D1	1000 mg/m² or 500mg/m²
L-ASPARAGINASE	D2	10000UI/m² or 5000UI/m²
METHOTREXATE IT	D1	15 mg
CYTARABINE IT	D1	40mg
METHYLPREDNISOLONE IT	D1	40mg
Consolidation course n°2, n°4 and n°6 (D1=D21)		
CYTARABINE	D1, D3, D5	1000 mg/m²/12h or 500mg/m²/12h*
Maintenance D1=D60 during the first 6 months and D1=D90 during 12 last months		
DEX	D1 and D2	40 mg/d
VINCRISTINE	D1	1 mg flat dose
Oral 6-Mercaptopurine	daily	60 mg/m²/d continuously
Oral Methotrexate	weekly	25 mg/m² 1/week continuously

* in patient aged more 70 years old

Figure 1.

Summary and Conclusions: In conclusion, the EWALL chemotherapy backbone yielded a high CR rate (76%) but a poor median survival, due to a high relapse rate during the first year. L-asparaginase seemed too toxic to be recommended during induction in these older patients. However, more intensive consolidations cycles appeared to be an interesting option that could be tested to prolong CR duration in these high-risk patients.

S1125

WEEKLY INOTUZUMAB OZOGAMICIN (INO) IN ADULT PATIENTS WITH RELAPSED OR REFRACTORY CD22-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Background: INO is a humanized anti-CD22 antibody conjugated to calicheamicin. CD22 is expressed on a majority of B-cell ALL. An initial study suggested INO efficacy and tolerability in ALL (*Lancet Oncol* 2012;13:403-11).
Aims: To optimize the INO dose and weekly schedule, and evaluate safety and efficacy in CD22+ relapsed/refractory ALL.
Methods: This phase1, dose-escalation study enrolled pts aged ≥18 y with CD22+ refractory/relapsed ALL and no central nervous system disease. INO was administered in 28-d cycles (Table 1), up to 6 cycles. The final dose was to be determined based on dose-limiting toxicities (DLT) and efficacy, using EffTox V2.10 software during dose escalation. Adverse event (AE) severity was assessed per CTCAE V3. DLTs included the following INO-related events in Cycle 1: gr ≥4 nonhematologic toxicity; prolonged myelosuppression (absolute neutrophil count [ANC] <500/μL or platelets <25,000/μL in bone marrow) with no evidence of leukemia persisting >45 d from last dose; gr 3 nonhematologic toxicity persisting ≥7 d from last dose; gr ≥3 increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), or bilirubin persisting >7 d; or any toxicity resulting in permanent INO discontinuation. Complete response (CR) was defined as <5% bone marrow blasts with absence of peripheral blasts, ANC ≥1,000/μL, platelets >100,000/μL, and no extramedullary disease; incomplete CR (CRi) permitted ANC <1,000/μL and/or platelets ≤100,000/μL.
Results: We report data for 24 pts (Table 1): median age was 47.5 y (range 23-69 y); 71% were male; 10 (42%) pts were in salvage1, 6 (25%) in salvage2, and 8 (33%) in salvage ≥3; 5 (21%) pts had prior allogeneic stem cell transplant

(SCT); 4 (17%) pts were Ph+; median CD22+ blasts was 98% (by central lab); median WBC was $3.9\times10^3/mm^3$ (range $0.5\text{-}29.1\times10^3/mm^3$). Median follow-up among surviving pts was 4.1 mo (range 0.5-12.6 mo). Twenty-three pts discontinued INO: 10 proceeded to SCT, 6 due to disease progression, 4 due to AEs (gr 3 acute renal failure, gr 2 ascites, gr 2 increased gamma-glutamyl transpeptidase [GGT], and gr 5 graft failure), and 3 due to other causes. One DLT of transient gr 4 elevated lipase occurred at INO dose level 3. The most frequent (≥20% of pts) treatment-related AEs were thrombocytopenia (38%; 33% with gr 3/4), increased AST (33%), nausea (25%), neutropenia (25%; 21% with gr 3/4), fatigue (21%), and increased GGT (21%). Fourteen (58%) pts had dose delays due to AEs; 5 (21%) pts had dose reductions due to AEs. Eight deaths were reported, including 1 death on Day 14 due to disease progression and 7 deaths during the post-treatment follow-up due to sepsis following transplant (n=3), ALL (n=3), and graft failure (n=1). Responses were observed for all INO doses (Table 1). The remission rate was 79% (n=19; 11 pts with CR, 8 pts with CRi); median time to remission was 28 d (range 20-78 d). A total of 18/19 (95%) pts with a CR or CRi achieved negative minimal residual disease (MRD; <1 blast out of 10^4 mononuclear cells by flow cytometry); median time to MRD negativity was 33.5 d (range 22-141 d). Minimum INO concentrations in responders were higher than in pts failing treatment.

Table 1.

	INO dose level			
	1 (n = 3)	2 (n = 12)	3 (n = 9)	Total (N = 24)
Total dose/cycle, mg/m²	1.2	1.6	1.8	
Day 1, mg/m²	0.8	0.8	0.8	
Day 8, mg/m²	0	0.4	0.5	
Day 15, mg/m²	0.4	0.4	0.5	
Day 28	Bone marrow aspirate and/or biopsy for disease assessment			
DLTs	0	0	1	1
Median no. of cycles initiated (range)	4 (1-6)	2 (1-4)	3 (1-4)	2 (1-6)
Response rate (CR + CRi + PR), n (%)	2 (67)	11 (92)	8 (89)	21 (88)
Remission rate (CR + CRi), n (%)	2 (67)	9 (75)	8 (89)	19 (79)
MRD negative, n/n with remission (%)	2/2 (100)	8/9 (89)	8/8 (100)	18 (95)
Median time to MRD negativity (range), d	98.5 (98-99)	32 (22-64)	30 (22-141)	33.5 (22-141)

Summary and Conclusions: INO had a tolerable safety profile consistent with prior reports, primarily characterized by hematologic, gastrointestinal, and hepatic AEs. Single-agent INO demonstrated encouraging clinical activity in this relapsed/refractory population; preliminary efficacy results appear dynamically related to exposure and circulating blasts. Further exploration in CD22+ ALL is warranted.

Myeloid leukemogenesis

S1126

A STUDY OF THE LEUKEMOGENIC INTERACTION BETWEEN MUTANT NPM1 AND NRASG12D IN CONDITIONAL KNOCK-IN MICE

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Background: Very recently, whole genome and targeted sequencing of individual cases of AML have raised the possibility that in many instances as few as three specific mutated genes may be sufficient to promote leukaemia. Appreciation of the biological synergy between the complement of leukaemogenic mutations in driving disease progression is key to the development of new treatments. The largest subgroup of AML which constitutes 35% of all cases, namely a karyotypically normal sub-group of AML, exhibits a mutation in the Npm1 gene ($Npm1^c$). A humanised conditional knock-in mouse model of the main form of $Npm1^c$ mutations, Type A, has lately been described in which one third of mice developed AML after a long latency (median17.5 months), suggesting a requirement for cooperating mutations. An insertional mutagenesis screen in the same mice revealed rapid onset AML ~80% of mice (median days 99) with activating insertions in in *Csf2*, *Flt3*, *Rasgrp1* or *Kras*, suggesting that activation of these downstream pathways are key in leukaemogenesis. Furthermore, the aforementioned human sequencing studies, including the first AML genome to be sequenced, reveal commonly co-occurring mutations in Npm1 and Nras ($Nras^{G12D}$, which results in a constitutively active form of the protein). Notably, only ~25% of mice genetically engineered to harbour the $Nras^{G12D}$ ($NRAS^{LSL\text{-}G12D}$) mutation develop AML.
Aims: Comprehensive analysis of the molecular synergy between the leukaemogenic mutations, $Npm1^{cA}$ and $NRAS^{G12D}$, in the development of AML.
Methods: To analyse the combined effects of $Npm1^c$ with $Nras^{G12D}$ we crossed conditional $Npm1^{flox\text{-}cA/+}$; Mx-1^{Cre} with NRAS $^{LSL\text{-}G12D}$ mice to generate $Npm1^{flox\text{-}cA/+}$; NRAS $^{LSL\text{-}G12D}$; Mx-1^{Cre} double conditional heterozygous mice (RNMX mice). Mice were aged until moribund, tissue samples collected and preserved for histopathology and nucleic acid extraction. Comparative analysis of haematopoietic cell compartments in younger mice (*i.e.* 4 weeks post mutation induction) from combined and singular mutant cohorts was performed using standard FACS techniques. Similar comparative analyses were performed on bone marrow cell extracts on the Lineage^{-ve} transcriptome and on self-renewal, using microarrays and serial re-plating assays respectively.
Results: Histopathological analysis of RNMX mice revealed the development of AML with ~80% penetrance. These mice have a median survival of 89 days, much reduced when compared to $NRAS^{G12D/+}$ (250 days) or $Npm1^{cA/+}$mice (400 days). Peripheral blood counts of aged RNMX mice revealed significantly elevated leukocyte counts and reduced platelets. Cells isolated from diseased RNMX aged mice tested so far, were all transplantable. Subsequent EXOME analysis of RNMX AMLs so far, have revealed the accrual of yet further mutations including a particular instance of $IDH1^{R132Q}$, synonymous to the $Idh1^{R132H}$ mutation associated, and known to co-occur with $Npm1^{cA}$ and $NRAS^{G12D}$, in human AML. Analysis of younger mice reveals $Npm1^{cA}$ dependent skewing toward the myeloid lineage and a general increase in haematopoietic cells concomitant with the $Nras^{G12D}$ mutation. Combined, in RNMX mice, myeloid expansion is responsible for statistically significant increased haematopoietic cellularity. *In vitro* self-renewal properties of bone marrow cells are also enhanced in RNMX mice compared to other cohorts. Global transcriptome analysis has, so far, revealed a previously identified $Npm1^{cA}$ signature of HOX gene overexpression, absent from NRAS mice, but persistent in the RNMX cohort.
Summary and Conclusions: These observations in RNMX mice specifically emphasise the role of combinatorial somatic driver mutations particular to AML and provides a rationale for the co-occurrence of $Npm1^{cA}$ and $Nras^{G12D}$ mutations (given that, in isolation, $Npm1^{cA}$ or $Nras^{G12D}$ mutations have relatively subtle effects on mouse haemopoiesis and a prolonged latency/frequency of AML). Combined with the observed spontaneous accrual of other known co-occurring somatic mutations (IDH1), this model presents a valuable tool in studying the pathogenesis and treatment of AML.

S1127

MOLECULAR SYNERGY BETWEEN MUTANT NUCLEOPHOSMIN AND FLT3-ITD TO DRIVE ACUTE MYELOID LEUKEMIA IN MICE

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Background: Acute myeloid leukemia (AML) is the commonest myeloid malignancy, yet there has been little therapeutic progress for this disease in decades. Recently, advances in DNA sequencing have led to the identification of recur-

rent somatic mutations in AML, but have also demonstrated that individual cases are driven by a small number of co-occurring mutations. Our ability to interpret these findings and address this important medical need relies on an improved understanding of the pathways corrupted by such mutations and particularly the basis of the molecular synergy between them. Here, we report that the two most commonly co-occurring somatic mutations in AML, namely NPM1 exon 12 mutations (NPM1c) and internal tandem duplications of FLT3 (FLT3-ITD), cooperate explosively to induce AML. Mice succumbed to AML after a median of 49 days, and also many cases exhibited loss-of-heterozygosity for Flt3, as seen in human AML. In revealing this striking molecular synergy, our work offers a basis for the frequent co-occurrence of these two mutations and provides a model for the detailed study of AML.

Aims: To study the combined effects of NPM1c with FLT3-ITD we crossed conditional Npm1flox-cA/+ with constitutive Flt3ITD/+ to generate Npm1flox-cA/+; Flt3ITD/+ double heterozygous mice.

Methods: Mice strains have been previously described.

Mouse blood, femurs and spleens were stained with Gr1-PE and CD11b-FITC, B220-APC-Alexa750, CD3-PerCP-Cy5.5 and cKit-APC, run on a BD Fortessa cytometer and analysed with FlowJo 7.6.5. Bone marrow cells were plated in M3434 and colonies counted after 7-10 days. For re-plating, 30,000 bone marrow cells were plated and counted after 8 days.

Results: In this study we observed universal and rapid emergence of AML in *Npm1c;FLT3ITD* mice. All mice developed AML and became moribund aged 31-68 days (Figure 1).Weekly blood showed a progressive increase in blood leukocyte counts in Npm1c/Flt3-ITD mice, to more than 25-fold that of control littermates, whilst hemoglobin and platelet counts were significantly reduced. Flow analysis of blood samples demonstrated, in Npm1c/Flt3-ITD mice, a population of blast cells and a large number of single Mac1+ precursors. Additionally, we also observed an increased number of mature myeloid (Gr1+/Mac1+) cells in Npm1c/Flt3-ITD mice indicating that any maturation block was incomplete.To assay their self-renewal potential, bone marrow cells were studied in serial re-plating assays. Npm1c/Flt3-ITD cells gave rise to significantly more colonies than any other genotype demonstrating their increased self-renewal potential. Interestingly, Npm1c/Flt3-ITD littermates often progressed to AML at different rates or developed more/less aggressive disease. To explain this we hypothesized that, as seen in human AML, LOH for Flt3 may be responsible. We found significant spontaneous loss of the wild-type Flt3 allele in blood samples from Npm1c/Flt3-ITD mice and a tendency for higher blood leukocyte counts when LOH was present. LOH was also seen in bone marrow and spleen but not tail DNA, in keeping with somatic loss of the wild-type allele in leukemic cells.

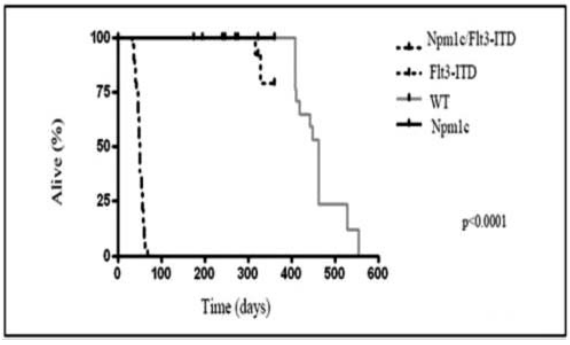

Figure 1.

Summary and Conclusions: Recent studies have revealed that AML heterogeneity is derived, to a large extent, from the specific combinations of somatic driver mutations. Here, we show that the combination of Npm1c and Flt3-ITD, is rapidly and universally leukemogenic in knock-in mice These findings are particularly striking in light of the fact that, in isolation, both Npm1c and Flt3-ITD mutations have relatively subtle effects on mouse haemopoiesis.

Our observations emphasize the remarkable complementarity between Npm1c and Flt3-ITD. In the context of a stochastic model for AML pathogenesis, this potent molecular synergy goes some way towards explaining why NPM1c and FLT3-ITD co-occur so frequently and makes the model described here a valuable tool for the study of the pathogenesis and treatment of AML

S1128

UNDERSTANDING THE PHYSIOLOGICAL FUNCTION OF THE TWO RNA-BINDING PROTEINS MSI1 AND MSI2 INVOLVED IN HETAMOLOGICAL MALIGNANCIES

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Background: Musashi homolog 2 (MSI2) is an RNA-binding protein (RBP) that was first associated with myeloid malignancies when it was shown to form a fusion protein with HOXA9 in chronic myeloid leukemia (CML). Subsequently it was found that MSI2 expression is highly uP-regulated during CML progression into blast crisis. Since then, high levels of MSI2 have been described in various malignancies including prostate cancer, pulmonary carcinomas and AML, in which it is predictive of unfavorable outcome.

Aims: Gene expression in mammals is extensively controlled at the post-transcriptional level by RBPs modulating all stages of the mRNA life cycle. This is achieved by covalent binding between RBPs and mRNAs depending on the unique RNA-recognition element (RRE) of each RBP. Neither the mechanism by which the overexpression of MSI2 contributes to leukemogenesis nor its physiological function is well understood. By identifying its transcriptome-wide mRNA targets we wish to uncover the physiological function of MSI2. This will allow us to understand the consequences of its deregulation in malignancies.

Methods: We applied PAR-CLIP, a method that was developed to identify the transcriptome-wide RBP binding sites by incorporation of a photoreactive nucleoside into nascent mRNAs, to MSI2. Next-generation sequencing (NGS) then allows the mapping of the exact crosslink sites by mapping thymidine to cytidine transitions (T2C) in the cDNAs of PAR-CLIP libraries. To approach the several tens of millions obtained NGS reads, extensive bioinformatic analysis was performed followed by biochemical methods to validate the findings.

Results: Since RBPs within the same protein family often have overlapping mRNA targets, we included MSI1 into our study as it is closely related to and highly co-expressed with MSI2. The roughly 200-300 million NGS reads of each PAR-CLIP library gave rise to around12,000 single linkage clusters meeting stringent quality criteria: (1) more than 25 overlapping reads and (2) at least one T2C transition in at least 20% of the cluster reads. We found that both MSI1 and MSI2 -despite their cytoplasmic localization- bind intronic, exonic and 3'UTR mRNAs at roughly equal distribution. We defined the RRE for MSI1 and MSI2 using a motif discovery approach (MEME). In both proteins it consists of the six nucleotides *TTTTAG*. As expected -given the shared RRE- the targeted genes of MSI1 and MSI2 overlap to a large degree (~75%). We recombinantly expressed MSI2 to perform biochemical validations. Gene ontology analysis of both the RRE and the targeted mRNAs separately indicated a role for MSI2 in MAPK and TGF-beta signaling. We also performed siRNA knockdowns of both MSI1 and MSI2 and correlated the regulated genes with those carrying PAR-CLIP clusters. This resulted in 136/70 genes for MSI1/MSI2, respectively. These genes are currently subject to further functional analysis.

Summary and Conclusions: The RBP MSI2 is frequently overexpressed in (hematological) malignancies. Its physiological function remains unclear since its mRNA targets are unknown. By applying PAR-CLIP to MSI1 and MSI2 we defined their respective direct mRNA targets as well as their RRE. We included MSI1 in our study since RBPs of the same protein family often target identical mRNAs. Gene ontology analysis indicated a role for MSI2 in MAPK and TGF-beta signaling pathways. Knockdown analysis revealed a total of 136/70 regulated genes for MSI1/MSI2, respectively. As predicted, the target genes of MSI1 and MSI2 overlap to a large extent (~75%). So far, neither MSI1 expression nor mutational states in MSI2-related malignancies have been analyzed. However, this might be of interest, considering that overlapping target genes are associated with similar biological functions.

S1129

HES1 IS RESPONSIBLE FOR NOTCH SIGNALING-MEDIATED SUPPRESSION OF ACUTE MYELOID LEUKEMIA DEVELOPMENT

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Background: The transcription factor Hairy enhancer of split1 (Hes1) is well characterized as a downstream target of Notch signaling. Hes1 is a basic helix-looP-helix-type protein, and represses target gene expression. Notch signaling has been proposed to play both pro- and anti-tumorigenic roles; it promotes development of T-cell acute lymphoblastic leukemia (T-ALL), while serves as a tumor suppressor for acute myeloid leukemia (AML). Meanwhile, Hes1 has been proven as an essential mediator of Notch signaling in T-ALL development, but its mediator for AML suppression remains to be elucidated.

Aims: To explore whether Hes1 is responsible for Notch signaling-induced suppression of AML development.

Methods: Common myeloid progenitors (CMPs) purified from RBP-J$^{f/f}$ mouse bone marrow (BM) were serially transduced with MLL-AF9 and Cre recombinase (iCre) using retroviral vectors, and transplanted into lethally irradiated syngenic mice. CMPs from Hes1$^{-/-}$ mouse fetal liver were also retrovirally transduced with MLL-AF9 and transplanted after multiple rounds of replating, Expression levels of downstream targets were evaluated by cDNA array and quantitative RT-PCR.

Results: Mice transplanted with MLL-AF9/RBP-J$^{-/-}$ cells developed leukemia at shorter latencies than those with MLL-AF9/RBPJ$^{+/+}$cells (MLL-AF9/RBPJ$^{-/-}$, 3-8 weeks, n=14 *vs* MLL-AF9/RBPJ$^{+/+}$, 4-10 weeks, n=13; P<0.01). MLL-AF9-transduced Hes1$^{-/-}$ cells formed higher number of colonies at third replating compared to MLL-AF9-transduced Hes1$^{+/+}$ cells. When infused into irradi-

ated syngenic mice, MLL-AF9/Hes1$^{-/-}$ cells developed leukemia at shorter latencies than MLL-AF9/ Hes1$^{+/+}$cells (MLL-AF9/Hes1$^{-/-}$, 7-10 weeks, n=18 *vs* MLL-AF9/Hes1$^{+/+}$, 10-14 weeks, n=18; P<0.001). Both MLL-AF9/Hes1$^{-/-}$ and MLL-AF9/Hes1$^{+/+}$ leukemia cells prepared from bone marrow of the first recipients were transplantable and caused leukemia in the secondary recipients. When Hes1 was retrovirally re-expressed in MLL-AF9/Hes1$^{-/-}$ cells, these cells developed leukemia in recipient mice at longer latencies than mock-transduced MLL-AF9/Hes1$^{-/-}$ cells (Hes1/MLL-AF9, 12 weeks, n=8 *vs* Mock/MLL-AF9, 5-7 weeks, n=7 P<0.001). Taken together, these results indicate that Notch signaling indeed suppresses MLL-AF9-triggered AML development and that Hes1 is a definitive downstream mediator for this Notch function. MLL-AF9/Hes1$^{-/-}$ and MLL-AF9/Hes1$^{+/+}$ leukemia cells were then compared for mRNA expression by cDNA microarray. Among the genes with different expression levels between MLL-AF9/Hes1$^{-/-}$and MLL-AF9/Hes1$^{+/+}$ leukemia cells, Flt3 was expressed at significantly higher levels in MLL-AF9/Hes1$^{-/-}$ leukemia cells. It was also demonstrated that Flt3 was phosphorylated with Flt3 ligand stimulation in the MLL-AF9-immortalized cells specifically with the Hes1$^{-/-}$ background.
Summary and Conclusions: Canonical Notch signaling serves as a tumor suppressor in MLL-AF9-induced AML through upregulation of Hes1.Hes1 is an essential Notch signaling mediator for AML suppression. At least a part of Hes1 function might be explained by repression of Flt3.

S1130

AML1-ETO COLLABORATES WITH THE TALE HOMEOBOX GENES MEIS1 AND MEIS2 IN INDUCING AML

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Background: *AML1-ETO (AE)* is the most frequent fusion gene in human AML. Previously, we and others have demonstrated that the fusion is not able to cause leukemia on its own in experimental murine models, but that it needs as a class II mutation collaborative partners such as *FLT3-length mutation* (class I) to generate AML in the murine bone marrow transplantation model (BMT model) (Schessl *et al*., JCI 2005).
Aims: We aimed at determining the functional role of the Hox co-factors *Meis1* and *Meis2* belonging to TALE family of homeodomain proteins. Meis1 is one of the strongest known co-factors for Hox associated leukemogenesis, but was not linked to CBF leukemias so far. There are no data so far on the role of Meis2 in human AML.
Methods: Taqmann analysis was done for AE positive samples to determine the expression of both MEIS genes. Expression of Meis genes and of AE were achieved by retroviral gene transfer. To test the functional relevance of *MEIS* genes in AE positive AML, lethally irradiated mice were transplanted with BM cells solely expressing the fusion gene (n=8), EGFP (n=8, control) or with BM expressing both genetic alterations AE+Meis1 (n=14) and AE+Meis2 (n=4). To test dependence of human AE positive AML cell lines on MEIS expression lentiviral based knock down of *MEIS1* & *2* were performed. DNA binding of AE in human AML cell lines with or without MEIS depletion was assessed by ChIP-Seq.
Results: Gene expression analysis revealed that *MEIS1* is expressed at high levels in a subgroup of AE positive AML patients. Furthermore, *MEIS2* was highly and aberrantly expressed virtually in all AE patients (n=70) compared to normal bone marrow. Expression patterns of both *MEIS* genes correlated with their promoter methylation in the t(8;21) patients and cell lines. Murine transplantation experiments showed that none of the mice in the AE as well as in the control group developed disease. In contrast, mice transplanted with BM co-expressing AE+Meis1 and AE+Meis2 developed lethal disease after a median latency of 102 and 255 days respectively. AE+Meis1 induced MPS in three, AML in seven and ALL in three cases, whereas AE+Meis2 induced AML in all cases. Functional relevance of *MEIS* expression was further confirmed in human AML cell lines as shRNA mediated depletion of *MEIS1* as well as *MEIS2* impaired cell growth in AE positive AML cell lines and so far one primary AE AML sample. To understand the mechanisms of AE/MEIS collaboration co-immunoprecipitation assays were performed documenting interaction of Meis1 as well as Meis2 with AE. ChIP-Seq has been performed and data on changes in DNA binding of AE in dependence of MEIS expression will be presented.
Summary and Conclusions: Our data demonstrate for the first time that AML1-ETO can collaborate with Meis1/2 and identify a novel collaborative partner in t(8;21) positive AML. It furthermore shows that a class II mutation can be converted into an overt oncogene by collaborating with its own class.

Lymphoid leukemogenesis

S1131

OVEREXPRESSION OF THE EMT REGULATOR ZEB2/SIP1 RESULTS IN A BLOCK IN T CELL DIFFERENTIATION AND IDENTIFIES THIS GENE AS A NEW DRIVER FOR T CELL LYMPHOBLASTIC LEUKEMIA

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Background: Zeb2 is a member of the ZEB family of transcriptional regulators. Its expression was correlated with the formation and/or function of cancer stem cells in solid tumors. We have previously demonstrated that Zeb2 is highly expressed in the hematopoietic system and evidence from mouse retroviral mutagenesis studies points to a role for Zeb2 in initiation and/or progression of leukemia/lymphoma.
Aims: Here, we examined the roles of Zeb2 in the hematopoietic system and in leukemia formation through a conditional gain-of-function approach.
Results: Bi-allelic overexpression of Zeb2 from the ROSA26 locus resulted in altered T cell development: a delay and partial block in differentiation was observed at the DN3 pre-T cell stage. In addition, Zeb2 overexpressing mice spontaneously develop thymic lymphomas starting at 5 months of age, indicating that Zeb2 acts as a driver in T cell malignancies. Next we have bred these mice onto a tumor-prone background (p53$^{flox/flox}$) and observed a significant decrease in tumor latency and an increase of the stem/progenitor markers c-Kit and CD44, suggesting an increase in leukemic stem cells. Using a minimal dilution series of tumour cells into NOD/SCID mice we could demonstrate a 10-100 fold increase in leukemia-initiating cells in the Zeb2 overexpressing tumors. To assess the relevance of these findings with human disease, we screened a cohort of T-ALL patients and found increased expression of ZEB2 predominantly associated within immature/ETP-ALL patients. Importantly, we could identify two ETP-ALL patients who presented a unique chromosomal translocation t(2;14)(q22;q32) on karyotype. This new translocation involves the ZEB2 locus and the BCL11B locus as confirmed by FISH analysis. Such BCL11B-associated translocations lead to the overexpression of the partner gene, thereby identifying ZEB2 deregulation as a molecular driving force for the development of ETP-ALL in these two patients.
Summary and Conclusions: In conclusion, we have shown that Zeb2 overexpression affects early T cell development and predisposes mice to develop an aggressive form of T-ALL with increased stem cell properties. This mimics patients with aggressive ETP-ALL driven by ZEB2 translocations, demonstrating that the EMT regulator ZEB2 is an oncogene for T-ALL.

S1132

ETV6-RUNX1 ALL, INSIGHTS FROM INTEGRATIVE GENOMIC ANALYSIS

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Background: Approximately 25% of pediatric B-cell precursor ALL are characterized by the *ETV6-RUNX1* fusion and are associated with a favorable prognosis. Monozygotic twin studies with concordant ALL and 'backtracking' studies using archived neonatal blood spots established that *ETV6-RUNX1* is a likely initiating event arising prenatally in a committed B-cell progenitor. However, the fusion gene is not sufficient on its own to cause overt leukemia and a number of subsequent studies have now provided strong evidence that additional mutations, arising post-natally, are essential for the clinical development of ALL.
Aims: To obtain a detailed portrait of the composite genetic events that, in concert with the *ETV6-RUNX1* fusion gene, drive this subtype of ALL, we have carried out extensive genomic analysis in patients with ETV6-RUNX1 ALL.
Methods: A total of 57 cases with diagnostic (leukemic) cell DNA paired with matched, remission samples as a source of constitutive DNA were used for exome sequencing (n=56) and low-depth whole genome sequencing for structural variation analysis (n=51). Integrative analysis of exome and whole genome

data was performed and data were evaluated for recurrent gene mutations, copy number alterations and genomic rearrangements. Signatures of somatic mutation and structural variation were studied for insights into operative mutational processes and exposures that may drive *ETV6-RUNX1* pathogenesis. Single cell analysis in two patients was performed using a PCR-based, microfluidic platform, to study timing of mutational processes and how these affect the patterns of subclonal segregation of mutations and clonal phylogeny.
Results: We confirmed 775 somatic substitutions and 16 indels across 715 protein coding genes and 3 micro RNAs. Each patient had on average 14 gene coding mutations consistent with the low number of acquired somatic mutations identified by systematic sequencing screens of haematological malignancies and other childhood cancer. Whole genome profilings identified 524 SVs (average:11, range 0-49) including 34 tandem duplications, 66 inversions, 106 intrachromosomal translocations and 317 deletions. Oncogenic mutations in *KRAS*, *NRAS*, *CTCF*, *DAXX*, *EZH2*, and *KDM6A* are described as well as patterns of complex rearrangement including the balanced chain of chromosomal rearrangements seen in prostate cancer. Most mutations are subclonal to *ETV6-RUNX1*. Integrative analysis of whole-genome and exome data identify genes with deletions and inactivating mutations including *MGA* and *ZMYM2* that would have not been identified by either dataset alone. Sequence context analyses on the observed mutations identifies two distinct mutational signatures that are operative in *ETV6-RUNX1* ALL, and collectively account for the majority of the acquired mutations observed.
Summary and Conclusions: We report the identification of a novel spectrum of somatic mutations in *ETV6-RUNX1* ALL and present the first detailed characterization of the genomic landscape of this common ALL subtype. We provide new insights into the molecular pathogenesis of *ETV6-RUNX1* ALL and discuss the potential biological and clinical implications.

S1133

THE ROLE OF JAK3 MUTATIONS IN THE DEVELOPMENT OF T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: DEVELOPMENT OF IN VITRO AND IN VIVO MODELS

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Background: JAK3 is a tyrosine kinase that associates with the common gamma chain in different cytokine receptors in which JAK1 tyrosine kinase is an essential signaling protein. In acute lymphoblastic leukemia (ALL) and acute megakaryoblastic leukemia mutations have been identified in JAK1 and JAK3. In our exome sequencing study of 67 T-ALL cases, we identified six patients with heterozygous mutations in the pseudokinase or kinase domain of JAK3. Two samples showed evidence for bi-allelic JAK3 mutation.
Aims: The aim of this work was to determine the signaling properties of these JAK3 mutant proteins, to study their *in vitro* and *in vivo* leukemogenic properties, and to test the efficacy of JAK kinase inhibitors for the inhibition of the different mutants.
Methods: We selected six JAK3 pseudokinase domain mutations (A573V, R657Q, A572T, V674A, V678M, M511I), and one JAK3 kinase domain mutation (L857Q). We expressed these proteins in Ba/F3 (B-cell) and MOHITO (T-cell) models through viral transduction. We performed western blot experiments to evaluate downstream signaling, and tested the efficacy of the kinase inhibitors tofacitinib and ruxolitinib for the inhibition of JAK3. RNAi experiments were used to identify essential components for the transforming capacities of the JAK3 mutants.In order to study the effect of JAK3 mutations on T-cell development *in vivo*, we expressed the mutant JAK3 proteins in mouse hematopoietic cells through viral transduction.
Results: Expression of the JAK3 kinase mutant and the six JAK3 pseudokinase mutants in Ba/F3 and MOHITO cells resulted in transformation to IL3 or IL7 independent growth. All mutants led to phosphorylation of JAK1, STAT5 and ERK, while only some mutants showed strong phosphorylation of the JAK3 kinase. Expression of the JAK3 mutant proteins in cells lacking the common gamma chain receptor did not result in auto-phosphorylation of the JAK3 mutant proteins. Based on RNAi experiments and the expression of JAK1 kinase deficient proteins, we were able to show that JAK1 kinase activity is essential for the transforming capacities of all except one (L857Q) JAK3 mutant. The JAK kinase inhibitors tofacitinib and ruxolitinib were able to inhibit the proliferation of the transformed cells with IC50 values around 300 nM and 150 nM, respectively. The kinase domain mutation was significantly less sensitive to ruxolitinib compared to the other JAK3 mutants, while such difference was not observed with tofacitinib. To determine the *in vivo* transforming properties of the JAK3 mutants, we performed bone marrow transplant assays. Mice that received bone marrow cells transduced with JAK3 mutants, developed a fatal T-cell leukemia within 100 days post transplant. The disease was characterized by increased white blood cells counts (>60,000/mm^3) and splenomegaly, with massive infiltration of CD8 single positive T-cells in bone marrow, spleen and thymus. Secondary transplanted mice developed leukemia within three weeks post transplant, confirming the disease to be an acute leukemia.
Summary and Conclusions: *In vitro* and *in vivo* experiments confirm the oncogenic properties of JAK3 mutant proteins and show that JAK1 is an essential kinase for most JAK3 mutants. Therefore, both JAK1 and JAK3 specific inhibitors could be tested for the treatment of JAK3 mutation positive leukemias. We demonstrate the efficacy of tofacitinib and ruxolitinib for the inhibition of various JAK3 mutants.

S1134

LEUKEMIA INITIATING CELLS (LICS) IN ACUTE LYMPOBLASTIC LEUKEMIA (ALL) ARE ENRICHED IN EARLY CELL CYCLE

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Background: Leukemia initiating cells (LICs) in acute lymphoblastic leukemia (ALL) were initially supposed to be organized in a hierarchical fashion similarly to acute myeloid leukemia (AML) with only a minority of phenotypically defined cellular sub-fractions that are able to propagate leukemia in immunodeficient mice. Recent data have challenged this view and clearly showed that LICs in ALL are not restricted to few phenotypically defined cells but that both immature and mature B-cell precursor (BCP) -ALL cells are equally able to reconstitute leukemia *in vivo* pointing to a stochastic model for the LICs in ALL. Nevertheless, the identification of a distinct leukemia initiating cell population in ALL is still an open issue.
Aims: In order to further characterize LICs in BCP-ALL we employed an alternative approach to determine leukemia initiating activity of ALL cells of distinct cell cycle stages by xenotransplantation assays using our NOD/SCID/huALL mouse model.
Methods: Patient derived xenograft B-cell precursor (BCP-) ALL samples were simultaneously stained for their DNA and RNA content and sorted according to distinct cell cycle phases, *i.e.* G0/G1 and G2-M. Sorted cells were transplanted onto NOD/SCID mice (10^5cells per recipient, 3 recipients per cell cycle subgroup). In addition, sorted cells were analyzed for surface expression of CD19, CD45, CD10, CD38, CD34 and Ly-5.
Results: No difference in the surface expression of markers earlier reported to characterize ALL initiating cells such as CD10, CD38, CD34 and CD19 was detected comparing BCP-ALL cells sorted according to distinct phases of the cell cycle. Most interestingly, functionally all ALL cell subpopulations showed leukemia initiating activity irrespective of their cell cycle stage and led to development of leukemia in the recipient mice. However, among the 4 cell cycle compartments analyzed, the cells isolated from the G0/G1 phase showed the shortest time to leukemia appearance in the recipients compared to cells from later/G2-M cell cycle phases. Importantly, cells belonging to the G2-M phase were always the last engrafting in the recipient mice. Even more interestingly, the differences in the engraftment activity were maintained also when cells were further transplanted onto secondary recipients: cells (unsorted) isolated from primary mice transplanted with G0/G1 cells engrafted earlier compared to cells (unsorted) isolated from mice primarily transplanted with G2/M cells. This increased leukemia initiating activity of ALL cells originating form G0/G1 phases clearly indicates an enrichment of cells with ALL initiating and propagating activity within early cell cycle phases.
Summary and Conclusions: In summary, ALL cells of all cell cycle phases are able to reconstitute leukemia in the recipient animals indicating LIC activity irrespective of cell cycle phases and do not differ in expression of surface markers. This is in line with recent findings describing that LICs in ALL are ubiquitous and frequent. Importantly, despite leukemia reconstitution by cells of all cell cycle subphases, a higher leukemia repopulating activity of cells within G0/G1 was identified indicating an enrichment of LICs in this early phase of the cell cycle which was maintained upon secondary transplantation. Taken together, these data indicate that all BCP-ALL cells posses leukemia initiating potential with cells in the G0/G1 phase representing the driving leukemia initiating cell compartment.

S1135

TISSUE AND DIFFERENTIATION STAGE-SPECIFIC EXPRESSION OF THE CALM/AF10 FUSION PROTEIN IS REQUIRED FOR LEUKEMOGENESIS

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Background: The translocation t(10;11)(p13;q14), which results in the formation of the CALM/AF10 fusion gene, is associated with variety of hematological malignancies including T-cell acute lymphoblastic leukemia (T-ALL), acute myeloid leukemia (AML), undifferentiated leukemia, and T cell lymphoma. In most cases, this translocation is associated with a very poor prognosis.

Aims: To study the mechanism of leukemic transformation by the CALM/AF10 fusion, we established tissue-specific knock-in mouse models.
Methods: In the Rosa26-CALM/AF10 mouse line, the CALM/AF10 cDNA preceded by a loxP flanked transcriptional stop cassette is integrated into the Rosa26 locus. Using tissue restricted or inducible expression of the Cre recombinase, the stop cassette can be removed, resulting in the expression of the CALM/AF10 fusion from the Rosa26 promoter.
Results: We crossed the Rosa26-CALM/AF10 mouse line with a Vav-Cre transgenic line to express CALM/AF10 in the entire hematopoetic compartment starting in the fetal liver, and with Cd19-Cre and Mb1-Cre lines to express CALM/AF10 in early and mature B cells. Expression of CALM/AF10 using Vav-Cre-mediated recombination led to the development of AML with a median latency of 1 year and 100% penetrance. Leukemic mice showed splenomegaly, leukocytosis, and diffuse infiltration of myeloperoxidase positive blast cells in several organs. In 40-50% of the cases the leukemic cells had a biphenotypic character, staining positive for both the myeloid marker Mac1 and the B lymphoid marker B220. In the leukemic cells, we could detect overexpression of *Hoxa* cluster genes (*Hoxa5*, *Hoxa7*, *Hoxa9*, *Hoxa10*) and the *Hox* cofactor *Meis1*. Stable expression of CALM/AF10 from the Rosa26 locus in B cells starting from the very early pro B cell stage using Mb1-Cre and Cd19-Cre-mediated recombination did not result in leukemia development in any of the animals after more than 1.5 years. CALM/AF10 expressing B cells from those healthy mice do not show *Hoxa* gene or *Meis1* overexpression.
Summary and Conclusions: This study demonstrates that CALM/AF10 is only leukemogenic when expressed at the right stage of hematopoetic development in the correct cell type and that the overexpression of *Hoxa* genes and of *Meis1* plays important roles in the leukemic transformation. Furthermore, the relatively long median latency of 1 year in the Vav-Cre induced cohort strongly supports the hypothesis that CALM/AF10 requires additional genetic lesions for leukemia development.

Platelets

S1136

T-CELL GENE EXPRESSION PROFILING SUGGESTS THAT "ACUTE" AND CHRONIC ITP ARE TWO SEPARATE DISEASE ENTITIES

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Background: Immune thrombocytopenia (ITP) is an organ specific autoimmune disease where platelets and megakaryocytes are targeted. Although the immunopathogenic cause of ITP has not been fully clarified, there is overwhelming evidence to suggest that a dysfunction of autoreactive T cells could represent the critical event in ITP. A substantial fraction of children with ITP undergo spontaneous remission within 6 months after the initial diagnosis. These patients have formerly been referred to as "acute" while those with persistent low counts beyond 12 months are referred to as chronic ITP. However, it is still unclear if chronic ITP is a natural course of the "acute" form or if these conditions are two different and distinct disorders.
Aims: T-cell dependent processes are central in the pathophysiology of ITP. Our aim was to study if the global gene expression signatures in T-cells differ between "acute" and chronic ITP in childhood.
Methods: Heparin-anticoagulated peripheral blood was obtained from 8 acute (2 boys, 6 girls, mean age 4.9±1.6 yrs; mean time to CR 1.7±0.3 month) and 9 chronic (7 boys, 2 girls, mean age 10.6±2.3 yrs; mean disease duration:56±14 month) ITP-patients and 7 healthy controls (6 boys, 1 girl, mean age 9.9±1.7 yrs). T-cells were isolated by immunomagnetic cell sorting (Miltenyi Biotec, Surrey, UK) and RNA was prepared using the Chomczynski method, followed by RNeasy mini-elute clean-up (Qiagen, Hilden, Germany). Twenty ng RNA was amplified with the Ovation RNA Amplification System V2 (NuGEN Techn., Inc, San Carlos, CA), and cDNA was synthesized using the Encore Biotin Module kit (NuGEN Techn., Inc, San Carlos, CA). After standard labeling, each sample was hybridized to Affymetrix U133Plus 2.0 Human Genome array (Santa Clara, CA). We verified some differentially expressed genes and corresponding protein with real-time PCR and ELISA.
Results: In the DNA microarray analysis we identified 4194 differentially expressed genes in "acute" and 168 in chronic childhood ITP *versus* controls, respectively. From these genes, 125 genes were differently expressed in both ITP forms compared with controls. Interestingly, 4069 and 43 differently expressed genes between ITP patients and controls were specific to "acute" and chronic ITP, respectively. A dendrogram plot showed that the two forms of ITP clustered separate from each other. Functional enrichment analysis based on GO classifications identified several pathways that differed between "acute" and chronic ITP patients compared with controls. Since ITP is an autoimmune disease we focused on immune related genes classified according to Immune Systems Gene Ontology. The analysis showed that enriched T-cells genes in "acute" ITP patients compared with controls were involved in T-cell receptor signaling pathway and regulation of T-cell activation. In chronic ITP patients compared with controls genes involved in regulation of macrophage and neutrophil chemotaxis were found. The GO classifications identified increased T-cells genes in "acute" ITP patients compared with controls such as IL-16 and CX3CR1 and real-time PCR confirmed the regulation. We also measured protein levels of TGFB1, which showed significant regulation between "acute" and chronic ITP compared with controls.
Summary and Conclusions: Our findings show that genes involved in T-cell receptor signaling and activation are differently expressed in "acute" ITP patients compared with chronic ITP. Furthermore, the clustering of T-cell gene expression profiles suggests that "acute" and chronic ITP are two separate disease entities.

S1137

DIFFERENTIAL REGULATION OF THE APOPTOTIC MACHINERY DURING MK DIFFERENTIATION AND PLATELET PRODUCTION BY IAP LIVIN

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Background: The exact mechanism of platelet production is poorly understood. A relationship between activation of the apoptotic cell machinery and the formation of pro-platelets has been established. In this study we show that Livin plays a role in this process. Livin is a member of the Inhibitor of Apoptosis Proteins (IAP) family of intracellular anti-apoptotic proteins that acts by binding and inhibiting caspases. We found that Livin is unique among the IAP members as upon strong apoptotic stimuli it is specifically cleaved by caspases to produce a truncated protein (tLivin) with a paradoxical pro-apoptotic activity.

Aims: To explore Livin expression during MK differentiation and better understand its role inplatelet production
Methods: In this work we studied Livin expression in normal bone marrow (BM) and in BM from patients with hematological diseases using immunohistochemistry (IH) staining. To evaluate the potential role of Livin in thrombopoiesis we used cord blood CD34+ cells that were grown under MK differentiating conditions (SCF and TPO). In addition, an *in vitro* model was established to evaluate the potential role of Livin in thrombopoiesis. The human BCR-ABL positive cell line, LAMA-84, was induced by a phorbol ester (PMA) to differentiate to MK. Increased cell size, ploidy and DNA synthesis, all markers of MK differentiation, were detected by flow cytometry (FACS).
Results:: Interestingly, Livin protein is clearly detected in MK in normal mature BM (by IH staining) and is expressed in platelets. *Down-regulation of Livin* in $CD34^+$ progenitor cells and LAMA-84 decreased the ability of MKs to produce functional platelets. Upon differentiation induced by PMA, LAMA-84 cells formed pro-platelets and produced functional platelets capable of aggregation. This differentiation of LAMA-84 cells into MKs was accompanied by Livin protein expression. In contrast to Livin, the levels of the anti-apoptotic proteins Bcl-2, XIAP and Survivin decreased upon MK differentiation. Livin over-expression in $CD34^+$ progenitor cells induced differentiation of these cells into MK and increased the ability of these primary MK to produce platelets. At the terminal stage of differentiation we observed accumulation of the pro-apoptotic tLivin concomitant with increased caspase 3 activity and apoptosis.
Summary and Conclusions: The IAP Livin is upregulated upon MK differentiation and is then cleaved to pro-apototicc tLivin at the end of this process to produce functional platelet. We suggest that Livin plays a role in thrombopoiesis by regulating the apoptotic cell machinery in MK.

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X-LINKED THROMBOCYTOPENIA WITH THALASSEMIA (XLTT) DISPLAYS INCREASED BONE MARROW FIBROSIS AND ANGIOGENESIS–COMPARISONS WITH PRIMARY MYELOFIBROSIS AND GATA1LOW MICE

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Background: GATA1 is a transcription factor that controls erythropoiesis and thrombopoiesis by binding to DNA targets directly or via its co-factor FOG-1. In man, several hypomorphic mutations in the *GATA1* gene localized on the X-chromosome are associated with thrombocytopenia and anemia. In mice, the hypomorphic $Gata1^{low}$ mutation causes a phenotype similar to human primary myelofibrosis (PMF) with thrombocytopenia, defective megakaryocyte (MKC) maturation with increased proliferation, progressive bone marrow (BM) fibrosis, and increased angiogenesis with dilated BM micro-vessels (MV). Although *GATA1* mutations have not been detected in PMF as yet, MKC from these patients exhibit abnormalities similar to MKC from $Gata1^{low}$ mice and have low expressions of GATA1 by immuno-histochemistry (IHC)/immuno-electronmicroscopy. X-linked thrombocytopenia with thalassemia (XLTT) is a rare inherited disorder caused by the 216 R>Q mutation in exon 4 of *GATA1*. The phenotype of male patients includes macrothrombocytopenia, splenomegaly and a beta-thalassemia trait. We describe two families with XLTT in whom three males were initially falsely diagnosed withPMF and all five investigated males showed BM fibrosis ranging grades I-II/III.
Aims: To enhance diagnostic accuracy and improve the knowledge on mechanisms of BM fibrosis by comparing the natural course of BM fibrosis and features of BM angiogenesis between XLTT patients, PMF patients and $Gata1^{low}$ mice.
Methods: Members of the two apparently non-related XLTT families (5 M reported here, 2 F not reported here) were studied. Red blood cell and platelet indices were measured with Siemens ADVIA 2120. BM biopsies from the five XLTT males, varying numbers of patients with PMF or post-PV or –ET-MF with grade II or III BM fibrosis, healthy controls and $Gata1^{low}$ micewere stained by IHC/immuno-fluorescence for several markers.
Results: BM fibrosis never exceeded grades I-II/III in the XLTT males who were aged 29-67 years at sampling, with increased reticulin staining but no collagen fibers (negative van Gieson staining). Also spleen sizes seemed stable over time (lengths by ultrasound 13-20 cm). Mild anemia, anisocytosis, reticulocytosis and low MCV (median 78 fL, ref 82-98 fL) were typically seen. Platelet counts were mostly in the range 22-87 $\times$ 10^9/L (ref 145-387$\times10^9$/L), with high MPV 11.1-12.0 fL (ref 7-9 fL) and PDW 63-74%. Some platelets were pale, and deficiency of alpha-granule was verified by EM. Blood CD34+ cells were increased. MKC were increased in BM, sometimes hypolobulated and showed reduced expressions of GATA1, VEGF and AGGF1 (angiogenic growth factor-1) but normal CD61. PMF MKC had reduced GATA1 expression but increased VEGF and CTGF (connective tissue growth factor). High micro-vessel density (MVD) in XLTT BM, with small MV and poor pericyte coverage of the vessel walls contrasted with large MV having high pericyte coverage in PMF and $Gata1^{low}$ mice. AGGF1 and CTGF were expressed in pericytes as shown by double-stainings for CD34, SMA-alpha and the growth factors. None of the XLTT patients showed the *JAK2* 617 V>F mutation.
Summary and Conclusions: Apparently non-progressive BM fibrosis is a clinically important feature in two XLTT families. Other findings in XLTT were low VEGF and AGGF1 expressions in MKC and enhanced angiogenesis with small MV and low pericyte coverage, as opposed to large MV and high pericyte coverage in PMF. These features together with mutation analyses may aid in the differential diagnosis against PMF.

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MUTATIONS RESPONSIBLE FOR ANKRD26-RELATED THROMBOCYTOPENIA INCREASE THE RISK OF HEMATOLOGICAL MALIGNANCIES BUT ARE NOT FREQUENTLY INVOLVED IN *DE NOVO* ACUTE LEUKEMIAS

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Background: It has been recently shown that mutations in the 5'UTR of *ANKRD26* result in an autosomal dominant form of thrombocytopenia with normal size platelets that has been named *ANKRD26*-Related Thrombocytopenia (*ANKRD26*-RT) (Am J Hum Genet 2011;88:115-20). Gene overexpression seems to be the consequence of mutations. It has been suggested that *ANKRD26*-RT is one of the less rare forms of inherited thrombocytopenias in that it was identified in 21 of 210 families with genetically transmitted low platelet counts (Blood 2011;117:6673-80). Analysis of 78 patients revealed that thrombocytopenia and bleeding tendency were usually mild or moderate, and bone marrow examination suggested that thrombocytopenia was derived from dysmegakaryopoiesis. Unexplained high values of hemoglobin and leukocytes were observed in some cases. The most important clinical remark of *ANKRD26*-RT was the association with acute leukemia, which affected 6% of patients.
Aims: The purpose of this study was to confirm the clinical and laboratory characteristics of *ANKRD26*-RT that have been identified in the first case series of patients. In particular, we wanted to confirm that the disease increases the risk of hematological malignancies. Moreover, we wanted to ascertain whether the 5 'UTR of *ANKRD26* is mutated in *de novo* acute leukemias.
Methods: We searched for mutations in the 5'UTR of *ANKRD26* in patients with inherited thrombocytopenias who had remained without a definite diagnosis at the end of the diagnostic procedure. The search for mutations in the 5'UTR of *ANKRD26* was also performed in 254 subjects with acute leukemia.
Results: We identified 11 heterozygous single nucleotide substitutions and 1 small deletion in the 5'UTR of *ANKRD26* in 65 patients from 21 pedigrees with inherited thrombocytopenia. Four of these mutations (c.-116C>G, c.-126T>C, c.-127delAT, c.-128G>C) had not been detected previously. Bleeding tendency was mild (most patients with grade 1 or 2 of the WHO bleeding scale), platelet count was moderately reduced (mean value/SD: 51/29 $\times10^9$/L) and mean platelet volume was usually normal (mean value/SD: 9/2 fL). Hemoglobin and leukocyte levels higher than normal were observed in 5 and 13 cases, respectively. Considering also 27 affected family members who died before our study, the extended series of 92 patients includes 6 subjects with AL, 3 with myelodysplastic syndrome (MDS) and 2 with chronic myelogenous leukemia (CML). Mutation screening in patients with *de novo* acute leukemias identified mutations in the 5'UTR of *ANKRD26* in one subject. However, medical history revealed that he was thrombocytopenic before developing leukemia and that other family members were known to be affected by *ANKRD26*-RT.
Summary and Conclusions: Our study brings the number of families with *ANKRD26*-RD described in the literature to 42 and makes this form of inherited thrombocytopenia one of the most frequently reported. Analysis of this new case series confirmed that the typical picture of *ANKRD26*-RT is that of an autosomal dominant disorder characterized by moderate thrombocytopenia with normal sized platelets and mild bleeding diathesis. It also confirmed

that mutations in 5'UTR of *ANKRD26* predispose to hematological malignancies, in that 11 of 92 affected subjects developed AL, MDS or CML. However, our study excluded that these mutations are frequently involved in *de novo* AL.

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EXTENT AND CLINICAL RELEVANCE OF BONE MARROW FIBROSIS IN IMMUNE THROMBOCYTOPENIA (ITP) PATIENTS TREATED WITH THROMBOPOIETIN RECEPTOR AGONISTS (TPO-RA)–A SINGLE CENTRE LONG-TERM FOLLOW-UP

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Background: Treatment of ITP patients with TPO-RA increases platelets through stimulation of the TPO receptor. One reported side effect of TPO-RAs is induction of bone marrow fibrosis (BMF)
Aims: To determine the magnitude and clinical significance of BMF and risk of developing phenotypical and/or karyotypic clonal abnormalities in TPO-RA-treated ITP patients
Methods: This single-centre study was carried out at the Platelet Disorders Centre of Weill Cornell Medical College (WCMC), New York, USA. Eligibility criteria were the presence of ITP diagnosis, treatment with TPO-RA and availability of at least one bone marrow biopsy (BMB) performed on treatment with one of the following TPO-RA: romiplostim, eltrombopag, AKR 501/E5501 (Eisai) or the Shionogi agent. BMB were performed every 1–2 years as part of the standard follow-up procedure for ITP patients on TPO-RA. The grade of BMF was assessed in 121 BMBs (13 pretreatment, 103 on-treatment, 5 post-treatment) acquired from 64 patients. Forty disease-free staging BMBs served as controls. All BMBs were separately reviewed by 3 pathologists to assess the grade of marrow fibrosis (MF); discordant cases were reviewed simultaneously to reach consensus. Fibrosis was graded from MF-0 to MF-3 according to the European Consensus Grading System. No additional bone marrows were performed as part of the study. The study was approved by the IRB of the WCMC; informed written consent to include their previously obtained material was acquired from all patients.
Results: Median age (interquartile range=IQR) of 64 patients (33 males/31 females) at the time of first BMB was 38 years (IQR 18-63). Twenty-six patients had ≥2 BMBs. The distribution of MF-grades is shown in the Table 1. The proportion of MF-0 decreased from 70% in pretreatment biopsies to 22% in the first set of BMBs (P=0.03) indicating that TPO-RA induces reticulin in the BM of most treated patients. Despite stable dosing, there was a significantly greater number of MF-2/MF-3 in the last available BMBs as compared to control BMBs, in which no MF-2/MF-3 was found (P=0.003). In the 26 patients having two or more BMBs the number of MF2/3 increased from 2 (8%) in BM1 to 8 (30%) in the last available BMB (P=0.03), which indicates progressive fibrosis in some patients. At time of last available BMB, median hemoglobin level, absolute neutrophils counts, platelet counts and lactic dehydrogenase in those with MF0/1 were 13.6 g/dl, 4.6×10^9/L, 98×10^9/L, 225 U/L and in patients and with MF-2/3 were 12.5 g/L, 6.6×10^9/L, 139×10^9/L, 223 U/L, respectively. No statistically significant differences were found. Comparing factors as age, duration of disease, duration of treatment, splenectomy status, type and dose of agent, only age was found to be significantly higher in patients with MF-2/3 as opposed to MF0/1 at time of last BMB [54 y *vs.* 38 years in (P=0.02].

Table 1.

	TPO-RA treated ITP patients						Controls
	BM 0 n=13	BM 1 n=64	BM 2 n=26	BM 3 n=10	BM 4 n=3	BM Last n=64	n=40
Median (IQR) duration of treatment in years		1.3 0.9-1.7	2.5 1.9-3.4	3.6 2.6-3.7	5.5 5.0-5.8	1.9 1.3-3.5	
MF-0	9 (70%)	14 (22%)	4 (15%)	1 (10%)	0	14 (22 %)	20 (50%)
MF-1 (%)	3 (23%)	45 (70%)	14 (54%)	5 (50%)	1 (33%)	39 (61 %)	20 (50%)
MF-2 (%)	1 (8%)	4 (6%)	7 (27%)	3 (30%)	2 (67%)	11 (17 %)	-
MF-3 (%)	0	1(2%)	1 (4%)	1 (10%)	0	0	-
Trichrome (n +/-)	0/10	1/64	1/26	2/8	0/3		
Karyotype (n of abnor/nor)	0/3	2/25	0/12	0/7	0/2		
Immunophenotype (n of abnor/nor)	0/6	0/41	0/16	0/6	0/2		
BM 0 = pretreatment biopsies; BM 1= 1st set; BM 2 = 2nd set; BM 3 = 3rd set; BM 4= 4th set; BM last= last available BM biopsy							

Summary and Conclusions: This large single centre experience indicates that TPO-RAs induce some degree of BMF, which although it was progressive in some, remained stable in the majority of patients and within the range that is found in normal individuals. The high grades of BMF (MF-2/3) observed in some did not appear to be clinically significant. Age was the only factor associated with higher grades of fibrosis. No neoplastic immunophenotypic or karyotypic abnormalities emerged during treatment with TPO-RA. Annual follow-up with BMB is recommended in TPO-RA-treated patients. Discontinuation should be considered in those who develop MF-3 or possibly even MF-2.

Cellular immunotherapy and vaccination

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EXPLORING LEUKEMIA- AND VIRUS-SPECIFIC HUMAN CD8+ T LYMPHOCYTES WITH STEM-CELL-LIKE AND CENTRAL MEMORY PROPERTIES FOR EFFECTIVE IMMUNOTHERAPY USING HUMANIZED MICE

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Background: Adoptive transfer of *in vitro* generated tumor- and virus- reactive T cells has evolved as a promising strategy in cellular immunotherapy to treat cancer and virus-associated neoplasia such as Epstein-Barr Virus (EBV)-mediated post-transplant lymphoproliferative disorder (PTLD) observed after solid organ and hematopoietic stem cell transplantation. However, terminally differentiated, high avidity effector T cells exhibit limited homing and self-renewal capacity to establish sustained memory.
Aims: In this study we thus explored the modulation of the canonical Wnt-signaling pathway previously shown to affect T cell differentiation to generate HLA-A*0201 restricted, EBV- and Wilms Tumor 1 (WT1) antigen-specific cytotoxic T lymphocytes (CTL) from naïve $CD8^+$ $CD45RA^+$precursors with stem-cell (T_{SCM}) and central-memory (T_{CM}) properties.
Methods: Naïve $CD8^+$ T cells isolated from peripheral blood mononuclear cells (PBMCs) of healthy donors using the *Naive CD8⁺ T-Cell Isolation Kit* (Miltenyi Biotec) were first primed by autologous dendritic cells loaded with EBV-peptides (LMP2, BRLF1, BMLF1) or WT1-peptides ($WT1_{126-134}$ or WT1 PepMix® (Jerini Peptide Technology)) and then restimulated using peptide presenting autologous PBMCs or $HLA\text{-}A2^+$ T2-cells in the presence of an optimized cytokine cocktail comprising interleukin (IL)-12, -7, -15, and -21. To modulate Wnt-signaling the glycogen synthase kinase-3β (Gsk-3β) inhibitor TWS119 was added to the culture. Phenotypic and functional analyses were performed by flow cytometry, IFN-γ ELISpot and ^{51}Cr-release assays, respectively, at different time points of culture. In addition, homing and persistence of different T cell subsets was tested in NOD/scidIL2Rcg-null (NSG) mice while adoptive transfer studies to evaluate antitumor reactivity of these T cells were performed in NSG recipients engrafted with autologous EBV-transformed B cells (B-LCL) or patient-derived AML blasts prior to T cell transfer.
Results: Upon repetitive stimulation we obtained strong expansion of EBV- and WT1-reactive CTL expressing a $CD8^+CD45RA^+CD45RO^-CD95^+CD27^+CD28^+CD62L^+CCR7^+$ T_{SCM} and $CD8^+CD45RA^-$ $CD45RO^+CD95^+CD27^+CD28^+CD62L^+CCR7^+$ T_{CM} phenotype when compared to CTL stimulated without TWS119. In support of our phenotypic analyses additional studies revealed elevated ß-catenin mRNA and eomesdermin expression levels suggesting a Tcf-7 mediated transcriptional effect on T cell differentiation. Interestingly, whereas both TWS119 treated and control CTL populations elicited comparable reactivity against B-LCL *in vitro* as shown by IFN-y ELISPOT and chromium-release assays, adoptive transfer of EBV-reactive, TWS119-treated CTL in B-LCL engrafted NSG mice resulted in potent reduction of EBV-induced B-cell hyperplasia and prolonged engraftment as compared to non TWS119 treated controls. Similar *in vivo* studies of WT1-specific CTL using AML-engrafted NSG mice are currently in progress.
Summary and Conclusions: In conclusion, this study demonstrates that stem-cell-like and central memory EBV- and WT1-reactive CTLs with enhanced survival and sustained effector functions can be generated from naïve $CD45RA^+$ T cells by modulating Wnt-signaling for improved immunotherapy.

S1142

A NOVEL METHOD FOR SUBRETINAL TRANSPLANTATION OF HUMAN ADULT BONE MARROW MESENCHYMAL STEM CELLS AMELIORATES THE DETERIORATION OF RETINAL STRUCTURE AND FUNCTION IN A RAT MODEL OF RETINAL DYSTROPHIES

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Background: Retinal dystrophies, including age related macular degeneration (AMD) and retinitis pigmentosa (RP) are the leading cause of vision incapacitation and blindness worldwide. Currently, there are no effective treatments for most of these progressive diseases. Stem cell-based therapies represent a promising treatment avenue, with recent studies in animal models demonstrating enhanced retinal function following subretinal transplantation of adult or embryonic human stem cell. Preliminary results in clinical trials showed some promising positive effects. However in both animal models and patients, transplanted cells were confined to the injection area, significantly limiting the potential therapeutic effect.
Aims: To develop an improved subretinal transplantation system of human-derived bone marrow mesenchymal stem cells (hBM-MSCs).
Methods: hBM-MSC cells (CD73+; CD90+, CD105+, CD45-) from healthy human donors were transplanted into the sub-retina of one eye of 69 RCS rats at p28 (0.25 million cells/eye). Ten RCS rats were subretinally injected with medium as control. Retinal function was tested by electroretinogram before and following transplantation for 22 weeks. Visual function was examined by an object recognition test. Eyes were inoculated for histology and immunofluorescence analyses. To demonstrate efficiency and safety of the new transplantation method in a large animal model, cells were transplanted in New Zealand White Rabbits and Spectral Domain Optical Coherence Tomography (SD-OCT, Heidelberg) was used for eye imaging and detection of transplanted cells.
Results: Transplanted cells were identified shortly after transplantation as a nearly continuous sheet of cells covering most of the subretinal surface and in the choroid in the rat model. One week after transplantation, cells were confined to the subretinal space. A prolonged (up to p168) and statistically significant enhancement of retinal function following hBM-MSCs transplantation was demonstrated by electroretinogram analysis. Transplanted rats demonstrated improved visual performance following cell transplantation. These results correlated with histological analysis that revealed a significant preservation of retinal structure. Thus, increased number of photoreceptors were observed in the outer nuclear layer along most of the retina with concomitant preservation of rod photoreceptor cell structure. No immunosuppressants were used and long-term safety analysis demonstrated no gross or microscopic adverse effects of cell transplantation. Experiments in rabbits demonstrated efficient subretinal transplantation, with transplanted cells forming a uniform sheet of cells covering most of the subretinal surface. A shallower and less traumatic retinal detachment was produced by cell injection (Figure 1).

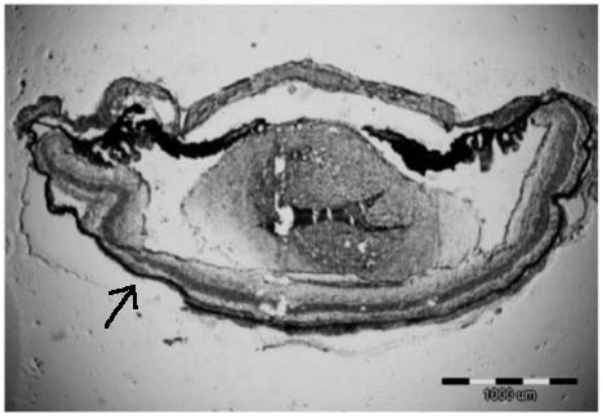

Figure 1. DiI-labeled (arrow) transplanted cells in rat eye.

Summary and Conclusions: In this study we showed for the first time that transplanting hBM-MSCs as a thin homogenous sub-retinal layer resulted in long-term protection of visual functions and significantly delayed photoreceptor degeneration throughout the retina. This new transplantation method may enhance host-graft interaction, cause less trauma to the host tissue and shallower retinal detachment. Our findings suggest that transplanting the cells as a thin layer enhances the therapeutic effect of these cells and safety of transplantation.

S1143

DENDRITIC CELL VACCINATION AS A POST-REMISSION TREATMENT IN ACUTE MYELOID LEUKEMIA: A REPORT OF 29 PATIENTS

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Background: Relapse remains a major problerm in acute myeloid leulemia (AML), especially in the majority of older patients who cannot undergo allogeneic hematopoietic stem cell transplantation. We have reported on the induction of complete and molecular remissions in 5/10 AML patients, following vaccination with dendritic cells (DC), electroporated with mRNA encoding the full-length sequence of the Wilms' tumor protein WT1 (PNAS 2010;107:13824-9). This clinical effect was correlated with CD8+ T-cell response directed against WT1.
Aims: We hypothesized that we could improve the clinical effects by inducing anti-WT1 CD4+ T-cell response in addition to the anti-WT1 CD8+ T-cell response already observed previously.
Methods: In addition to the original construct with the full-length WT1 (construct 1), we also used 2 other constructs, one with a Sig-DC-LAMP MHC class II-skewing signal and a deletion of the WT1 nuclear localization signal (construct 2) and one similar to it, but codon-optimized (construct 3). mRNA, derived from these 3 constructs by *in vitro* transcription, was electroporated into monocyte-derived DC.

Results: When mRNA derived from the 2 new constructs (constructs 2 and 3) with the MHC class II-skewing signal was electroporated into DC, there was a higher cytoplasmic WT1 protein expression and a stronger stimulation of WT1-specific CD8+ T-cells as compared to the original full-length sequence without MHC class II-skewing signal (construct 1). DC electroporated with mRNA derived from constructs1, 2 and 3 were used to vaccinate respectively13, 6 and 10 AML patients, at very high risk of relapse and who were in remission following chemotherapy and pre-DC vaccination. In those 3 groups, the clinical response rate, as measured by a normalization of *WT1* mRNA tumor marker levels in blood and/or marrow, occurred in respectively 7/13, 1/4 and 0/6 patients. In addition, there were 4 patients with evidence of stable disease, some of it late, during DC vaccination. All patients, who did not normalize the *WT1* mRNA tumor marker level relapsed and/or died. Globally, 8/29 patients have not relapsed yet. Of those 8 patients, 5 had an increased *WT1* mRNA tumor marker level which normalized following DC vaccination, 3 of them now more than 5 years after the start of DC vaccination and most probably cured. One of those 3 patients, was in partial remission of AML following chemotherapy and was brought into complete and molecular remission by the DC vaccination only. Mono- or poly-epitope WT1-specific tetramer-positive T cells were detectable in all evaluable patients, with the highest frequency in patients who remained in complete remission after DC vaccination.
Summary and Conclusions: In conclusion, *WT1* mRNA-transfected DC vaccination is emerging as a non-toxic and effective strategy to prevent relapse in AML. Contrary to expectations, the original full-length *WT1* construct without MHC class II skewing signal has demonstrated the highest clinical activity so far.

S1144

INHIBITION OF PROTEIN GERANYLGERANYLATION SPECIFICALLY INTERFERES WITH CD40-DEPENDENT B CELL ACTIVATION RESULTING IN A REDUCED CAPACITY TO INDUCE T CELL IMMUNITY

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Background: Antibody-independent effector functions of B cells, such as antigen presentation and cytokine production, have been shown to play an important role in a variety of immune-mediated conditions such as autoimmune diseases, transplant rejection and graft-*versus*-host disease. Most current immunosuppressive treatments target T cells and are relatively unspecific and result a profound immunosuppression that places the patients at an increased risk of severe infections and development of cancer. Therapeutic strategies which interfere with B cell activation could therefore be a useful addition to the current immunosuppressive armamentarium.
Aims: The aim of this study was to identify novel immunomodulatory agents, which could be used to inhibit B cell activation and effector functions.
Methods: Human B cells were stimulated with CD40L, which is one of the most potent B cell-activating stimuli and strongly enhances the antigen-presenting function of B lymphocytes. The gene expression profiles of the CD40-activated B cells were compared to the those of resting B cells in order to determine pathways which were upregulated after CD40-activation.
Results: The transcriptomic analysis revealed that many of the of the genes which belong to the mevalonate pathway were upregulated following CD40-mediated activation of B cells. Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate limiting enzyme of the mevalonate pathway, by lipophilic statins such as simvastatin and atorvastatin resulted in a specific inhibition of B cell activation via CD40 and impaired their ability to act as stimulatory APC for allospecific T cells. Furthermore, statin-treated B cells acquired tolerogenic features and preferentially expanded regulatory T cells. Mechanistically, the inhibitory effect resulted from the inhibition of protein geranylgeranylation subsequent to the depletion of mevalonate, the metabolic precursor for geranylgeranyl.
Summary and Conclusions: Inhibition of geranylgeranylation either directly through geranylgeranyl transferase inhibitors or indirectly through statins represents a promising therapeutic approach for the treatment of diseases where antigen-presentation by B cells plays a role.

S1145

CELLULAR DYNAMICS OF THE INDUCTION OF T CELL-MEDIATED ANTITUMOR IMMUNITY BY A B CELL-BASED CANCER VACCINE

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Background: B cell-based cellular vaccines are a promising platform for cancer immunotherapy. Compared to dendritic cells little is known about the precise mechanisms of the induction of CD8 T cell responses by B cells.
Aims: The aim of this study was to determine the safety and effectiveness of B cell-based tumor immunotherapy in the murine B16 melanoma tumor model and to analyze the dynamics of the T cell-B cell interactions with regard to the clinical application of B cell-based cancer vaccines.
Methods: The kinetics of T-B cell interactions in a 3-D collagen matrix was studied using time lapse videomicroscopy. Mice where vaccinated by injection of *ex vivo* generated CD40-activated B cells either subcutaneously or intravenously.
Results: CD40-activated B cells express the full lymph node homing triad including CD62L, CCR7/CXCR4 and LFA1. Murine and human CD40-activated B cells migrate towards cognate ligands such as CCL19, CCL21 and CXCL12. Furthermore, such CD40-activated B express several T-cell chemoattractants and induce T-cell chemotaxis *in vitro*. To dissect T cell/APC interaction on a single cell we analyzed three-dimensional migration in collagen matrix. Interestingly, antigen-loaded CD40-B differ from immature and mature DC by displaying a rapid migratory pattern undergoing promiscuous, short-lived (7.5min) but stable interactions with cognate T cells. Furthermore, upon injection GFP+ CD40-activated B cells home to secondary lymphoid organs. Taken together, these data suggest that CD40-B are equipped with the receptors and migratory capacity necessary to home to secondary lymphoid organs and have the property to attract and enter into stable contacts with T cells. Preclinical evaluation of CD40-actived B cell-based tumor immunotherapy demonstrated the safety and effectiveness of this approach. Even the injection of high numbers of CD40-activated B cells did not result in any detectable toxicity. Furthermore, the B cell-based vaccine led to the induction of antigen-specific antitumor immunity.
Summary and Conclusions: In summary, we show fundamental differences in the mechanisms of cytotoxic T cell activation induced by DC and B cells. T cell stimulation by CD40-activated B cells, unlike T cell activation induced by DCs, primarily takes place through short-lived sequential interactions.we demonstrate that vaccination with peptide-loaded CD40-activated B cells elicits antigen-specific T cell-mediated antitumor immunity without causing toxicity. Thus CD40-activated B cell-based immunotherapy represents a promising approach to cancer immunotherapy.

Chronic lymphocytic leukemia - Clinical studies

S1146

THE BCL-2 INHIBITOR ABT-199 (GDC-0199) IS ACTIVE AND WELL-TOLERATED IN ULTRA HIGH-RISK RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: Patients (pts) with CLL and deletions of chromosome 17p (del(17p)) or whose disease is refractory to fludarabine (F) are considered to have ultra high-risk CLL. The median life expectancy of these pts is less than 2 to 3 years with standard therapy; therefore novel agents are urgently needed, particularly for this subgroup. Overexpression of BCL-2 and failure to trigger upstream pro-apoptotic pathways underpin resistance to apoptosis in these patients. *In vitro*, this block to apoptosis can be overcome by targeting mitochondria through BCL-2 inhibition. The first-generation BCL-2 inhibitor, navitoclax, achieved partial remissions (PRs) in 35% of pts with relapsed/refractory (R/R) CLL, however concomitant inhibition of BCL-X_L resulted in dose-limiting thrombocytopenia (TCP). ABT-199 is a second generation inhibitor with greater affinity for BCL-2, but 500-fold less affinity for BCL-X_L, than navitoclax. We hypothesized that selective BCL-2 inhibition with ABT-199 would demonstrate significant clinical efficacy, including in pts with ultra high-risk disease.
Aims: The primary objectives of this phase-I dose-escalation study are to evaluate the safety and pharmacokinetics (PK) of ABT-199, determine a maximum tolerated dose and recommended phase-2 dose, and assess efficacy and biomarkers in pts with R/R CLL. In this analysis, we have sought to determine if pts with ultra-high risk CLL have similar response rates to the overall study population.
Methods: Pts with ECOG performance status ≤1 and adequate marrow function received a single dose of ABT-199 on Week1 Day -3 or -7 (W1D-3, W1D-7), followed by continuous once-daily dosing from W1D1, until disease progression or unacceptable toxicity. After cohort1, the initial dose was reduced and daily dosing modified to include a 2 or 3 step dose-escalation to the target dose for each cohort. Evaluations included adverse events (AE; NCI-CTCAE-V4), PK parameters and disease response (IWCLL 2008).
Results: As of January 2013, 56 pts have been enrolled (see Table 1) in cohorts assessing doses from 150mg to 1200mg. Sixteen (29%) had del(17p) and 18 (32%) F-refractory CLL (see Table 1). The most common non-haematologicalAEs (>15% pts) were nausea (36%), diarrhea (30%), fatigue (25%), upper respiratory tract infection (23%), and cough (16%). Grade 3/4 AEs occurring in ≥5 pts were neutropenia 21(38%), thrombocytopenia 6 (11%) and tumour lysis syndrome (TLS) 5 (9%). The thrombocytopenia was not dose-related. With initial dosing, TLS occurred in 3/3 pts in cohort 1 and 2/53 pts with the modified dosing schedule (both were DLTs). Additionally, 1 fatal AE occurred within 48 hrs of dose-escalation to 1200 mg in a pt with laboratory evidence of TLS (DLT). 13 pts have discontinued, 7 due to progression, 6 for other reasons: TLS (2), other illness (2), thromboembolic event (1), consent withdrawal (1). After a single dose of ABT-199 with a low-fat meal, T_{max} and $T_{1/2}$ values were approximately 7 and 17 hrs, respectively, a pattern supporting daily dosing. Preliminary efficacy data are summarised (Table 1).

Table 1.

Characteristic	All CLL n=56	del (17p) n=16*	F-refractory n=18*
Age (yr)†	67 [36-86]	69 [47-80]	66 [36-78]
Male#	41 (73)	11 (69)	12 (67)
Number of prior therapies†	3.5 [1-10]	4 [2-9]	5 [1-10]
Bulky disease >= 5 cm#	28 (50)	6 (38)	10 (56)
Bulky disease >= 10 cm#	8 (14)	0 (0)	4 (22)
Time on study (days)†	190 [1-495]	159 [33-495]	203 [1-484]
Response			
Time to first 50% reduction in nodes (days)†	43 [20-417]	43 [33-81]	44 [20-88]
Best response‡	n=54	n=16	n=18
CR/CRi	7 (13)	1 (6)	3 (17)
PR	39 (72)	13 (81)	11 (61)
SD	4 (7)	1 (6)	1 (6)
PD	0 (0)	0 (0)	0 (0)
D/C prior to first (W6) assessment	4 (7)	1 (6)	3 (17)
Response Rate (CR + PR)	85%	88%	78%

†median [range]; #n(%);*6 pts had both del(17p) and F-refractory disease
‡Evaluable pts either 1) completed at least a W6 assessment or 2) discontinued (D/C) prior to W6

Summary and Conclusions: ABT-199 is highly active in ultra high-risk CLL, achieving a response rate of 88% in del(17p) and 78% in F-refractory pts, comparable to the 85% response rate in the whole study population Additional dosing and scheduling modifications are currently being explored to minimise the risk of TLS while preserving efficacy. (study status see clinicaltrials.gov)

S1147

RANDOMISED COMPARISON OF FCR-LITE AND CLR (CHLORAMBUCIL PLUS RITUXIMAB) REGIMENS IN ELDERLY PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chlorambucil is still considered as standard of care in elderly and physically unfit patients with chronic lymphocytic leukemia (CLL). However, a considerable number of patients have insufficient or short responses to chlorambucil and there is a need to develop safe and more efficacious treatment approaches. Two regimens have been proposed for treatment of patients with comorbidities and elderly patients: FCR-Lite and CLR, both showing relatively high efficacy and good tolerability.
Aims: To compare in randomized trial FCR-Lite and CLR regimens in treatment of elderly patients with chronic lymphocytic leukemia.
Methods: Untreated CLL patients older than 71 years and physically unfit patients aged 61–70 years with cumulative illness rating score (CIRS) 7 and more were included into the trial. After Informed consent the patients were randomly assigned to receive either FCR-Lite (F oral 32 mg/m2 for three days, C oral 150 mg/m2 for three days) or CLR (Chl 10 mg/m2 for 7 days). Rituximab was given once in each cycle in both regimens (375 mg/m2 i.v. day 0 at first cycle and 500 mg/m2 day 1 all subsequent cycles). Cycles were repeated every 4 weeks.
Results: 97 patients have been included into the study. The patient's characteristics were relatively well balanced between arms with regard to age, stage, genomic aberrations and VH status. 72% were Binet B, 16% Binet C and 12% Binet A. The median age was 71 years (range 60 to 84), 47 patients were females (48%), the median CIRS score was 8 (range 1-18). The overall incidences of trisomy 12 and abnormalities of 13q, 11q23, and 17p13 detected by FISH were8,5%, 50%, 21,3%, and6,4%, respectively, with no statistically significant differences between treatment arms. One patient withdraw consent, 1 patient died from pneumonia before initiation of treatment, and 3 patients discontinued treatment due toxicity or worsening of concomitant condition. Ninety two patients were available for assessment. At the time of analysis, February 2013, the median observation time was 29.8 months. A mean number of 5.1 courses was given in the CLR arm *versus* 5.3 courses in the FCR-Lite arm. 72% (FCR-Lite) and 66% (CLR) of patients received 6 cycles. There was no significant differences in grade III–IV myelotoxicity in FCR-Lite and CLR arms: neutropenia was observed in 36% patients and 34% patients, anemia 4% and2,6% and thrombocytopenia in 8% and 13%, respectively. The incidence of CTC grade 3 or 4 infections was also not significantly different (17,7% in FCR-Lite arm *versus*14,8% in the CLR arm (P=0.9). The overall response rate of the FCR-Lite arm was 93% as compared to 85% in the CLR arm (P<0.35). The complete response rate was significantly higher in the FCR-Lite arm (42%; 19/45) compared to CLR (10,6%; 5/47) (P=0.001). Forteen patients (31%) in FCR-Lite arm achieved an MRD-negative CR while in CLR arm there were no MRD-negative cases. Median PFS in CLR arm was 25,3 months, while in FCR-Lite arm it was not reached (P=0,0042). PFS at 2 years in FCR-Lite arm was 72,6%. To date, 80 patients remain alive and 17 patients (17%) have died. In LR arm 6 patients died from progression, 2 from secondary tumors and 3 from other causes. In FCR-Lite arm there have been no deaths from progression, 3 patients died from secondary tumors and 3 from other causes.
Summary and Conclusions: Preliminary results show that FCR-Lite is more efficacious in terms of response and progression free survival without significant increase of toxicity.

S1148

RESULTS OF THE RANDOMISED PHASE II NCRI ADMIRE TRIAL OF FCR AND FCM-R IN PREVIOUSLY UNTREATED CLL: ORAL FCR IS HIGHLY EFFECTIVE AND SAFE BUT THE ADDITION OF MITOXANTRONE DOES NOT IMPROVE RESPONSES

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Background: Fludarabine, cyclophosphamide and rituximab (FCR) results in improved progression free (PFS) and overall survival (OS) compared to FC. Initial evidence from non-randomised Phase II trials suggest the addition of mitoxantrone to FCR improves remission rates. We previously demonstrated that the addition of mitoxantrone and rituximab to oral FC (FCM-R) was safe and potentially effective in previously treated patients with CLL.
Aims: To compare the primary end-point (complete response (CR) rates) and the short-term secondary endpoints (Minimal Residual Disease (MRD), ORR and safety) for FCR and FCM-R in previously untreated CLL.
Methods: ADMIRE was a phase IIB, randomised, controlled, parallel group trial in previously untreated CLL performed in 29 UK centres. 218 patients were planned to be randomised on a 1:1 basis to FCR or FCM-R, to provide 80% power to detect a 20% improvement in CR rate. Patients required therapy by IWCLL criteria and were considered fit for fludarabine-based therapy. The schedule for oral FCR was equivalent to iv FCR with iv rituximab given on Day 1 of each cycle (375mg/m^2 Cycle 1; 500mg/m^2 Cycles 2-6), oral fludarabine at 24mg/m^2/day for 5 days (Days 1-5) and oral cyclophosphamide at 150mg/m^2/day for 5 days (Days 1-5). FCM-R included intravenous mitoxantrone (6mg/m^2/day on Day 1 of each cycle). Six cycles were planned 28 days apart. Patients with neutropenia delaying any cycle of therapy received G-CSF (lenograstim 263mcg/day; Days 7-13) on all subsequent cycles. All patients received prophylaxis with co-trimoxazole and acyclovir.
Results: 215 patients were recruited June 2009 to April 2012 with 107 receiving FCR and 108 FCM-R. The treatment arms were well balanced for the following: median age of 62 (range 33–77) and 66% (n=141) under 65; 76% (n=163) male; 12% (n=26) progressive stage A, 52% (n=111) stage B and 36% (n=78) stage C; 56% (100/177) had unmutated Ig genes; 5% (10/187) were 17p deleted; and 19% (36/187) were 11q deleted. More 17p deleted patients were randomly allocated to FCR (8 *vs* 2). 28% (61/215) discontinued therapy early including 23% (n=25) for FCR and 33% (n=36) for FCM-R. Overall, 10% (n=21) received 1-3 cycles, 8% (n=8) FCR and 12% (n=13) FCM-R. 60% (n=128) of patients received G-CSF during therapy with a higher proportion on FCM-R compared with FCR (68% *vs* 60%). 62% (n=134) of patients experienced some form of dose modification including 61% for FCR and 64% for FCM-R; the most common modification was dose delay. 150 SAE's were reported from 95 patients; 69 events from 42 receiving FCR and 81 from 53 receiving FCM-R. 91 patients (43%) were hospitalised due to an SAE, 41 (39%) FCR compared with 50 (47%) FCM-R. There were no treatment related deaths. At the time of writing 172 patients (80%) had undergone independent central review for response, with 36 outstanding and 7 being ineligible or withdrawn. 151 patients are currently included in the primary endpoint analysis; with 21 excluded due to good clinical responses but missing trephine biopsies so that CR could not be assessed. 70% (105/151) of available patients achieved a CR; 71% (53/75) FCR and 68% (52/76) FCM-R. ORR was 97% (n=145/150) in available patients, 96% (72/75) FCR and 97% (73/75) FCM-R. MRD by flow cytometry (sensitivity<10^{-4}) in the bone marrow was assessed at 3 months post-therapy; of those assessable 57% (106/186) had undetectable MRD, 62% (58/93) FCR compared to 52% (48/93) FCM-R.
Summary and Conclusions: Oral FCR is well tolerated with acceptable toxicity. Dose intensity was supported with G-CSF in 60% of patients. The complete remission rate (CR+CRi) at 70% and the MRD negativity rate of 62% were very high compared to previous collaborative group trials. There was no improvement in the CR rate with the addition of mitoxantrone, fewer patients achieved an MRD negative remission and there was slightly increased toxicity; concluding that the addition of mitoxantrone to FCR will not be taken forward into a Phase III trial. The higher than predicted CR and MRD negativity rates for FCR compared to previous reports may be due to an increased use of growth factor support, close attention to dose intensity, routine use of prophylactic antibiotics and/or the use of oral fludarabine and cyclophosphamide.

S1149

HAIRY CELL LEUKEMIA: EVALUATION OF LONG-TERM OUTCOMES IN 487 PATIENTS

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Background: Hairy cell leukemia (HCL) is a rare hematologic disorder. Purine analogues (pentostatin and cladribine) have transformed the course of the disease with similar overall responses obtained in more than 85% of patients, with median relapse-free survival up to 15 years. With prolonged survival, HCL patients tend to develop further malignancies. Isolated case reports and single center series described the different malignancies observed.
Aims: We conducted a large, multi-center, retrospective survey in France to determine the frequency of malignancy in HCL patients (pts) and their families, and to analyze the long-term effects of the established purine nucleotide analogs (PNA) cladribine (C) and pentostatin (P).
Methods: Physician members of the Société Française d'Hématologie were surveyed on their management of HCL pts over the past 30 years. Clinicians completed a form that collected data concerning personal and familial medical history of pts, with particular focus on hematologic malignancies, solid tumors, clinical and biological presentation at HCL diagnosis, therapeutic options, response to treatment, time to relapse, second malignancies and cause of death.
Results: We studied 487 HCL patients (mean patient age 59y; range 29–90y) from 36 French clinical centers. HCL diagnosis was established after examination of peripheral blood and/or bone marrow and by immunophenotyping. Eighty-eight patients (18%) had familial history of cancers, with one familial HCL case. Forty-one patients (8%) presented solid tumors and hematologic malignancies before HCL diagnosis with a median time of 89 months (range 2–529). Twenty-three patients (5%) received no treatment. Three hundred and forty-five patients (71%) received just one first-line treatment (C: 235 pts, P: 82 pts, interferon: 13 pts, other: 15 pts), and 119 pts (24%) received two-to-seven lines of therapy. After first-line treatment, although complete response (CR) rates and median relapse free survival (RFS) were similar between C (CR:85.1%, RFS:164mo) and P (CR: 85.3%, RFS:159mo), overall survival (OS) is better with C than P (respectively 97% *versus* 89%, at 5 years). Whereas more patients achieved CR in second-line treatment with C than P, RFS and OS are significantly better when patients received P. Twenty-nine pts (6%) died (11 HCL-related, 6 related to second malignancies). Forty-eight pts (10%) developed second malignancies (34 solid tumors, 10 hematologic malignancies, 4 both) after a median time of 74 months (2-375). Excess of incidence of second malignancies, including solid and hematologic malignancies, is observed with a standardized incidence ratio (SIR) at 1.86 (IC95%: 1.34-2.51) with no significant difference between the two PNAs. SIR for second hematologic malignancies was particularly increased at 5.32 (IC95%: 2.90-8.92).
Summary and Conclusions: This study highlights the high frequency of cancers in HCL pts (18%) and their family members, suggesting a role for genetic factors in the development of the disease. First-line treatment based on PNAs, P or C, is very efficient with OS at 5 years at 95%. Frequency of second malignancies is particularly increased (10%) with a SIR at 1.86, especially concerning hematologic malignancies (SIR=5.32). The respective role of P and C in the development of second malignancies is debatable. PNA remains the reference treatment in HCL, as long as secondary effects, especially second malignancies, of emerging therapeutics are not clearly evaluated. Indeed secondary mutations in RAS pathway have been described in patients treated with BRAF inhibitors. We recommend the use of PNAs in first-line, reserving BRAF inhibitors exclusively in very high-risk HCL patients.

S1150

UPDATE ON A PHASE 1 STUDY OF THE SELECTIVE PI3K-DELTA INHIBITOR, IDELALISIB (GS-1101) IN COMBINATION WITH OFATUMUMAB IN PATIENTS WITH RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: PI3K-delta (δ) is critical for activation, proliferation and survival of B cells and plays a role in homing and retention in lymphoid tissues. Ide-

lalisib is a first-in-class, targeted, highly selective, oral inhibitor of PI3Kδ that has shown considerable monotherapy activity in patients with heavily pretreated CLL.

Aims: The primary objective of this study was the assessment of safety and the secondary objective was the evaluation of clinical response: overall response rate (ORR), progression-free survival (PFS), duration of response (DOR), overall survival (OS). The study is ongoing.

Methods: The study evaluated idelalisib (GS-1101) for relapsed/refractory CLL continuously given at 150 mg BID in combination with a total of 12 infusions of ofatumumab (O, 300 mg initial dose either on Day 1 or Day 2 relative to the first dose of idelalisib, then1,000 mg weekly ×7, then1,000 mg every 4 wks × 4). Pts who were benefitting from therapy after the 48 weeks of the primary study were eligible to continue idelalisib on an extension study. Clinical response was evaluated according to published criteria (Hallek 2008; Cheson 2012).

Results: 21 pts (6F/15M) with a median (range) age of 66 (43-79) years and a WHO performance status of 0 (13, 62%) or 1 (8, 38%) were enrolled. Adverse disease characteristics (n,%) included bulky lymphadenopathy (12, 57%), refractory disease (7, 33%), multiple prior therapies (median:2, range: 1-6). Almost all patients (20, 95%) had at least 1 prior therapy containing rituximab (R), and 9 (43%) were refractory to R. 3 (14%) had received prior O. Prior therapies also included alkylating agents (18, 86%, [bendamustine:11, 52%]) and purine analogs (16, 76%, [fludarabine:15, 71%]). 8/21 (38%) pts had evidence of del17p, and data available from 13 pts showed that 10 (77%) had unmutated IGHV. As of 22 Feb 2013, the median (range) treatment duration was 11 (0-18) months. 10/21 (48%) pts have completed the primary study and enrolled into the extension study. 8/21 (38%) discontinued: 3 for reasons of AE and 2 for other reasons. There were 3 deaths that occurred on study. 7/21 (33%) pts are continuing idelalisib treatment on the extension study. The ORR (ITT, as assessed by investigators) was 76% (16/21), with 3 CRs (14%, 1 unconfirmed), and a median (range) time to response of 1.9 (1.8-8.3) months. Median progression-free survival and duration of response were 17.8 months and 15.9 months, respectively. Median overall survival (OS) has not been reached; OS at 11 months was 80%. The most common TEAEs (any Grade/≥Gr3, regardless of causality) included diarrhea (48%/5%), cough (43%/0%), pyrexia (38%/0%), dyspnea (33%/0%), nausea (24%/0%), pneumonia (19%/19%), fatigue (19%/0%), and rash (19%/0%). Elevation of liver transaminases (TA, any Grade/≥Gr 3) was seen in 29%/14%. Of those, only 1 pt discontinued the study because of (recurrent) TA elevation.

Summary and Conclusions: The combination of idelalisib with ofatumumab represents a non-cytotoxic regimen with a favorable safety profile and high activity resulting in durable tumor control in pts with high-risk pretreated relapsed/refractory CLL. Phase 3 trials evaluating the efficacy of idelalisib in combination with therapeutic anti-CD20 antibodies such as ofatumumab or rituximab are ongoing (NCT01659021, NCT01539512).

Clinical studies in multiple myeloma 2

S1151

EFFICACY, SAFETY, AND QOL IN MM-003, A PHASE3, MULTICENTER, RANDOMIZED, OPEN-LABEL STUDY OF POMALIDOMIDE (POM)+LOW-DOSE DEXAMETHASONE (LODEX) VS HIGH-DOSE DEXAMETHASONE (HIDEX) IN RRMM

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Background: Relapsed/refractory multiple myeloma (RRMM) patients (pts) who are refractory to bortezomib (BORT) and lenalidomide (LEN) treatment (Tx) have a poor prognosis with short overall survival (OS) and reduced quality of life (QOL). HiDEX is commonly used to treat RRMM. POM was recently approved by the US FDA for the treatment (Tx) of RRMM patients who have received at least 2 prior therapies, including BORT and LEN.

Aims: MM-003 study compared POM+LoDEX *vs.* HiDEX in RRMM pts who failed BORT and LEN and progressed on their last Tx.

Methods: Pts must have been refractory to last prior Tx (progressive disease [PD] during or within 60 days) and failed BORT and LEN after ≥2 consecutive cycles (C) of each (alone or in combination). Pts were randomized 2:1 to receive 28-day C of POM 4 mg D1-21+LoDEX 40 mg (20 mg for pts aged >75 y) weekly or HiDEX 40 mg (20 mg for pts aged >75 y) D1-4, 9-12, and 17-20. Tx continued until PD or unacceptable toxicity. The primary endpoint was progression-free survival (PFS) and secondary endpoints included OS, overall response rate (ORR; ≥partial response [≥PR]), duration of response, safety, and QOL. For pt-reported QOL outcomes, change scores and minimal important differences were calculated as meaningful change from baseline through C5 (1 standard error of measurement) for the 5 clinically relevant EORTC QLQ-C30 domains (Global Health Status, Physical Functioning, Fatigue, Emotional Functioning, and Pain). Time to QOL worsening was compared between arms by the Kaplan-Meier method.

Table 1.

	POM + LoDEX (n = 302)	HiDEX (n = 153)	*P* Value	
All patients				
ORR, %	21	3	<.001	
Median duration of response, mos	10.1	Not estimable		
Pts ≥ 6 Mos Post-Enrollment	**n = 204**	**n = 99**		
ORR, %	24	3	<.001	
Median duration of response, mos	10.1	Not estimable		
Survival Outcomes, mos	**n = 302**	**n = 153**	**HR**	**Log-Rank *P* Value**
Median PFS	3.6	1.8	0.45	<.001
Median OS	Not reached	7.8	0.53	<.001
Median Time to First Worsening in QOL Domains, days (95% CI)			**Log-Rank *P* Value**	
Global Health Status	114 (71-143)	85 (37-140)	.058	
Physical Functioning	174 (123-288)	106 (57-NE)	.088	
Fatigue	113 (71-169)	60 (57-113)	.038	
Emotional Functioning	190 (145-361)	124 (64-235)	.023	
Pain	147 (89-NE)	113 (58-NE)	.203	

Results: 455 pts were randomized to POM+LoDEX or HiDEX. The median number of prior Tx was 5 (range 1-17). 72% were refractory to LEN and BORT. Median follow-up was 4 mos. POM+LoDEX significantly extended median PFS and OS *vs.* HiDEX, despite 29% of HiDEX pts receiving POM after PD (Table 1). At

this point, the Data Monitoring Committee recommended crossover from HiDEX to POM±LoDEX. As of Nov9, 2012, the ORR remained significantly higher for POM+LoDEX *vs.* HiDEX (Table 1). The most frequent grade 3-4 adverse events (AEs) for POM+LoDEX *vs.* HiDEX were neutropenia (42% *vs.* 15%), anemia (27% *vs.* 29%), and infection (24% *vs.* 23%); discontinuation due to AEs was infrequent (7% *vs.* 6%). Regarding QOL, favorable trends were observed for POM+LoDEX *vs.* HiDEX in each of the 5 relevant domains. QOL in responders *vs.* patients who have progressed by arm indicated statistically significant differences favoring POM+LoDEX responders in Physical Functioning (*P*=.005) and Fatigue (*P*=.032). In all pts, by Kaplan-Meier estimation, POM+LoDEX extended median time to meaningful symptom and QOL worsening *vs.* HiDEX for all 5 domains (Table 1). Updated results will be presented at the meeting.
Summary and Conclusions: POM has shown activity in MM pts refractory to LEN and BORT. In this study, POM+LoDEX significantly extended PFS and OS *vs.* HiDEX and resulted in improvements in clinically relevant QOL measurements over the course of Tx. POM+LoDEX should become a standard of care in these pts.

S1152

LENALIDOMIDE ASSOCIATED WITH CYCLOPHOSPHAMIDE AND DEXAMTHASONE (CRD) IS AN EFFECTIVE NON TOXIC OPTION FOR NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS

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Background: The advent of new drugs used in triplet combinations with alkylating agents and steroids has dramatically improved the outcome of MM patients, however peripheral neuropathy (PN) and the potential for myelosuppression and second primary malignancies (SPMs) have made their use in some settings difficult. Among the new drugs, lenalidomide (Len), due to his oral availability and the lack of treatment related PN, is particularly attractive.A recent phase III study has combined Len with Melphalan and Prednisone followed by Len maintenance (MPR-R) obtaining good results, but myelosuppression and SPMs were seen.[1] Combining Len with Cyclophosphamide (Cy) and Dexamethasone (Dex) is particularly attractive because of its reduced cytotoxicity and mutagenicity. We investigated the CRD triplet in relapsed patients in phase I/II studies.[2-3] A total of 52 heavily pre-treated patients were treated in the 2 studies with good response rate (CR+PR 65%>81%) and manageable toxicities (Table 1).

Table 1.

	Phase I	Phase I/II	Phase III
AR	% pts	% pts	% pts
Neutropenia	38%		24.9%
Thrombocytopenia	NA	32%*	7.6%
Anemia	NA		12.9%
Infections	29%	6%	NA
PN	NA	0%	0.6%
DVT/PE	14%	6%	5.6%
Others	NA	19%	30.9%**

*all cytopenias
**including infections

Aims: The positive results of these studies led us to design a phase III study (MRC Myeloma XI trial) which compared CTD with CRD in newly diagnosed MM patients. As part of the safety analysis we have looked at the toxicity of pts receiving CRD.
Methods: The trial is ongoing and has recruited 1054 patients (pts) in the younger intensive pathway and 828 in the non intensive one. Based on the dose finding studies CRD doses were: Cy 500 mg on days 1 and8, Len 25 mg on days 1-21 and Dex 40 mg on days 1-4, 12-15 given for a minimum of 4 to 6 cycles until maximum response or unacceptable toxicity. Older or frail pts received attenuated doses of Dex (20 mg on days 1-4 and 15-18). The response rates with the combination in studies up to know have been excellent, with OR rates of 81% and CR rates of 29% in relapsed patients, with maximum response developing in a median of 3 cycles. Response rates in presenting patients will be higher and updated results will be presented.
Results: Of the 1882 pts that entered the trial 937 have been randomised to CRD treatment. Data for pts receiving at least 1 cycle are available on 711 pts (75.9%), of which 495 (69.6%) have completed induction. Mean number of cycles received has been 4.8 (±1.99), with a median of 5 (range 1-18). A dose modification of any of the 3 drugs was needed in 62.2% of pts (Cy in 27.6%, Len in 47.4% and Dex in 40.6%), but only 39 pts (5.5%) stopped induction due to toxicity before receiving the minimum number of cycles required. More than 60% of the pts (63.3%) were able to receive the prescribed therapy without any delay. Grade 3-4 hematologic adverse events were reported in 24.9% (neutropenia), 7.5% (thrombocytopenia) and 12.6% (anaemia) of the patients, respectively. The incidence of any extra-hematologic adverse reaction (AR) grade 3-4 was 30.3%. No grade 4 PN was reported; PN grade 3 (sensory or motor) was seen in 0.6% of the patients. Deep vein thrombosis (DVT) was reported in 3.3% of patients and the incidence of pulmonary embolism (PE) was 2.3%. SAEs are available for all the randomized population, and were reported in 535/937 pts (57.1%). About half of the SAEs were related to at least one of the study drugs; the most frequent were infections (39.1%) and cytopenias (18.5%). Treatment related mortality was 0.6%. With a median follow up of 1.3 years from beginning of therapy the incidence of SPMs has been 0.3% (n=3).
Summary and Conclusions: Overall our data confirm that CRD treatment has a good safety profile, and is therefore a valid option for both young and old newly diagnosed MM patients.

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S1153

SECOND PRIMARY MALIGNANCIES (SPM) IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS ACCORDING TO LENALIDOMIDE EXPOSURE: A META-ANALYSIS OF 6383 INDIVIDUAL PATIENT DATA

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Background: Three large randomized trialsrecently reported an increased risk of second primary malignancies (SPMs) in newly diagnosed myeloma (MM) patients treated with lenalidomide. However, patients with MM have multiple risk factors for SPM and the excess risk with lenalidomide compared with non-lenalidomide has not been described.
Aims: We performed an individual patient data meta-analysis to estimate the incidence of SPM according to lenalidomideexposure.
Methods: Relevant studies, from PubMed and ASCO/IMW/ASH abstracts (after 2000), that met the following criteria were included: (1) randomized trials of newly diagnosed MM patients; (2) randomization to treatment with lenalidomide in at least one arm (lenalidomide-trials); (3) randomization to treatment to at least one new drug but not lenalidomide (no-lenalidomide-trials); (4) available data of SPMs. The primary aim was the cumulative incidence of SPMs,that was estimated accounting for competing events (Gooley *et al.*).
Results: 6383 patients were included in the analysis. The median follow-up was 30 months. Median age was 69 years, 45% of patients aged 65-74 years and 22% ≥75 years. Total cases of SPMs were 420 (6.6%), including 188 (2.9%) hematologic and 232 (3.6%) solid cancers. The cumulative incidence at 3 and 5 years of SPMs and of death were summarized in the Table 1. 3218 patients were enrolled in the lenalidomide-trials and were available for adirect comparisons between lenalidomide *vs* non-lenalidomide treatments.Solid

tumors occurred with similar incidence in all treatment groups. The risk of hematologic SPM was significantly higher in patients receiving lenalidomide (5-year cumulative incidence 3.2% *vs* 1.1%, P=0.04) and increased linearly over time. Therisk is limited to patients receiving lenalidomide plus melphalan (4.1, 95%CI: 2.4-5.8) with no excess in other combinations (lenalidomide without melphalan: 1.2, 0.0-2.6; melphalan without lenalidomide: 1.1, 0.0-2.7) (P=0.003).The risk of death for adverse events and for progression was higher than the risk of SPM.

Table 1. Cumulative incidence (%) at 3 and 5 years of SPMs and death (95% CI).

	3-year estimation			5-year estimation		
	Lenalidomide Trials		No lenalidomide Trials	Lenalidomide Trials		No lenalidomide Trials
Cumulative incidence	Lenalidomide Arms	No lenalidomide Arms		Lenalidomide Arms	No lenalidomide Arms	
SPMs						
Overall	5.2 (4.0-6.3)	3.8 (2.2-5.4)	3.2(2.4-4)	10.2 (8.0-12.4)	7.2 (4.1-10.3)	6.2(5.1-7.4)
Hematologic	1.4 (0.8-2.1)	0.3 (0.0-0.8)	1(0.6-1.5)	3.2 (2.0-4.4)	1.1 (0.0-2.7)	2.6(1.8-3.3)
Solid	3.7(2.8-4.7)	3.5(1.9-5.1)	2.2(1.5-2.8)	7(5.1-8.9)	6.1(3.4-8.8)	3.7(2.8-4.5)
Death						
All Causes	23.3 (21.2-25.6)	24.8 (21-29.2)	36.3 (34.5-38.8)	47 (43.1-51.2)	64.8 (54-75.5)	56.2 (53.5-58.6)
Toxicity (infections, cardiac, etc)	6.6(5.4-7.8)	6.0(4.2-8.9)	8.2(7-9.4)	9.8(8-11.7)	17.5(11.4-23.6)	10.8(9.4-12.2)
MM	13.2(11.4-15)	14.9(11.6-18.1)	14.8(13.2-16.4)	25.9(22.6-29.2)	35.4(25.6-45.2)	24.4(22.4-26.3)

The source trials have been registered with the following identifier numbers: EudraCT 2005-004714-32; EudraCT 2007-004007-34; ASTAT 2004-000665-36; ISRCTN 90632740; NCT 00551928; NCT 01160107; NCT 01091831; NCT 00134101; NCT 00098475; NCT 01093136; NCT 00232934; NCT 00378105; NCT 00405756; NCT 00218855; NCT 01063179.

Summary and Conclusions: An increase of SPMs was observed in patients receiving lenalidomide compared with controls. The observed difference was attributed to the increased occurrence of hematologic SPMs, mainly with combination including lenalidomide plus melphalan. In the context of the observed survival benefit, the benefit/risk profile of lenalidomide treatment remains positive.

S1154

SUBCUTANEOUS VELCADE PLUS PREDNISONE (VP) OR PLUS CYCLOPHOSFAMIDE (VCP) OR PLUS MELPHALAN (VMP) IN FRAIL, ELDERLY, NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: A PHASE II COMMUNITY-BASED STUDY.

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Background: Bortezomib-Melphalan-Prednisone (VMP) is the standard of care for the treatment of newly diagnosed elderly multiple myeloma (MM) patients. Subcutaneous and weekly bortezomib infusions were both associated with a significant reduction in adverse events (AEs), such as peripheral neuropathy, without affecting efficacy. Frail elderly patients are more susceptible to AEs with subsequent treatment discontinuation that significantly affect efficacy and dose-intensity, thus suggesting the need for a reduced-dose strategy.
Aims: To assess the safety and the efficacy of 3 reduced-dose intensity subcutaneous (sc) bortezomib-based treatments in elderly, frail, newly diagnosed MM patients unsuitable for protocol with standard inclusion criteria.
Methods: Treatment included nine 28-day cycles of bortezomib 1.3 mg/m^{2}sc days1,8,15, 22 plus oral prednisone 50 mg every other day (VP) or VP plus oral ciclophosphamide 50 mg every other day (VCP) or oral melphalan 2 mg every other day (VMP) for 28 days, followed by maintenance with sc bortezomib every 2 weeks until progression.
Results: A total of 152 patients were enrolled, including 51 patients in the VP, 51 in the VCP and 50 in the VMP group. Median age was 78, 76 and 77 years, and 40%, 24% and 30% of patients were older than 80 years in the VP, VCP, and VMP groups, respectively. Overall, 76%, 71% and 72% had ISS stage II/III disease and 18%, 21% and 20% of the patients were defined at high risk by FISH with at least one chromosomal abnormalities among del17, or t (4;14), or t(14;16). Frail patients defined as Charlson comorbidity index ≥2 or Activity of Daily Living score (ADL) ≤4 or Instrumental Activity of Daily Living score (IADL) ≤5 or age ≥80 years were 66%: 78% in the VP, 57% in the VCP and 62% in the VMP group. Median follow-up was 10 months. Patients received a median of 6 VP, 8 VCP, and 7 VMP treatment cycles. All three induction regimens exhibited substantial activity, with an overall response rate (≥partial response) of 60% in the VP, 60% in the VCP, and 70% in the VMP group (P=0.67). At least 1 grade 3-5 adverse event was reported in 31% of patients in the VP group, 41% in the VCP group and 42% in the VMP group (P=0.41). Hematologic grade 3 AEs occurred in less than 10% patients in all groups. The incidence of non hematologic grade 3 AEs was similar in the 3 groups and were mainly infective (12%), cardiovascular (8%), and neurologic (7%), including 5% of peripheral neuropathy. Discontinuation rate due to AEs was 14% in the VP, 16% in the VCP and 22% in the VMP group (P=0.30, VP *vs* VMP). In frail patients, the overall response rate was 60% in VP, 52% in VCP, and 77% in the VMP group (P=0.13). At least 1 grade 3-5 adverse event was reported in 30% of the VP, 52% of the VCP and 48% of the VMP group (P=0.14). Discontinuation rate due to AEs was 15% in the VP, 21% in the VCP and 23% in the VMP group (P=0.53, VP *vs* VMP) (Table 1).

Table 1.

	Fit (N=52)			Frail* (N=100)		
	VP (N=11)	VCP (N=22)	VMP (N=19)	VP N=40	VCP N=29	VMP N=31
≥PR	82%	68%	58%	60%	52%	77%
At least 1 grade 3-5 adverse event	36%	27%	32%	30%	52%	48%
Discontinuation rate for toxicity	9%	9%	21%	15%	21%	23%

* Charlson comorbidity index ≥2 or Activity of Daily Living score (ADL) ≤4 or Instrumental Activity of Daily Living score (IADL) ≤5 or age ≥80 years.

Summary and Conclusions: In frail elderly newly diagnosed MM patients, the doublet VP regimen showed a similar efficacy compared to VCP and VMP triplet combinations. Rate of discontinuation due to AEs was lower in the VP group. Longer follow-up is needed to assess long-term outcomes.

S1155

IMPACT OF BORTEZOMIB INCORPORATED INTO AUTOTRANSPLANTATION ON OUTCOMES OF MM PATIENTS WITH HIGH-RISK CYTOGENETICS: AN INTEGRATED ANALYSIS OF 1610 PATIENTS ENROLLED IN FOUR EUROPEAN PHASE 3 STUDIES

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Background: Over the last decade, integration of novel agents into ASCT for younger patients with newly diagnosed MM has significantly improved their clinical outcomes. However, whether the novel agents are able to improve or overcome the adverse prognosis related to several genomic aberrations is still an open question.
Aims: The European cooperative groups HOVON/GMMG, IFM, PETHEMA/GEM and GIMEMA conducted 4 phase III studies aimed to compare bortezomib-based (B-b) induction regimens (VD or VTD or PAD) *vs* non-bortezomib-based (nB-b) ones (VAD or TD) before single or double ASCT. We performed an integrated analysis of data from these studies to evaluate the role of B-b ASCT *vs* nB-b ASCT, in particular in patients with or without cytogenetic abnormalities (cyto pos or neg) detected by FISH.
Methods: Out of a total of 2169 patients who were enrolled, 74% had available data on the percentage of cyto pos or neg CD138-purified plasma cells and were included in the current analysis. A high-risk cytogenetic profile (HR-cyto) was defined by the presence of t(4;14) and/or del(17p).
Results: In comparison with control treatment, B-b induction significantly increased the rate of CR before ASCT in the overall population (4% *vs* 15%, P<.001) and in the HR-cyto subgroup (0% *vs* 18%, P<.001). With a median follow-up of 37 months, median PFS was significantly longer for patients randomly assigned to receive B-b ASCT (41.5 mos *vs* 33 mos for the control group) (HR=0.76, P<.001), a gain retained across subgroups lacking (HR=0.79, P=0.010) or carrying HR-cyto (HR=0.58, P<.001). A marginal OS benefit favoring B-b ASCT was observed in comparison with nB-b ASCT (HR=0.85,

P=0.058). In a stratified multivariate analysis, PFS and OS in the overall population were favorably influenced by the lack of the following variables: cytogenetic abnormalities, thrombocytopenia and high β2-m. Additional independent variables significantly predicting for extended PFS and OS in the overall population included achievement of CR after induction therapy (HR=0.46 and 0.44 for PFS and OS), double ASCT (HR=0.46 and 0.33 for PFS and OS) and B-b ASCT (HR=0.74 and 0.79 for PFS and OS). These latter variables independently affected PFS and OS in the HR-cyto subgroup (CR after induction: HR=0.49 and 0.43 for PFS and OS; double ASCT: HR=0.34 and 0.25 for PFS and OS; B-b ASCT: HR=0.61 and 0.69 for PFS and OS). B-b ASCT was independently associated with prolonged PFS (HR=0.60), but not with OS, in patients with positive del(17p) but negative t(4;14). Within the group assigned to receive B-b ASCT, patients who carryied both t(4;14) and del(17p) (ultra HR-cyto) had the worst outcomes (median PFS: 21 mos) and in a multivariate analysis were likely to mostly benefit from novel-agent-based double ASCT (HR=0.13 and 0.28 for PFS and OS).

Summary and Conclusions: Results from this integrated analysis of different studies including different B-b induction regimens demonstrated the superiority of B-b ASCT over nB-b ASCT in terms of higher rate of CR and extended PFS in newly diagnosed MM. PFS benefit with B-b ASCT was retained across subgroups of patients with and without HR-cyto. In comparison with the control group, B-b ASCT significantly improved the outcome of patients with HR-cyto, but did not overcome the adverse prognosis related to t(4;14) and/or del(17p). Results of OS analysis should be cautiously interpreted due to the still short follow-up. Finally, apparent benefits observed with novel agents integrated into double ASCT in the subgroup of patients with HR-cyto and ultra HR-cyto need to be confirmed in prospective randomized studies.

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SINGLE-AGENT LENALIDOMIDE IN PATIENTS WITH RELAPSED/ REFRACTORY MANTLE CELL LYMPHOMA FOLLOWING BORTEZOMIB: EFFICACY, SAFETY AND PHARMACOKINETICS FROM THE MULTICENTER PHASE II MCL-001 "EMERGE" TRIAL

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Background: Patients (pts) with relapsed/refractory mantle cell lymphoma (MCL) have limited treatment options, particularly when progressing after bortezomib. Lenalidomide is an immunomodulatory agent with antitumor activity in B-cell non-Hodgkin lymphoma, including a subset of relapsed/refractory MCL pts previously treated with bortezomib.

Aims: A phase II multicenter, single-arm, open-label trial evaluated the efficacy, safety, and pharmacokinetics (PK) of lenalidomide in pts with MCL who relapsed or were refractory to bortezomib.

Methods: Pts were treated with lenalidomide 25 mg/day PO days 1-21 every 28 days. Eligible pts had received prior rituximab, cyclophosphamide, and anthracycline (or mitoxantrone) and relapsed or progressed within 12 months of last bortezomib dose or were refractory to bortezomib (at least 2 cycles). The primary endpoints were overall response rate (ORR) and duration of response (DOR), assessed by independent central review committee. Secondary endpoints included complete response (CR)/unconfirmed CR (CRu), time to response (TTR), progression-free survival (PFS), overall survival (OS), and safety. PK analysis was an exploratory endpoint. Response and time-to-event data were evaluated according to modified International Working Group criteria and Kaplan-Meier estimates, respectively. Optional blood samples for PK analysis were obtained 1-12 hours post-lenalidomide days2,4, and 15 for cycle 1 and on day 15 of cycle 2. Plasma concentration data were combined with data from multiple myeloma (MM) and myelodysplastic syndrome (MDS) pts, analyzed using population PK methodology.

Results: 134 pts (median age 67 years; range, 43-83) were enrolled; 2/3 of pts were ≥65 years, and 93% had stage III or IV disease. Pts had received a median of 4 prior therapies (range, 2-10); 78% had ≥3 prior chemotherapy regimens, and 60% were refractory to prior bortezomib. ORR was 28% (CR/CRu in 7.5%), with a median DOR of 16.6 months (95% CI, 7.7-26.7). Median TTR was 2.2 months (range, 1.7-13.1), and median PFS was 4.0 months (95% CI, 3.6-5.6). With a median follow-up of 9.9 months, estimated median OS was 19.0 months (95% CI, 12.5-23.9). Mean lenalidomide dose intensity was 20 mg/day, and median treatment duration was 94.5 days (range, 1-1,002). The most common grade ≥3 AEs (reported in >10% of pts) included neutropenia (43%), thrombocytopenia (28%), and anemia (11%). A total of 24 MCL pts were included in the population PK analysis. In these pts, mean lenalidomide clearance and central distribution volume were estimated to be 9.5 L/h and 46.1 L, respectively. Terminal half-life was 3.4 hours. Creatinine clearance was the only significant covariate predictive of lenalidomide clearance, while age and sex did not affect lenalidomide clearance. The lenalidomide PK profile in MCL was similar to MM and MDS pts. Among 24 pts in the PK study, 6 pts had grade 3/4 neutropenia; no apparent relationship was observed between lenalidomide exposure and grade 3/4 neutropenia.

Summary and Conclusions: Single-agent lenalidomide treatment showed rapid and durable responses in heavily pretreated pts with MCL who were relapsed/refractory to bortezomib. The PK profile of lenalidomide in MCL pts was comparable to that in other patient populations.

S1157

OUTCOME AFTER RIC ALLO-STEM CELL TRANSPLANTATION FOR PATIENTS WITH MANTLE CELL LYMPHOMA WHO RELAPSED AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION, A RETROSPECTIVE STUDY OF THE SFGM-TC GROUP.

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Background: Induction poly-chemotherapy plus rituximab followed by autologous stem-cell transplantation (ASCT) for newly diagnosed young patients with mantle cell lymphoma (MCL) is current standard of care. However, transplanted patients remain highly exposed to relapse. Reduced intensity conditioning allogeneic stem-cell transplantation (RIC-allo) is among therapeutic options for these young relapsed/refractory patients.
Aims: Several retrospective studies addressed the question of RIC-allo in MCL but authors reached to various conclusions. In addition, any study addressed the question of RIC-allo in the setting of previously auto-transplanted patients. Therefore, we performed a national survey conducted on behalf of the SFGM-TC (société française de greffe de moëlle et de thérapie cellulaire).
Methods: Inclusion criteria were as followed: patients with MCL who relapsed after ASCT and for whom RIC-allo has been performed. Patients who underwent RIC-allo within 3 months after ASCT were excluded. Local investigators were asked to confirm diagnosis and update follow-up. All SFGM-TC centers (except one) accepted to participate.
Results: According to the SFGM-TC database, 105 patients matched these criteria. Patients' characteristics were as followed: median age at diagnosis was 54y [range, 30-65], 68% patients were male, 93% of patients presented with stage IV disease at diagnosis. Median PFS after ASCT was 4y [range, 0.4-10]. Median age at RIC-allo was 60y [range, 39-68] and median time from ASCT to RIC-allo was 4y [range, 0.4-10]. Before RIC-allo, 85% of patients had received at least 2 lines of treatment. Sources of donor were a matched related donor in 35 cases (33%), a matched unrelated donor in 50 cases (38%) or a mismatched donor in 20 cases (19%). Conditioning regimens for RIC-allo were cyclophosphamide-based, fludarabine-based or busulfan-based in 20%, 33% and 47%, respectively. A low-dose TBI (Maximum dosing of 2Gy) was used in 27 patients. At time of RIC-allo, 63% (n=63) of patients were in CR, 7% (n=7) in PR and 20% (n=20) with Progressive Disease (data missing in 10%). Twenty patients (19%) experienced grade III/IV aGVHD and Extensive cGVHD was reported in 17 cases (16%). After RIC-allo, 81 (94%) patients reached at least PR. Median FU after RIC-allo was 52 months [range, 12.6-166]. At time of the present analysis, median PFS was 21 months whereas median OS calculated from time of RIC-allo was 51months. Median PFS and OS according to disease status at transplantation were 59m and 62m for patients in CR; 10m (PFS and OS) for patients in PR, 4.1m and 5m for patients in PD; respectively. Seventeen patients (17%) relapsed. Median time from RIC-allo to relapse was 6 months [range, 2-57] and 46 patients (44%) died. Causes of death were related to toxicity in 29 cases, lymphoma progression in 9 patients and other causes in 6 patients (missing data=2).
Summary and Conclusions: Our ongoing analysis shows that incidence of relapse after RIC-allo remains high for MCL patients who have previously relapsed after ASCT. As expected, patients in CR at time of RIC-allo experience a longer PFS duration, underlying that new salvage therapies are highly warranted prior to RIC-allo.

S1158

RESPONSE RATES USING STANDARD CRITERIA, FDG-PET AND MRD MEASUREMENT AFTER 4 COURSES OF R-DHAP AND AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN MCL, RESULTS FROM THE LYMA TRIAL

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Background: Induction chemotherapy followed by autologous stem cell transplantation (ASCT) is the current standard of care in younger patients with mantle cell lymphoma (MCL) (<65 years). Cytarabine-containing chemotherapy regimens (R-DHAP) plus R-CHOP prior to ASCT are associated to longer overall survival (OS) (Hermine *et al.*, ASH 2012). Response rates before and after ASCT can predict patients' outcome. Therefore, induction chemotherapy has a major impact.
Aims: We analyzed response rates after 4 courses of R-DHAP and after ASCT in the Lyma trial, a prospective phase III trial.
Methods: In 2008, the GOELAMS and GELA groups (now Lysa) launched a prospective phase III trial addressing the question of rituximab maintenance after ASCT (Lyma trial) in younger MCL patients. Before ASCT, all patients were planned to receive 4 courses of R-DHAP (without R-CHOP). Transplanted patients were randomized between two study arms (rituximab maintenance *versus* observation). After 4 courses of R-DHAP and after ASCT, patients were monitored for response according to the Cheson 99 criteria. At the same time points, minimal residual disease (MRD), assessed by quantitative PCR, was measured in peripheral blood (PB) and bone marrow (BM). FDG-PET analysis was also performed. From September 2008 to August 2012, 299 patients were enrolled.
Results: Herein, we present response rates for the first 200 patients enrolled in the trial (response assessment for the 99 remaining patients are ongoing and will be presented at the time of the meeting). Patients presented the following characteristics: Median age,57.2 years; male, 79.5%; MIPI score, low (l-MIPI) 53%, intermediate (i-MIPI) 27% and high (h-MIPI), 20%. Blastoid variant was diagnosed in 12.5% of the patients.. 182 patients (91.5%) received 4 courses of R-DHAP and 164 (82%) proceeded to transplant. 156 (78.5%) patients were randomized. After 4 courses of R-DHAP, CR/CRu rate was 76.5% (l-MIPI 74.5%, i-MIPI 83.5%, H-MIPI 70%) while 13% of the patients reach PR. 162 patients underwent FDG-PET and no abnormal FDG uptake was observed for 79.6% of the patients (l-MIPI 83%, i-MIPI 81%, H-MIPI 66.6%). Regarding MRD status, 79.2% and 64.4% of patients reached negativity in PB and BM,respectively. After ASCT, CR/CRu rate was 93.5% (l-MIPI 93%, i-MIPI 98%, H-MIPI 89,5%) and 95.3% of transplanted patients reached MRD negativity in PB *versus* 78.1% in BM. According to FDG-PET (n=121). CR after ASCT was 89%.
Summary and Conclusions: These results show for the first time that CR/CRu and MRD negativity rates after only 4 courses of R-DHAP are similar to those reported after 6 courses of R-CHOP/R-DHAP. Furthermore, the Lyma trial is the first large phase III trial that prospectively evaluates FDG-PET response in MCL. Because it has been demonstrated that response before and after ASCT can predict response duration after ASCT, response rates after 4xR-DHAP alone are warranted to design next generation clinical trials in MCL.

S1159

ROLE OF 18FDG-PET/CT IN POST-CHEMOTHERAPY EVALUATION OF PRIMARY MEDIASTINAL LARGE B CELL LYMPHOMA (PMLBCL): RESULTS OF THE INTERNATIONAL EXTRANODAL LYMPHOMA STUDY GROUP (IELSG)-26 TRIAL (NCT00944567)

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Background: PET/CT is widely used in the definition of response and as a prognostic indicator, in Hodgkin lymphoma and diffuse large B cell lymphoma, but its role in PMLBCL remains to be defined.
Aims: The IELSG 26 study was defined to prospectively evaluate the role of PET, after rituximab and anthracycline-containing immunochemotherapy (R-CHT) in patients (pts) with PMLBCL.
Methods: Between 2007 and 2010, 125 pts gave their consent to be enrolled and were treated with R-CHOP(-like) (20 pts), R-VACOP-B (34 pts) or R-MACOP-B (71 pts) regimens; consolidation radiotherapy (RT) as indicated by local guidelines was allowed. PET/CT scans were planned at baseline, at 3-4 weeks after R-CHT and at 12 weeks after RT. Central PET/CT review was performed at the end of treatment using the Deauville score (Meignan *et al.* Leuk Lymphoma 2009) and complete metabolic response (mCR) was defined as a negative PET scan or one having minimal residual uptake lower than mediastinal blood pool (MBP) activity in regions which were positive at baseline, according to the IHP criteria (International Harmonization Project in Lymphoma, Juweid *et al.* JCO 2007).
Results: Treatment was administered as initially planned in 119 pts; there were 6 early withdrawals and central PET/CT review was done in 115/119 pts; 102 of 115 had consolidation RT. PET/CT visual assessment at 3-4 weeks post-R-CHT showed mCR in 54/115 pts (47%; 95% CI, 36%>56%): in 12 cases (10%; 95% CI, 6%>18%) PET/CT scan was completely negative (Deauville score 1), while in 42 (37%; 95% CI, 28%>46%) there were small residual masses with an uptake less than MBP (score 2). PET/CT scans showed a positive residual

mass after R-CHT in 61/115 pts (53%; 95% CI, 44%>62%). The residual uptake was higher than MBP but below the liver uptake (score 3) in 27 pts (23%; 95% CI, 16%>32%), slightly higher than the liver uptake (score 4) in 24 pts (21%; 95% CI, 14%>29%) and markedly higher than the liver uptake (score 5) in 10 pts (9%; 95% CI, 4%>15%). Despite only 47% of pts achieving a mCR by IHP criteria after R-CHT, at a median follow-up of 3 years, the estimated 5-year overall survival (OS) and progression-free survival (PFS) rates in the whole population (n=125) is 92% (95% CI, 86%>96%) and 86% (95% CI, 79%>91%), respectively. The achievement of a mCR by the IHP criteria at 3-4 weeks after R-CHT predicted a longer PFS (P=0.004) and OS (P=0.029) with high sensitivity but poor specificity. Pts with Deauville score 3 had a clinical outcome identical to that of 'PET negative' (score 1-2) pts and ROC analysis suggested that moving the cut-point for the definition of mCR from the MBP to the liver uptake, will increase specificity while maintaining sensitivity. Indeed, the liver uptake as a cut point is a better predictor for both PFS (P<0.001) and OS (P=0.003) (Figure 1).

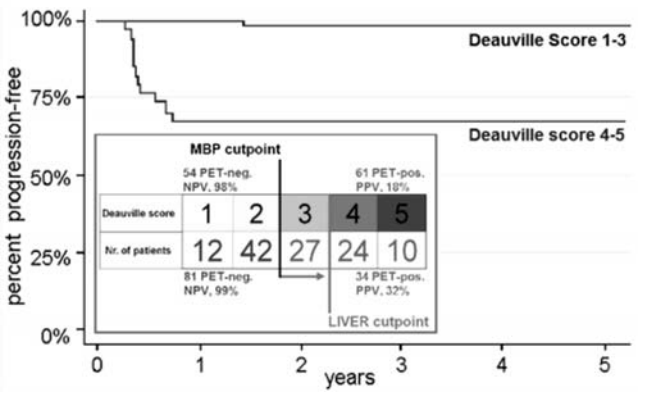

Figure 1.

Summary and Conclusions: This study provides the first prospective validation on the use of a 5-point visual scale, the Deauville criteria, for the PET response definition after R-CHT in large cell non-Hodgkin lymphomas. Using the MBP cut-point, the mCR rate after R-CHT in PMLBCL was lower than reported in DLBCL, but approximately 90% of pts are projected to be alive and progression-free at 5 years post-treatment and only the pts with score 4 and 5 had a significantly worse outcome. Hence, the liver uptake may represent a more appropriate cut-point than MBP to identify poor-risk pts who may need early intensification. The design of this study precludes any conclusion about the RT role, but the ongoing IELSG-37 randomized trial will assess whether RT can be safely omitted in PMLBCL pts in mCR after R-CHT.

S1160

EXTRALYMPHATIC CRANIOFACIAL DIFFUSE LARGE B-CELL LYMPHOMA: ROLE OF RADIOTHERAPY AND INTRATHECAL CNS PROPHYLAXIS

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Background: The role of radiotherapy and intrathecal prophylaxis in extralymphatic craniofacial involvement of aggressive B-cell lymphoma remains to be determined in the rituximab era.

Aims: To study the relevance of radiotherpay and intrathecal CNS prophylaxis to extralymphatic craniofacial DLBCL.

Methods: In a retrospective subgroup analysis of 9 consecutive prospective DSHNHL trials covering all DLBCL risk groups from 18 to 60 years of age, patients with and without craniofacial involvement were compared with respect to clinical presentation, event-free and overall survival.

Results:: 336 sites of extralymphatic craniofacial involvement were observed in 284/3840 (7.4%) patients (orbita: 30, paranasal sinuses: 90; main nasal cavity: 38, tongue: 26, remaining oral cavity: 99, salivary glands: 53). In a multivariable analysis adjusting for IPI risk factors the addition of rituximab improved EFS and OS in both patients with and without craniofacial involvement. The 141 responding patients who received radiotherapy to sites of craniofacial involvement had a similar 3-year event-free (79% *vs* 79%; P=0.835) and 3-year overall survival (88% *vs.* 85%; P=0.311) when compared with the 56 patients who did not receive radiotherapy. Without rituximab, the 2-year-rate of cumulative risk of CNS disease was increased in 205 patients with compared to 2586 patients without craniofacial involvement (4.2% *vs.* 2.8%; P=0.038), while this difference disappeared in patients who received CHOP(like) chemotherapy in combination with rituximab (1.7% in 77 patients with compared to 2.9% in 946 patients without craniofacial involvement; P=0.868). Of 85 patients with craniofacial involvement who received intrathecal prophylaxis with methotrexate, the 2-year-rate of cumulative risk of CNS disease was 4.3% compared to 2.3% in 189 patients who did not (P=0.995).

Summary and Conclusions: Rituximab eliminates the increased risk for CNS disease in patients with craniofacial involvement. As a practical consequence intrathecal prophylaxis and radiotherapy to sites of craniofacial involvement should not be given any more.

Myelodysplastic syndromes - Biology

S1161

SPECTRUM OF GENETIC ALTERATIONS IN ACQUIRED APLASTIC ANEMIA

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Background: Acquired aplastic anemia (AA) is a prototype of idiopathic bone marrow failure, which is caused by autoimmune destruction of hematopoietic progenitors. However, its natural course could be more complicated than expected for a simple autoimmune disease, which is related in large part to the development of apparently clonal disorders such as paroxysmal nocturnal hemoglobinuria (PNH) and myelodysplastic syndrome (MDS) or acute myelogenous leukemia (AML), during its course. In addition, clonality in AA is one of the long standing issues, which is evident from the cytogenetic abnormalities and skewed pattern of X chromosome inactivation in some patients. Although these evidences suggest a pathogeneic link between these disorders, clonal architecture in AA has not been fully explored.
Aims: To genetically define the origin of clonal hematopoiesis in patients with acquired AA in terms of gene mutations.
Methods: We analyzed peripheral blood DNA samples from 192 patients with AA for genetic alterations, in which coding sequences of 51 genes, which include common target of somatic mutations in myeloid malignancies, such as *RUNX1*, *ASXL1*, *TET2*, *IDH1/2*, *DNMT3A* and major components of splicing factors, were captured by liquid phase hybridization and subjected to high-throughput sequencing using Illumina HiSeq 2000. Copy number alterations have been also examined using SNP arrays analysis in all cases.
Results: About 40% were severe or very severe diseases. PNH-type cells and uniparental disomy at short arm of 6th chromosome (6pUPD) were found in 55% and 12%, respectively. Immunosuppressive therapies were performed in 70% of the cases. 32% of the cases had ATG. Response was obtained in 81% of the informative cases. 169 single nucleotide variants (SNV) are detected by target sequencing, which were subjected to deep sequencing. Finally 43 somatic mutations were detected in 34 patients (18%). Mutations were most frequently found in *DNMT3A* (7 mutations), followed by *ZRSR2* (6), *ASXL1* (5), *BCOR* (4), however *TET2* mutations, which are most commonly observed in MDS, were not identified. Mutations were more biased to nonsense (11 mutations), frameshift (6), splice site changes (3) and non-frameshift indel (5), compared to missense (18). There was no significant difference in severity, positive PHN-type cells, 6pUPD, or copy number abnormalities between mutation positive and negative cases. Mutations were associated with older age (P=0.014) and higher response to immunosuppressive therapies (P=0.040). SNP array analysis revealed copy number abnormalities in 24/192 cases (12.5%), in whom 6pUPD were observed most commonly (6.3%), therefore 51/192 cases (26.6%) had evidence of clonal evolution. In most cases with more than 5 years observation periods, no progression to AML or MDS has been reported.
Summary and Conclusions: Although the role of observed mutations in evolution from AA to AML/MDS is still unclear, we revealed a new insight of clonality in AA. In the meeting, we will introduce our findings including clinical course in some typical AA cases with mutations.

S1162

MOLECULAR SPECTRUM OF JUVENILE MYELOMONOCYTIC LEUKEMIA IDENTIFIED BY WHOLE EXOME SEQUENCING

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Background: Juvenile myelomonocytic leukemia (JMML) is a rare pediatric myelodysplastic/myeloproliferative neoplasm, of which molecular pathogenesis is poorly understood except for mutations of RAS pathway genes including *PTPN11, NF1, N/KRAS* and *c-CBL*. Clinical management of patients with JMML according to genetic status is of great international interest, but remains controversial among researchers.
Aims: To obtain a complete spectrum of gene mutations in JMML.
Methods: Whole exome sequencing was performed for paired tumor-normal DNA from 13 JMML cases. Candidate somatic mutations were detected through in house pipeline for whole exome sequencing. All the candidate germline and somatic nuncleotide changes were validated by Sanger/deep sequencing. A total of 92 JMML tumor specimens were screened for mutations in previously reported genes (*PTPN11*, *NF1*, *KRAS, NRAS*, and *c-CBL*) and newly identified 3 genes with deep sequencing. Moreover, to clarify the clinical significance of aberrant DNA methylation in JMML, we evaluated quantitative CpG methylation pattern of 10 candidate genes by pyrosequencing in the same cohort.
Results: Only 11 non-silent somatic mutations were detected in whole exome sequencing (0.85/sample), of which 6 involved the known RAS pathway genes (2 *PTPN11*, 1 *NRAS*, 1 *KRAS*, and 1 *NF1*). Germline involvement was suspected in 7 cases (54%), of which 6 had germline mutations of RAS pathway genes (2 *PTPN11*, 2 *NF1*, and 2 *c-CBL*). Totally, the RAS pathway mutations were found in 11 out of 13 discovered cases (85%). Five out of the 11 somatic mutations were in three non-RAS pathway genes (3 *SETBP1*, 1 *JAK3*, and 1 *SH3BP1*). The mutation of *SETBP1* has recently been reported in Schinzel-Giedion syndrome (Nat Genet. 2010) and adult atypical chronic myeloid leukemia (Nat Genet 2012). By deep sequencing, RAS pathway mutations were found in 82 out of 92 cases (89%), where *PTPN11* mutations were predominant (n=39, 42%) followed by *N/KRAS* (n=28, 30%), *c-CBL* (n=14, 15%), and *NF1* (n=9, 10%) mutations. The other 10 (11%) patients were negative for known RAS pathway mutations. In addition, genetic mutations of *SETBP1* and *JAK3* were recurrently found in 16 patients (17%). These mutations had lower allele frequencies compared to the accompanying RAS pathway mutations, indicating that they were responsible for disease progression rather than the establishment of JMML. Clinically, these mutations were associated with poor overall survival (P=0.10). By epigenetic analysis, aberrant methylation in any of *CDKN2A, BMP4*, *CALCA*, and *RARB* was also a significant poor prognosis factor for transplantation free survival (P=0.009) (Figure 1).

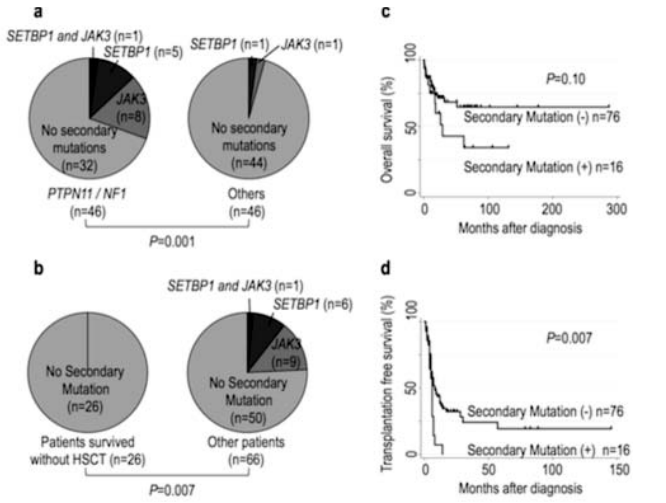

Figure 1. Clinical features of JMML cases with or without secondary mutations.

Summary and Conclusions: Our whole-exome sequencing revealed the spectrum of gene mutations in JMML. Together with high frequency of RAS pathway mutations, the paucity of non-RAS pathway mutations is a prominent feature of JMML. Mutations of SETBP1 and JAK3 were common recurrent secondary events presumed to be involved in tumor progression and associated with poor clinical outcomes. Our finding provides an important clue to understand the pathogenesis of JMML that helps to develop novel diagnostics and therapeutics for this leukemia.

S1163

ABT-737 PROLONGS SURVIVAL IN AN NRASD12/BCL2-MEDIATED MOUSE MODEL OF MYELODYSPLASTIC SYNDROME (MDS) BY TARGETING LEUKEMIA INITIATING CELLS (LIC), PRIMITIVE LIN-/SCA1+/KIT+ (LSK) AND PROGENITORS

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Background: MDS are characterized by apoptosis of bone marrow (BM) progenitors, associated in particular to BCL-2 family gene deregulation. In our transgenic acute myeloid leukemia (AML) mouse model expressing NRAS and BCL2 with 90% BM blasts and reduced apoptosis (Omidvar Cancer Res, 2007), ABT-737, a mimetic inhibitor of the BH3 domain of BCL2 protein family, prolonged mouse survival (Gorombei, ASH 2012).
Aims: Here we address the effect of ABT-737 on survival and LICs in our MDS mouse model with and pro-apoptosis (Omidvar Cancer Res, 2007).
Methods: Transgenic MRP8NRASD12/MMTVLTRtTA/TetoBCL-2 MDS mice were treated or not after genotyping and hBCL-2 expression by flow, and the disease confirmed by blood counts. Treatment consisted of 75 mg/kg ABT-737 (Abbott)×3 /week for 15 injections. Mice (treated or not) were followed for survival (n=27 untreated and n=23 treated) or sacrificed (n=60) and BM harvested and Giemsa stained for BM analysis, by microscopy (n=4 in each group), characterized for LICs by transplantation of spleens into lethally irradiated syngeneic recipients (n=3 per group), primitive cells as Lin-/Sca1+/cKit+ (LSK) by flow cytometry (12 untreated, 8 treated), and for progenitor assays (11 untreated, 5 treated). BM apoptosis was measured by annexin V staining; SPECT (12 untreated, 3 treated) and liver sections examined by TUNEL (n=2). RAS activity was measured by RAS-pulldown assays. RNA was extracted from Sca1+ enriched spleen cells (3 treated and untreated) and assayed for gene expression profiling (GEP) using exon specific arrays (Affymetrix).
Results: Median survival was 15 days in 27 untreated mice and 70 days, (two thirds surviving >60 days) in the 23 treated mice (P<0.0005). This correlated with reduction of marrow blasts from 10±2.1% in untreated to 6±2.8% in treated mice (P<0.05) and clearance of tissue infiltration by myeloblasts. After ABT-737 treatment 2 of 3 transplanted mice remain alive after 5 months whilst the untreated mice died by day 50; the BM LSK cell population decreased to nearly normal levels (normal 1.8±0.6%; 8.5±3.6% in untreated *versus* 4.5±2.4% in treated, P<0.05) with complete restoration of colony growth to normal range (35±10 in wild type normal mice, 45±9 in untreated *versus* 38±8in treated mice p~0.1). In treated mice, increased apoptosis was observed in the bone marrow and liver. RAS-GTP ad BCL-2 expression remained unchanged following treatment. Exon specific gene expression arrays showed 399 genes differentially expressed between treated and untreated mice; 305 and 94 genes were upregulated and downregulated respectively including genes important for stem cell development such as ALDH2, ALDH3B2, CTBP2, possibly reflecting partial restoration of normal stem cell function, consistent with reduced LSK, progenitor cell numbers and apparent transplantation of normal cells. Downregulation of anti apoptotic genes such as Map3K14 and upregulation of pro apoptotic genes such as Apaf1 and Pik3Cg was observed, consistent with increased apoptosis data. Restoration of normal hematopoiesis was confirmed by upregulation of myeloid differentiation genes (CD14, CSF1) and genes involved in the cell cycle (Ccna2, Ccnb2, E2F2, Cdkn2C, Ttk, Ccne2).
Summary and Conclusions: ABT-737 extends lifespan in our mutant NRAS BCL-2 MDS transgenic mice. ABT-737 targets LIC and primitive progenitors, and regulates cell cycle, differentiation and apoptosis pathways. These data suggest that clinical trials with BCL-2 targeting reagents in MDS patients are warranted.

S1164

MUTATIONS IN SETBP1 GENE ARE RECURRENT IN MYELODYSPLASTIC SYNDROMES AND OFTEN COEXIST WITH CYTOGENETIC MARKERS ASSOCIATED WITH POOR PROGNOSIS

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Background: The molecular basis of disease progression in the myelodysplastic syndromes (MDS) and chronic myelomonocytic leukemia (CMML) remains poorly understood. SETBP1, a regulator of SET nuclear oncogene, has recently been shown to be mutated in myeloid malignancies, including MDS, secondary AML (sAML) and atypical chronic myeloid leukemia (aCML). aCML patients with *SETBP1* mutations have a poor prognosis.
Aims: We sought to investigate whether *SETBP1* plays a role in disease progression in MDS/CMML. We screened a large series of MDS cases, and sAML cases evolving from MDS/CMML, for *SETPB1* mutations. We also studied serial samples from MDS/CMML cases in evolution to AML.
Methods: DNA was isolated from patient bone marrow or peripheral blood samples. The region surrounding the SKI homology domain was sequenced using standard Sanger-sequencing methods. Serial samples were also screened for mutations in *ASXL1* and *TET2*. In addition, one of the MDS cases in progression to AML with del(7)(q21) from onset was subjected to whole exome sequencing (WES) both at diagnosis and at the leukemic stage.
Results: We screened a cohort of 328 patients with a range of myeloid malignancies (122 MDS, 12 CMML, 2 MDS/MPN, 115 *de novo* AML and 77 sAML) for *SETBP1* mutations. Overall, 4.9% of patients had *SETBP1* mutations and these were all heterozygous and missense. Sixteen mutations were detected in 16 patients; G870S, I871S and R627C alterations were most frequently observed (n=4, n=4 and n=3, respectively), while G870R, P637R, E863K, D868N, T873R were less prevalent (n=1 each). Mutations were detected in 4.1% cases with MDS (5/122), 50% with MDS/MPN (1/2), 16.7% with CMML (2/12), 0.9% with *de novo* AML (1/115) and 9.1% with sAML (7/77). Among the sAML cases, mutations were present in 23.1% of cases secondary to CMML (3/13) and in 6.3% secondary to MDS (4/64). Strikingly, the majority of the *SETBP1* mutations were found in samples with specific cytogenetic features: 8 mutations in cases with monosomy 7 (-7) or del(7q), 3 mutations in cases with abnormalities including isochromosome 17 [i(17)(q10)], and 3 mutations in cases with normal karyotype. The remainder of the patients with *SETBP1* missense mutations had del(20q) or inv16 (Table 1). Chromosome 7 abnormalities [-7/del(7q)] and i(17)(q10) are associated with shortened overall survival in MDS and risk of leukemic evolution. The increased frequency of *SETBP1* mutations observed in these cytogenetic subgroups and in sAML prompted us to analyse serial samples from 22 MDS/CMML cases in transformation to AML. Four of these cases showed *SETBP1* mutations, 3 of which acquired the mutation during leukemic evolution. Notably, in the fourth case the *SETBP1* mutation was detectable before the cytogenetically aberrant clone [harboring a i(17)(q10) marker] arose. In addition to *SETBP1*, we also screened the serial samples for mutations in *ASXL1* and *TET2*. Overall, 15 of the 22 cases (68.2%) showed mutations in at least one of the three genes during the disease course. *ASXL1* and *TET2* were mutated in 11 and 5 cases, respectively, with 3 cases harboring mutations in both genes. *ASXL1* mutations were present in 2 of the 4 cases with *SETBP1* mutations. No clear pattern of mutation acquisition was noted, likely due to the small number of cases with more than one mutation. One MDS case with del(7)(q21) in progression to AML was subjected to WES both at diagnosis and at the AML stage. Only the AML sample showed a G870R somatic mutation.

Table 1.

Karyotype	MDS/CMML	De novo AML	Secondary AML	SETBP1 mutated cases No (%)
monosomy 7/del(7q)	15	2	3	8 (40.0)
i(17)	1	1	2	3 (75.0)
inv16	0	6	0	1 (16.7)
del(20q)	14	2	3	1 (5.3)
normal	34	51	33	3 (2.5)
del(5q)	18	1	1	-
loss Y	17	2	0	-
t(15;17)	0	11	0	-
t(8;21)	0	8	0	-
trisomy 8	19	0	4	-
complex	17	10	17	-
others	1	19	6	-
No data available	0	2	8	-
TOTAL	136	115	77	16 (4.9)

Summary and Conclusions: Somatic *SETBP1* mutations are recurrent in MDS, CMML and sAML, and frequently coexist with cytogenetic markers associated with poor prognosis. Analysis of serial samples from MDS and CMML cases in transformation showed that nearly 70% of the cases presented mutations in at least one of three genes screened (*SETBP1, ASXL1* and *TET2*). *SETBP1* mutations were frequently acquired during leukemic evolution.

S1165

P53 PROTEIN EXPRESSION PREDICTS OUTCOME AND CYTOGENETIC RESPONSE IN PATIENTS WITH LOW-/INT-1 RISK MYELODYSPLASTIC SYNDROMES TREATED WITH LENALIDOMIDE: RESULTS FROM THE MDS004 CLINICAL TRIAL.

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Background: International Prognostic Scoring System (IPSS) Low- or Intermediate (INT)-1 risk (low-risk) myelodysplastic syndrome (MDS) with del(5q) was considered to have an indolent course, but recent data have indicated a subgroup of patients with more aggressive disease and shorter overall survival (OS). Using deep sequencing, it was previously demonstrated that 18% of such patients carry small *TP53* mutated subclones at diagnosis, rendering them at significantly higher risk for disease progression (Jädersten *et al.*, JCO 2011). The presence of *TP53* mutations was associated with strong p53 protein expression by immunohistochemistry (IHC) indicating that p53 IHC may be a sensitive biomarker for outcome in lower-risk MDS.
Aims: To assess the association between p53 protein expression by IHC in patients with lower-risk del(5q) MDS treated with lenalidomide and response to treatment, outcome, and *TP53* gene mutations.
Methods: Bone marrow (BM) biopsies obtained at screening and during follow-up in 85 of 205 (41%) patients from the MDS004 clinical trial (Fenaux *et al.*, Blood 2011) were retrospectively assessed for p53 protein expression by IHC in a blinded fashion by manual and automated image analysis using p53 DO1 and DO7 monoclonal antibodies. Sections were assessed for the percentage and intensity of p53 nuclear staining based on a total count of 1000 BM haematopoietic cells (lymphocytes and lymphoid aggregates excluded) at high magnification (40×/60× objectives), and p53 protein expression was graded as 0 (negative), 1+ (faintly positive), 2+ (moderately positive) and 3+ (strongly positive). Results for strong p53 staining were correlated to clinical outcome and treatment response. A subset of BM samples was reviewed by three independent investigators to test reproducibility. The *TP53* mutational status was assessed by deeP-sequencing in nine patients and in microdissected cells with strong, moderate and negative p53 staining by using pyrosequencing.
Results: Strong p53 protein expression in ≥1% BM cells was detected in 30 of 85 patients (35%). Baseline demographics, clinical characteristics and WHO subgroups did not differ between the IHC cohort and the MDS004 study patients without trephine biopsies. Results for the two p53 monoclonal antibodies, manual *versus* automated measurements, and across three independent investigators were highly concordant (P<0.01, for all three). The presence of ≥1% BM cells with strong p53 expression ($p53^{+++}$) was significantly associated with shorter OS (P=0.0104) and higher risk for progression to AML (P=0.0003). The P53 IHC status showed no association with transfusion independence or response duration (P=0.636 and P=0.4421), but was significantly associated with cytogenetic response (CyR) (complete and partial response taken together) with: 18/35 (51%) CyR for the p53-negative patients as compared to 3/21 (14%) CyR for p53-positive patients (P=0.009). As CyR rates differed for 5 mg *vs* 10 mg lenalidomide (len) in the entire MDS04 study, CyR rates were also specifically evaluated for patients randomized to each dose level. For patients randomized to 10 mg len, CyR occurred in 1 of 8 (13%) and in 16 of 19 (84%) p53-positive and negative patients, respectively. Eight of 21 patients with serial BM samples had an increased percentage of $p53^{+++}$ cells at three months, which was associated with higher risk for progression to AML (P<0.01) and shorter OS (P=0.0005) as compared to patients who remained negative for p53 by IHC. Cytogenetic evolution, including del(17p), was observed in three of the eight patients with increased $p53^{+++}$ by IHC. Pyrosequencing analysis of microdissected, p53-immunolabeled cells confirmed *TP53* mutation in cells with strong p53 staining, while cells with moderate staining where predominantly of wild-type TP53.
Summary and Conclusions: The present study validates p53 IHC as a predictive tool in del(5q) lower-risk MDS patients treated with lenalidomide, and as a biomarker for underlying *TP53* mutations. *TP53* mutational status should be included in risk assessment systems for MDS with del(5q).

Molecular markers in acute myeloid leukemia

S1166

WHOLE EXOME SEQUENCING OF PRIMARY REFRACTORY AML PATIENTS REVEALS A DIVERSE MUTATIONAL PATTERN WITH HIGH PREVALENCE OF MDS RELATED MUTATIONS

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Background: A critical step during treatment of patients with acute myeloid leukemia (AML) is the achievement of complete remission (CR). Although the majority of younger patients with AML will achieve CR, about 5-50% will fail to respond sufficiently to induction treatment, with highest CR-rates in CBF leukemias and worst response in complex karyotypes and secondary AML. Treatment of AML patients with induction failure (IF-AML) is difficult and outcome is dismal, with long-term survival mainly restricted to patients who are successfully salvaged and transplanted. The molecular mechanisms of resistance are still poorly understood, especially in patients with normal karyotype, a subgroup mainly associated with good CR rates. Since improved understanding of resistance mechanisms might help to develop novel treatment strategies, we aimed to study genetic alterations in IF-AML patients using whole exome sequencing (WES).
Aims: Since improved understanding of resistance mechanisms might help to develop novel treatment strategies, we aimed to study genetic alterations in IF-AML patients using whole exome sequencing (WES).
Methods: We performed WES (TrueSeq enrichment, Illumina) of FACS-sorted leukemic blasts (CD34/33/117+) and normal T-cells (CD4+; as germline reference) after whole genome amplification (WGA) in 29 patients (median age 44 years; range, 21-63) with *de novo* AML who had IF. Primary refractory disease was defined as failure to achieve CR after conventional double induction chemotherapy using standard anthracycline/cytosine arabinoside containing chemotherapy, with the majority of patients showing blast persistence at high levels or even increased blast counts. Patients had primarily intermediate-risk cytogenetics according to MRC criteria (N=24), of whom 16 patients had a cytogenetically normal (CN) AML. Paired-end 100 bP-sequencing was performed on a HiSeq2000 instrument (Illumina). Bioinformatic analysis included BWA-mapping (Hg19 reference) and somatic single nucleotide variants (SNVs) calling using SAMTOOLS. Only variants not specified as simple nucleotide polymorphisms (SNP) in db135 with a minor allele frequency of >1% were included in the final analysis.
Results: With an average target base coverage of 46- fold (38-56 inter quartile range, IQR) we identified a median number of 22 high confident SNVs per patient (range 9-310). Ongoing validation of SNV-calls using Sanger sequencing confirmed somatic status in the majority of cases. Frequently mutated, recurrently affected known genes included TET2 (24.1%), IDH1 and 2 (27.6%), DNMT3A (13.8%), WT1 (13.7%) and RUNX1 (13.8%); mutations were also less frequently found in SF3B1 (10.3%) and BCOR (10.3%). Rarely found genes in AML include ATM (N=2), BRCA2 (N=2) and MYC (N=2), whereas several novel recurrent alterations currently undergo further validation, *e.g.* CREBBP (N=2), and MYCBP2 (N=2).
Summary and Conclusions: Combining the mutational data, we so far did not identify a specific molecular lesion in IF-AML. However, although we focused on *de novo* AML, we observed a high prevalence of alterations previously associated with MDS, including TET2, RUNX1, SF3B1 and DNMT3A, which might point to an unrecognized underlying secondary development of these leukemias. Especially alterations in epigenetic modifier proteins were common, which might point towards potential new treatment strategies in these patients.

S1167

INTERACTIONS BETWEEN GEMTUZUMAB OZOGAMICIN TREATMENT AND ACUTE MYELOID LEUKEMIA PATIENT SUBSETS DEFINED BY CYTOGENETICS, GENE MUTATIONS, AND SINGLE-NUCLEOTIDE POLYMORPHISM ARRAY-BASED KARYOTYPING

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Background: We recently reported the results of the ALFA-0701 Phase 3 tri-

al, showing that the addition of fractionated doses of gemtuzumab ozogamicin (GO) during induction and consolidation chemotherapy may significantly improve outcome of acute myeloid leukemia (AML) patients aged 50-70 years. In this trial, as well as in Medical Research Council AML 15 and 16 trials, it appeared that patients with favorable or intermediate cytogenetics, including those with normal karyotype (NK) AML, may benefit from GO, while not those with adverse cytogenetics.

Aims: Our objectives were to evaluate the prognostic impact of molecular abnormalities and lesions detected by single-nucleotide polymorphism array (SNP-A)-based karyotyping on clinical outcome in AML patients treated in the ALFA-0701 trial, and to analyze potential interactions between GO treatment and patient subsets defined by cytogenetics, gene mutations, and SNP-A lesions.

Methods: We performed mutational analysis of 11 genes (*FLT3*, *NPM1*, *CEBPA*, *MLL, RUNX1*, *WT1*, *ASXL1*, *TET2*, *IDH1/2,* and *DNMT3A*), *EVI1* overexpression screening, and 6.0 SNP-A analysis in the diagnostic samples of the 278 patients with previously untreated *de novo* AML enrolled in the ALFA-0701 trial. The screening for *TET2*, *IDH1/2*, and *DNMT3A* mutations was restricted to NK-AML.

Results: Karyotype and SNP-A data were available for 254 and 248 patients, respectively. SNP-A analysis revealed 450 genomic abnormalities (245 losses, 117 gains, 88 uniparental disomies) in 135/248 (54%) patients. In NK-AML (n=146), 38% of the patients had ≥1 SNP-A lesion and 89% had ≥1 molecular abnormality, *NPM1* mutations (52%), *DNMT3A* mutations (34%), and *FLT3* internal tandem duplication (ITD) (25%) being the 3 most frequent molecular abnormalities. The median duration of follow-up from time to randomization was 25 months. As expected, karyotype had a strong influence on overall survival (OS). The presence of SNP-A lesions conferred a significantly shorter OS in the whole cohort (HR, 2.52 [95%CI, 1.68-3.77]; P<0.0001) and in the subset of 132 NK-AML patients analyzed by SNP-A (HR, 2.45 [1.39-4.31]; P=0.002). In the NK-AML subset, *DNMT3A* mutations (HR, 2.33 [1.35-4.01]; P=0.002) and, to a lesser extent, *FLT3*-ITD (HR, 1.77 [1.01-3.1]; P=0.045) were associated with a significantly shorter OS. In multivariate analysis, only the presence of unfavorable karyotype (HR, 2.47 [95%CI, 1.61-3.79]; P<0.001) and SNP-A lesions (HR, 2.05 [95%CI, 1.33-3.10]; P=0.001) were retained as significantly associated with OS in the whole cohort, while the presence of SNP-A lesions (HR, 3.14 [95%CI, 1.74-5.68]; P<0.001), *DNMT3A* mutations (HR, 1.90 [95%CI, 1.08-3.35]; P=0.027), and randomization in the control arm (HR, 2.50 [95%CI, 1.35-4.55]; P=0.003) independently predicted shorter OS in NK-AML patients. The benefit of GO treatment was predominantly observed in NK-AML. In this subset, the negative impact of *FLT3*-ITD was observed in the control arm (HR, 1.77 [95%CI, 1.04-2.99]; P=0.005) but not in the GO arm (HR, 0.89 [0.32-2.44]; P=0.82), while such an effect of GO treatment was not found for SNP-A lesions and *DNMT3A* mutations.

Summary and Conclusions: SNP-A lesions and *DNMT3A* mutations represent adverse independent prognostic factors in AML. Our results suggest that addition of GO to standard chemotherapy might overcome the poor prognosis of *FLT3*-ITD, but not that of SNP-A lesions and *DNMT3A* mutations. However, further studies based on larger patient cohorts are required to formally identify the molecular determinants of response to GO.

S1168

HIGH EXPRESSION OF DNMT3B NEGATIVELY IMPACTS ON CLINICAL OUTCOME OF OLDER PATIENTS (PTS) WITH PRIMARY CYTOGENETICALLY NORMAL (CN) ACUTE MYELOID LEUKEMIA (AML) [CALGB 20202 (ALLIANCE)]

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Background: DNA methyltransferases (DNMT1, DNMT3A, DNMT3B) are involved in DNA methylation and epigenetic gene silencing. Aberrant DNA methylation appears to contribute to leukemogenesis, and increases with age. *DNMT3A* mutations (mut) have been reported to impact negatively on the clinical outcome of AML pts, and it recently was reported that *DNMT3B* overexpression adversely affected outcome of a clinically and cytogenetically diverse cohort of 195 adult AML pts (PLoS One 2012;7:e51527). However, the prognostic impact of *DNMT3B* expression has not yet been evaluated in similarly treated older [≥60 years(y)] pts with CN-AML, especially in the context of a comprehensive panel of molecular prognosticators that include mut in the *DNMT3A, TET2* and *ASXL1* genes, which are implicated in aberrant epigenetics in AML.

Aims: To test the clinical impact of *DNMT3B* expression in a large cohort of similarly treated and molecularly well-characterized CN-AML pts, and to derive *DNMT3B* expression-associated gene-expression (GEP), microRNA-expression (MEP) and gene promoter methylation profiles.

Methods: We analyzed 210 pts aged 60-83 y (median, 68). *DNMT3B, ERG* and *BAALC* expression was assessed by NanoString nCounter assay. Mutation analyses of genes known to affect outcomes of CN-AML were performed using PCR amplification and Sanger sequencing. Pts were dichotomized into low and high *DNMT3B* expressers using a median cut. The GEP (n=177 pts) and MEP (n=162) were assessed using, respectively, the HG-U133plus2.0 and The Ohio State University custom microRNA microarrays. Methylation was measured by the NGS MethylCaP-seq (Blood 2012;120:2466).

Results: High *DNMT3B* expressers had lower complete remission (CR) rates (*P*<.001, 56% v 79%), and worse disease-free (DFS; *P*=.03; 3y-rates, 14% v 21%) and overall (OS) survival (*P*<.001; 3y-rates, 11% v 28%) than *DNMT3B* low expressers. High *DNMT3B* expressers had higher WBC (*P*=.002), and% blood (*P*=.003) and marrow (*P*<.02) blasts, were more often in the European LeukemiaNet (ELN) Intermediate-I Genetic Group (*P*<.007), and had more often *FLT3*-ITD (*P*<.001), *IDH2* R172 mut (*P*=.02), and high *ERG* (*P*<.001) and *BAALC* (*P*=.001) expression. In multivariable analyses, high *DNMT3B* expression associated with worse CR [*P*=.01, hazard ratio (HR)=.42] once adjusted for *BAALC* expression, WBC and age, and shorter OS (*P*=.008, HR=1.52) once adjusted for ELN Groups, *DNMT3A* R882 mut, and *BAALC* expression.

By GEP, we found 2188 genes differentially expressed (*P*<.001, FDR<.01) between high and low *DNMT3B* expressers; 1465 were upregulated concordantly with *DNMT3B* including genes previously implicated in AML (*e.g.*, *KIT*, *FLT3*, *WT1*, *ERG*, *FANCL*, *TP53*, *HOXA6*, *NPM1*, *DNMT3A* and *GATA2*), and 723 downregulated (*e.g.*, *TGFBI*, *CEBPB*, *VEGFA*, *GATA3*, *TLR8* and *TLR4*). In contrast, only 4 *miRs* were differentially (*P*≤.001, FDR<.1) expressed between high and low *DNMT3B* expressers. *miR-133a* and *miR-133b*, targeting *SP1* and *MCL1,* were upregulated, and *miR-148a*, regulating *MITF*, and *miR-122*, repressing *SLC7A1* and modulating interferon-1, were downregulated. Notably, among 134 pts with genome-wide methylation data available, we did not observe any differentially methylated regions in gene promoters.

Summary and Conclusions: High *DNMT3B* expression is independently associated with adverse outcome of older CN-AML pts. The *DNMT3B* expression associated with distinct expression profiles that include many genes, but few *miRs*. Differential expression of these genes does not seem to be related to their methylation status, suggesting that *DNMT3B* may contribute to disease aggressiveness through other mechanisms yet to be elucidated.

S1169

CLINICAL RELEVANCE OF MINIMAL RESIDUAL DISEASE MONITORING IN NPM1 MUTATED AML: A STUDY OF THE AML STUDY GROUP (AMLSG)

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Background: Mutations in the *nucleophosmin 1* (*NPM1*^{mut}) gene represent the most common gene mutations in acute myeloid leukaemia (AML), with highest frequency in cytogenetically normal AML. We have previously shown the applicability and prognostic value of *NPM1*^{mut} based RQ-PCR assays for the detection of minimal residual disease (MRD).

Aims: To confirm our previous results on the prognostic relevance of *NPM1*^{mut}-transcript levels in an extended cohort of AML patients (pts) (18 to 77 years) harbouring *NPM1*^{mut} type A, B, D, JT,4, QM or KM, and to assess the impact of concurrent *FLT3* internal tandem duplications (ITD) and *DNMT3A* mutations.

Methods: All pts were enrolled in one of four AMLSG [AMLHD98A, AMLSG 07-04, AMLSG 09-09, AMLSG 16-10] treatment trials. Treatment comprised double induction therapy with ICE (idarubicin, cytarabine, etoposide) with or without ATRA or gemtuzumab ozogamicin or 1 cycle of daunorubicin and cytarabine followed by 1 to 4 cycles of high-dose cytarabine, autologous or allogeneic stem cell transplantation. *NPM1*^{mut} transcript levels (ratio of *NPM1*^{mut}/*ABL1* transcripts×10^4) were determined by RQ-PCR using TaqMan technology; the sensitivity of the assays was 10^{-5}to 10^{-6}.

Results: A total of 2421 samples [bone marrow (BM) n=2397; peripheral blood (PB) n=24] from 407 *NPM1*^{mut} pts were analysed at diagnosis, after each treatment cycle, during follow-up (FU) and at relapse. *NPM1*^{mut} transcript levels at diagnosis varied between 2.9×10^1 and 10.4×10^6 (median, 6.4×10^5). Pretreatment transcript levels were not correlated with clinical characteristics (*e.g.*, age, WBC, BM blasts, *FLT3* mutation status) and did not impact event-free survival (EFS), relapse-free (RFS) and overall survival (OS). In multivariable analyses, *NPM1*^{mut} transcript levels at different time points were significantly associated with shorter remission duration and shorter OS. After double induction therapy, the cumulative incidence of relapse (CIR) at 4 years was 7% for RQ-PCR-negative pts (n=43) *versus* 50% for RQ-PCR-positive pts (n=195) (P<0.00001); the lower CIR translated into a significant better OS (P=0.0003). After completion of therapy, CIR at 4 years was 20% for RQ-PCR-negative pts (n=106) and thus significantly lower compared with 62% in RQ-PCR-positive pts (n=97; P<0.00001). Again, the lower CIR translated into a significantly better OS (P<0.00001). Multivariable analysis at both time points revealed *NPM1*^{mut} transcript levels as a significant marker for shorter remission duration [(HR, 2.05; 2.20, respectively) and shorter OS (HR, 1.65; 1.62, respectively). During FU, 687 BM and 24 PB samples from 209 patients were analysed. The relapse rate at 2 years for pts exceeding the previously defined cut-off value of >200 *NPM1*^{mut} copies was 90%. Finally, we evaluated the impact of concurrent *FLT3*-ITD and *DNMT3A* mutations on *NPM1*^{mut} transcript levels. Following the first induction cycle, the median *NPM1*^{mut} transcript level was significantly lower in pts without concurrent *FLT3*-ITD or *DNMT3A* mutations. This effect could be observed throughout subsequent treatment cycles.

Summary and Conclusions: In our extended cohort we could confirm the two clinically relevant MRD time points, after induction and after completion of therapy, which allow for the identification of pts at high risk of relapse. During the follow-up period, we could confirm our previously defined cut-off value which is highly predictive for relapse. The reduction of *NPM1*^{mut} transcript levels strongly correlates with the *FLT3*-ITD and *DNMT3A* mutation status.

S1170

AMPLIFICATION OF CHROMOSOME 21 AS A MECHANISM FOR ERG OVEREXPRESSION IN PATIENTS WITH MYELOID MALIGNANCIES

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Background: *ERG* overexpression was shown to be related to adverse outcome in cytogenetically normal AML (CN-AML). The proto-oncogene *ERG* is situated on chromosome 21 (chr21). Acquired gain of chr21 (+21) is a recurrent cytogenetic abnormality in AML. The pathogenetic impact of +21 remains elusive, but data indicates that +21 could be a mechanism underlying the increased expression of genes located in the respective regions.

Aims: The aim of this study was 1) to ascertain the prognostic impact of *ERG* expression in context of other relevant molecular prognostic markers in CN-AML and 2) to analyze a possible relationship between +21 and variations in *ERG* mRNA expression levels.

Methods: *ERG* mRNA expression was analyzed using a TaqMan real-time PCR assay. 1) *ERG* expression was assessed in a cohort of 331 patients (pts) with *de novo* CN-AML. Female/male ratio was 168/163 and age ranged from 18.3-64.8 years (median, 52.7 years).The median%*ERG*/*ABL1* level was used to distinguish low from high *ERG* expressers. *BAALC* levels were analyzed accordingly. Expression levels were correlated with clinical outcome and with the presence of *FLT3*-ITD (n=124/331), *NPM1* mutations (*NPM1*mut, n=208/331), *RUNX1*mut (n=35/330), *CEBPA*mut (n=29/331), *MLL*-PTD (n=27/331), *ASXL1*mut (n=13/331), *FLT3*-TKD (n=32/331), *IDH1*R132mut (n=41/328), *IDH2*R140mut (n=48/328), *IDH2*R172mut (n=6/328), *NRAS*mut (n=53/329) and *WT1*mut (n=27/330). 2) To address *ERG* expression in relation to +21 the following cohorts were analyzed: 66 AML pts with a complex karyotype and +21 (CK+21); 44 AML pts with +21 as sole cytogenetic abnormality (+21s), 15 pts with different myeloid malignancies (6 *de novo* AML, 5 MDS, 1 s-AML, 2 t-AML,1 t-MDS) showing *ERG* amplification by use of interphase FISH (amp21) and 32 CK-AML pts without +21 (CK). Mean *ERG* expression levels were compared by t-test.

Results: 1) At diagnosis,%*ERG/ABL1* levels ranged from 0.078 to1,016.027 (median: 188.582). High *ERG* expression was associated with lower age (P=0.003), higher white blood cell (WBC) count (P=0.017), *FLT3*mut/wt ratio of at least 0.5 (50/165, 30.3% *vs* 25/166, 15.1%, P=0.001) and high *BAALC* expression (104/164, 63.4% *vs* 60/166, 36.6%, P<0.001), as compared to low *ERG* expression. In contrast, *NPM1*mut (91/165, 55.2% *vs* 117/166, 70.5%, P=0.004) and *IDH1*R132mut (11/164, 6.7% *vs* 30/164, 18.3%, P=0.002) were less frequent in high *ERG* expressers. Kaplan Meier analysis revealed inferior overall survival (OS at 3 years: 50.9% *vs* 67.3%, P=0.035) and event free survival (EFS at 3 years: 34.6% *vs* 42.9%, P=0.048) for high *ERG* expressers as compared to low *ERG* expressers. However, in a multivariate model adjusted for age, WBC, *BAALC* expression, *FLT3*mut/wt ratio of at least 0.5, *MLL*-PTD and *WT1*mut no significant impact of *ERG* expression was observed. 2) Mean±SEM expression levels of *ERG* were higher in pts with +21 (+21s and CK+21 combined, 336±27) as compared to pts with CN-AML or CK-AML (229±10, 177±36; P<0.001 and P=0.001, respectively). Moreover, mean *ERG* expression was even higher in pts with amp21 (613±159) as compared to pts with +21 (336±27, P=0.107), albeit this difference was not significant, probably due to small numbers of pts.

Summary and Conclusions: High *ERG* expression is associated with adverse outcome, as well as with the well established risk factors *FLT3*-ITD, high *BAALC* expression and the lack of *NPM1*mut. Furthermore, high levels of *ERG* observed in pts with +21 and moreover in pts with amp21 indicate ERG as an important factor contributing to the pathogenesis and progression of myeloid malignancies with gain of chr21.

Red blood cells clinics

S1171

SERUM FERRITIN FOR PREDICTING CLINICALLY RELEVANT LIC THRESHOLDS TO GUIDE MANAGEMENT OF PATIENTS WITH NON-TRANSFUSION-DEPENDENT THALASSEMIA TREATED WITH DEFERASIROX: THALASSA STUDY EXTENSION ANALYSIS

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Background: Although liver iron concentration (LIC) assessment by magnetic resonance imaging (MRI) to diagnose iron overload and guide iron chelation therapy is clinically more robust, limited access worldwide makes it practical to also use serum ferritin (SF) assessments. THALASSA assessed efficacy and safety of deferasirox in iron-overloaded non-transfusion-dependent thalassemia (NTDT) patients. Previously, we reported the utility of SF as a predictor of clinically relevant LIC thresholds in screened patients (Taher *et al. Haematologica* 2012;97 [Suppl 1]:abst 0927)–representing a patient population being evaluated for iron overload and considered for iron chelation therapy; these patients had a broad range of LIC and were deferasirox-naïve.
Aims: The objective of this analysis was to further evaluate the relationship between SF and LIC after up to 2-yrs' treatment with deferasirox, to help guide the management of iron chelation therapy in patients receiving treatment at clinically relevant thresholds, ie THALASSA thresholds for stopping treatment (LIC<3mg Fe/g dw) or dose escalation (LIC≥7mg Fe/g dw).
Methods: THALASSA enrolled iron-overloaded NTDT patients (LIC≥5mg Fe/g dw and SF>300ng/mL) aged ≥10 years. Patients completing the 1-yr core study could continue into the 1-yr extension, with placebo patients switching to deferasirox. Data from all patients completing the extension with available SF and LIC at the end of treatment (EOT) were used (EOT was end of extension or earlier if patients stopped treatment when LIC<3mg Fe/g dw [n=22]). A receiver operating characteristic analysis was conducted; positive predictive values (% of patients with LIC≥cutoff in all patients whose SF>cutoff) and negative predictive values (% of patients with LIC).
Results: 130 NTDT patients completed treatment and had SF and LIC measurements at baseline and EOT. There was a moderate-to-strong correlation between SF and LIC at baseline (R=0.6382); the magnitude of correlation increased slightly by EOT (R=0.7214). The predictive value of SF for LIC thresholds at EOT are shown in the Table 1. SF>300 is 86.1% predictive of LIC≥3, indicating a 13.9% probability of LIC<3 leading to potential over-treatment. SF<300 is 40% predictive of LIC<3, hence a 60% probability of LIC≥3 potentially leading to sub-optimal treatment when LIC≥3, but only 13.3% probability of LIC≥5. SF>1700 and >2000 are 100% predictive of LIC≥7. SF<1700 is 61.0% predictive of LIC<7, indicating a 39.0% probability of sub-optimal treatment.

Table 1. End-of-treatment predictive value of serum ferritin cutoffs for clinically relevant LIC thresholds used to guide treatment decisions in THALASSA.

Thresholds	End of treatment	
	PPV	NPV
LIC≥3 mg Fe/g dw		
SF cutoff of 300 ng/mL	86.1	40.0
• *SF>300 is 86.1% predictive of LIC≥3 → 13.9% probability of over-treating with LIC<3* • *SF<300 is 40% predictive of LIC<3 → 60% probability of sub-optimal treatment when LIC≥3*		
LIC≥7 mg Fe/g dw		
SF cutoff of 2000 ng/mL	100	59.5
• *SF>2000 is 100% predictive of LIC≥7 → 100% appropriate dose escalation* • *SF<2000 is 59.5% predictive of LIC<7 → 40.5% probability of sub-optimal treatment when LIC≥7*		
SF cutoff of 1700 ng/mL	100	61.0
• *SF>1700 is 100% predictive of LIC≥7 → 100% appropriate dose escalation* • *SF<1700 is 61.0% predictive of LIC<7 → 39.0% probability of sub-optimal treatment when LIC≥7*		

PPV; positive predictive value = % of subjects with LIC≥cutoff in all subjects whose SF>cutoff
NPV; negative predictive value = % of subjects with LIC<cutoff in all subjects whose SF≤cutoff

Summary and Conclusions: Data from patients exposed to deferasirox treatment for up to 2 yrs indicate that a SF threshold <300 ng/mL for stopping chelation treatment appears adequate to minimize the risk of over-chelation. This threshold potentially leads to some patients stopping chelation prematurely, but only a small proportion of these patients would have LIC≥5 mg Fe/g dw, ie at risk of iron-related complications. Using a SF threshold >1700 ng/mL for dose escalation, all patients would be appropriately dose increased (ie all would have LIC≥7 mg Fe/g dw). However, a good proportion of patients with SF<1700 ng/mL would not be dose-increased despite also having a LIC≥7 mg Fe/g dw. Although this analysis is limited by a small number of patients with the lowest levels of SF and LIC, these results confirm previous observations in screened patients and suggest that, if carefully considered, SF could be useful to guide iron chelation therapy management where LIC monitoring by MRI is limited.

S1172

MORBIDITY RISK IN UNTREATED PATIENTS WITH B-THALASSEMIA INTERMEDIA: A CLOSER LOOK AT THE ROLE OF IRON OVERLOAD

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Background: Patients with β-thalassemia intermedia (TI), a form of non-transfusion dependent thalassemia (NTDT), remain at risk of iron overload due to increased intestinal iron absorption. Although an association between iron overload and morbidity risk in this patient population is suggested by cross-sectional studies, data from longitudinal cohorts are limited.
Aims: To evaluate the association between iron overload and morbidity risk in transfusion-independent and chelation-naive patients with TI followed longitudinally for an extended time and thus representing the natural history of the disease.
Methods: This was a retrospective cohort study of patients from five comprehensive care centers in Italy, Lebanon, Oman, Iran, and Egypt. Beyond diagnosis of TI, inclusion criteria were: 1) no history of liver disease (biopsy confirmed fibrosis, cirrhosis, or cancer), osteoporosis, hypothyroidism, hypoparathyroidism, diabetes, hypogonadism, extramedullary hematopoiesis, thrombosis, or pulmonary hypertension, and 2) no history of transfusion, iron chelation, or fetal hemoglobin induction therapy. Patients were followed for eleven years (2000-2010), and occurrence of any of the aforementioned morbidities was recorded. Serum ferritin (SF) levels were recorded at baseline and annually during follow-up. Clinical outcomes were assessed for three distinct groups of mean SF: ≤300 ng/mL (n=8, 15.4%), >300 to <800 ng/mL (n=17, 32.7%), and ≥800 ng/mL (n=27, 51.9%); which are proposed thresholds for tailoring iron chelation therapy in NTDT patients.

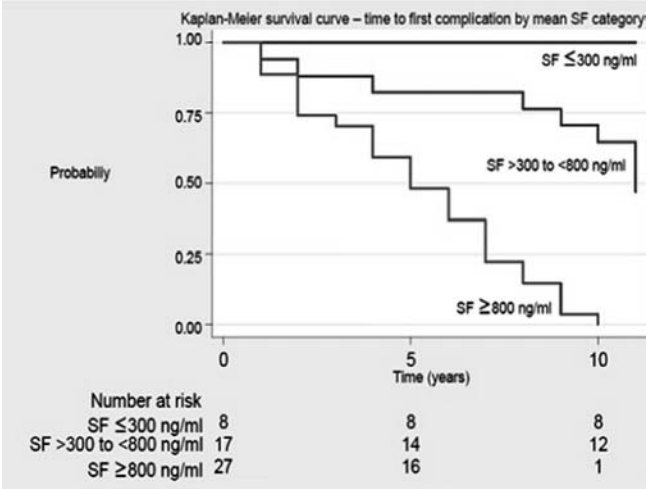

Figure 1.

Results: Fifty-two patients were included in this analysis. Their mean age at baseline was 24.1±1.6 years and 25 (48.1%) were men. No patients died or were lost to follow-up during the study period. There was a steady increase in mean SF level throughout the follow-up period, from a mean±SE of 513.2±51.2 ng/mL at baseline to 1209±103ng/mL at study end (mean annual increase of 9%).%). By study end, 36 (69%) of patients had at least one SF measure ≥800 ng/mL. The cumulative incidence of developing at least one morbidity was significantly higher in patients with mean SF levels ≥800 ng/mL than patients with mean SF levels >300 to <800 ng/mL and ≤300 ng/mL (100% *vs.* 52.9% *vs.* 0%, respectively; Chi-squared test P=0.001). A similar observation was made for

multiple="multiple" morbidity (59.3% *vs.* 5.9% *vs.* 0%, respectively; Chi-squared test P=0.001). Kaplan-Meier plots showed a statistically significant difference in morbidity-free survival between the three mean SF level groups (Figure1, Logrank test, P=0.001). Morbidity-free survival rates at 5- and 10-years were 48.2% and 0% in patients with a mean SF level ≥800 ng/mL, 82.4% and 64.7% in patients with a mean SF level >300 to <800 ng/mL, and 100% in patients with a mean SF level ≤300 ng/mL. A Cox proportional hazard model was developed (stepwise backward selection) that included baseline age and splenecomy status, sex, mean total hemoglobin level and mean SF level observed over the study period. Mean SF level was the only independent and statistically significant risk factor for the occurrence of at least one morbidity (P=0.001) as well as multiple morbidities (P=0.005) during the follow-up period.

Summary and Conclusions: Without chelation, SF levels continue to increase in transfusion-independent patients with TI. SF levels ≥800 ng/mL are associated with a significant increase in long-term morbidity risk; while levels ≤300 ng/mL are not associated with any risk. These data should help guide decisions for iron chelation therapy in this patient population.

S1173

NUCLEOSOMES AND NEUTROPHIL ACTIVATION AS MARKERS OF NEUTROPHIL EXTRACELLULAR TRAP FORMATION DURING SICKLE CELL DISEASE PAINFUL CRISIS

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Background: Sickle cell disease (SCD) is characterized by recurrent acute vaso-occlusive painful crisis frequently leading to SCD related complications, like acute chest syndrome. The complex pathophysiology of the vaso-occlusive painful crisis is mediated by activation of endothelial cells, adhesion of sickled erythrocytes and polymorphonuclear neutrophils (PMN), oxidative stress, coagulation activation and an increased release of inflammatory mediators, resulting in ischemic organ damage. Recently, PMN have been demonstrated to form Neutrophil Extracellular Traps (NETs) upon activation. During this process, DNA and DNA-binding proteins are extruded from neutrophils exposing a mesh consisting of nucleosomes, histones and neutrophil proteases. NET formation has been shown to propagate coagulation in sepsis and deep venous thrombosis. Moreover, nucleosomes and histones exposed on NETs have been shown to be highly cytotoxic to endothelial cells. Beside the exposure on NETs, nucleosomes can be actively released into the circulation from dead cells. Circulating nucleosomes detected in sepsis have been reported to correlate with markers of coagulation and inflammation as well as with organ dysfunction and mortality.

Aims: The aim of this case-control study was to assess plasma levels of circulating nucleosomes and neutrophil activation as evidenced by human neutrophil elastase–α_1-antitrypsin (EA) complexes, as indirect measure of NET formation in plasma.

Methods: After obtaining informed consent, nucleosomes and EA complexes were measured using ELISA in blood samples of race matched healthy controls (24), sickle cell patients during steady state (74) and sickle cell patients with painful crisis (70). Markers of inflammation and endothelial activation were also measured. For statistical analysis, patients were divided in two groups: patients with the relatively severe genotypes HbSS and HbSβ^0-thalassemia (HbSS/HbSβ^0-thal) and patients with the relatively milder HbSC and HbSβ$^+$-thalassemia genotypes (HbSC/HbSβ$^+$-thal). The study protocol was approved by the local medical ethical committee.

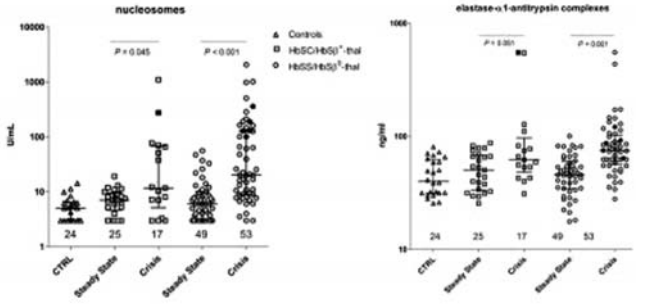

Figure 1.

Results: Plasma levels of nucleosomes in both patients with HbSS/HbSβ^0-thal and HbSC/HbSβ$^+$-thal were significantly higher during painful crisis (median; IQR, 20.2 U/mL; 8.9–129.0, *P*<0.001, and 11.7 U/mL; 5.1–67.7, *P*=0.045) as compared to those in steady state (6.0 U/mL; 3.0–9.8 and 7.1 U/mL; 4.6–9.6). EA levels were higher in HbSS/HbSβ^0-thal and HbSC/HbSβ$^+$-thal patients during painful crisis (75.1 ng/mL: 56.5–102.4, *P*<0.001; 62.0 ng/mL: 48.0–96.7; *P*=0.051) as compared to levels during steady state (45.7 ng/mL; 34.7–59.7 and 50.2 ng/mL; 33.3–67.7), but just failed to reach statistical significance in HbSC/HbSβ$^+$-thal patients. During painful crisis, EA levels correlated strongly with levels of nucleosomes in both HbSS/HbSβ^0-thal (Sr=0.55, *P*<0.001) and HbSC/HbSβ$^+$-thal patients (Sr=0.90, *P*=<0.001). In steady state HbSS/HbSβ^0-thal patients, levels of nucleosomes correlated with endothelial markers sVCAM-1 and vWF:Ag (Sr=0.421, *P*=0.003; Sr=0.452, *P*=0.001). Six patients who developed an acute chest syndrome during painful crisis were among the patients with the highest nucleosome and EA levels. A significant (Sr=0.441, *P*<0.001) correlation was found between levels of nucleosomes and duration of hospitalization (Figure 1).

Summary and Conclusions: We demonstrate for the first time increased levels of circulating nucleosomes in sickle cell patients with painful crisis reflecting NET formation which strongly correlates with PMN activation and disease severity.

S1174

MUTATIONS OF ABCG2 TRANSPORTER AS THE GENETIC BASIS OF HIGH FREQUENCY BLOOD ANTIGEN JUNIOR

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Background: Blood group antigen variants caused by genetic alterations may trigger severe diseases during pregnancy and blood transfusion, such as hemolytic disease of the fetus and newborn (HDFN) or adverse hemolytic transfusion reactions. Membrane proteins, comprising about 30% of the total number of human proteins, are crucial in many diseases, while currently no simple assays are available for the determination of their tissue levels. ABCG2 gene has recently been described as the the genetic basis of one of the high frequency red-blood-cell (RBC) blood group antigens, encoding Junior (Jun) blood group.

Aims: To identify the genetic background of low RBC ABCG2 protein expression level in healthy individuals.

Methods: 61 healthy volunteers (47 unrelated individuals and 14 family members of two volunteer probands with low ABCG2 transporter expression) were enrolled. RBC ABCG2 protein expression was measured by flow cytometry (FACS) using paraformaldehyde fixed RBC stained with three different ABCG2 specific monoclonal antibodies. Using whole genomic DNA, common ABCG2 SNPs V12M (c.34G>A, p.12Val>Met, rs2231137) and Q141K (c.421C>A, p.141Gln>Lys, rs2231142) were genotyped by LightCycler allelic discrimination system, while novel mutations were identified by direct sequencing of exons and flanking intronic regions.

Results: ABCG2 protein expression on RBC did not associate with age or sex. Among the 47 donors, there were 11 individuals with heterozygous Q141K variant (allele frequency: 11.7±6.6%), and 3 individuals with heterozygous V12M variant (allele frequency: 3.2±3.6%) genotypes. Individuals carrying the heterozygous ABCG2 Q141K variant exhibited significantly lower expression of ABCG2 (5.27±1.19) on RBC, as compared to homozygous wild-type individuals (6.13±0.61, P=0.011). There was no significant difference between homozygous wild-type individuals and heterozygous V12M carriers.Two unrelated individuals out of 47 exhibited lower RBC ABCG2 expression (2.65±0.29). Sequencing of the ABCG2 gene in these cases revealed two different heterozygous mutations; a nonsense mutation, causing an arginine to stop codon change in exon 7 (c.706C>T, p.R236X, rs140207606) and a small deletion also in exon 7 (c.791_792delTT, L264HfsX14). Both of these mutations result in premature termination. Low ABCG2 expression phenotypes were segregated in both families with the presence of the respective mutations.

Summary and Conclusions: Based on our results, FACS may be a rapid and reliable assay for the quantitative determination of membrane protein expression in human erythrocytes. We found significant differences between the expression levels of the wild-type ABCG2 protein and the heterozygous Q141K polymorphic variant. Our observations suggest that the ABCG2 genetic variants associated with the absence of high frequency blood group antigens may be more common than previously described.

S1175

THE SAFETY, TOLERABILITY AND EFFICACY OF FBS0701, AN IRON CHELATOR IN CLINICAL DEVELOPMENT FOR TRANSFUSIONAL IRON OVERLOAD: DATA FROM 72 WEEKS OF TREATMENT

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Background: Iron overload is a major concern in transfusion-dependent anemias. Many patients are inadequately treated owing to a lack of efficacy at established doses of iron chelators or inadequate dosing resulting from adverse events (AEs) and adherence issues. The efficacy, safety and tolerability of the iron chelator FBS0701 has been reported at 2 doses (16 and 32 mg/kg/day) over 24 weeks, from a randomized study in 51 patients. A clear dose-response shown by changes in liver iron concentration (LIC) from baseline (+3.1 mg/g dry weight [dw] *vs* −0.3 mg/g dw for the low- *vs* high-dose groups, $P<0.03$) was reported (Neufeld *et al. Blood* 2012). Excellent tolerability with the absence of dose-dependent AEs encouraged extension of the study up to a total length of 96 weeks.
Aims: To assess the efficacy, safety and tolerability of FBS0701 in adults with transfusional iron overload treated with assigned fixed doses of chelator for 24 weeks followed by dose adjustments until study end. Here we describe data from 72 weeks' treatment.
Methods: Patients with thalassemia syndromes and sickle cell disease completing the first 24 weeks with LIC ≥2 mg/g dw, serum ferritin (SF) ≥350 ng/mL and cardiac T2* ≥10 ms were eligible for a 24-week extension. Those completing this extension and still meeting eligibility criteria could enter a further 48-week extension. Dose adjustments were allowed based on efficacy parameters at/after week 24, at which point doses up to 60 mg/kg/day were permitted for the remainder of the study. Outcome measures included LIC determined by FerriScan® R2 MRI at weeks12, 24, 48 and 72, monthly SF levels, safety monitoring and laboratory measurements. All patients provided written informed consent.
Results: Fifty-one patients started the study (age, 18–48 years). Forty-two of 49 patients completing the first 24 weeks entered weeks 24–48; 39 completed 48 weeks. Of these, 37 patients entered the extension (weeks 49–96). Of the patients who had LIC measurements at week 72, 10/29 (34.5%) and 19/29 (65.5%) had high (>0.4 mg/kg/day) and low (≤0.4 mg/kg/day) baseline transfusion burden, respectively. LIC until week 72 is shown in the Figure 1. Median (range) SF reduced from 2766 (570–6667) ng/mL at week 24 (n=30) to 2396 (631–7037) ng/mL at week 72 (n=29). AEs related to study drug were reported in 31/51 patients (60.8%; safety set). Serious AEs occurred in 5 patients (9.8%), leading to discontinuation of study drug in 1 patient owing to a treatment-related case of hypoesthesia. Treatment-related increases in transaminases occurred in 8 patients (15.7%); in 3 cases this was due to new onset hepatitis C leading to discontinuation of FBS0701 in 1 patient. The other 5 patients had fluctuating levels that were not clinically significant, while continuing on FBS0701. Common treatment-related AEs were headache and flatulence (both n=6, 11.8%). Gastrointestinal related-AEs included diarrhea (n=3, 5.9%), nausea, constipation and abdominal pain (all <5%). Mean serum creatinine was consistent with baseline levels over the 72 weeks.

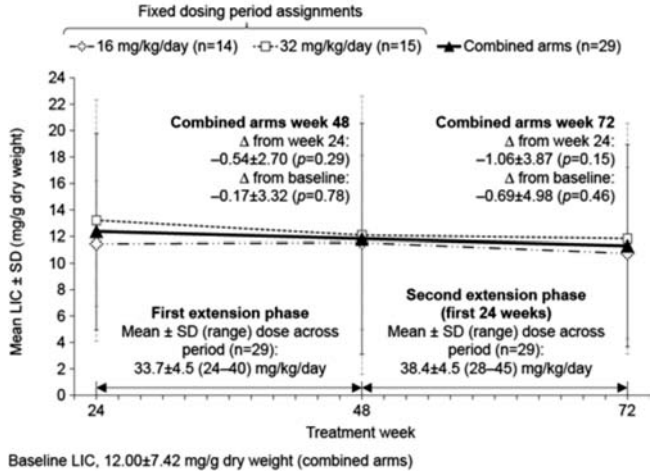

Figure 1. Liver iron concentration (LIC) from weeks 24 to 72 in patients with measurements at week 72.

Summary and Conclusions: FBS0701 doses up to 50 mg/kg/day continued to be well tolerated, with no evidence of renal or hematological effects or other safety concerns. FBS0701 at 32 mg/kg/day has been shown to maintain iron balance during the first 24 weeks. Safety data support making further dose escalations to achieve the ideal endpoint of negative iron balance in all patients.

Novel therapeutics

S1176

HIGH-THROUGHPUT DRUG SENSITIVITY AND RESISTANCE TESTING (DSRT) COMBINED WITH MOLECULAR PROFILING FOR THERAPEUTIC TARGETING OF NUP98-NSD1-POSITIVE AML

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Background: The *NUP98-NSD1* fusion gene is a recently discovered recurrent aberration in 17% of pediatric and 2-3% of adult cytogenetically normal (CN) AML patients. The occurrence of the fusion is strongly associated with *FLT3-ITD* mutations. The transforming property of *NUP98–NSD1* involves a block in cellular differentiation and enforced leukemic progenitor self-renewal through persistent overexpression of distinct HOX-genes (*HOXA5, HOXA7, HOXA9, HOXA10* and *MEIS1*). The fusion is associated with very poor outcome and a 4-year event-free survival of less than 10% in both pediatric and adult patients (Iris *et al.* Blood 2011).
Aims: Our aim was to identify novel therapies for *NUP98-NSD1*-positive AML.
Methods: We performed deep molecular profiling (exome and RNA sequencing at multiple time points throughout the disease) and *ex vivo* high-throughput drug sensitivity and resistance testing (DSRT) on a patient with relapsed, chemorefractory CN-AML. DSRT was performed using fresh BM MNCs (>50% blasts) with a panel of all FDA/EMA-approved small molecule and conventional cytotoxic oncology drugs, as well as investigational compounds (n=202) over a10,000-fold concentration range. A leukemia specific drug sensitivity score (sDSS) derived from an area under the dose response curve calculation was used as the efficacy assessment by comparing drug sensitivity profiles of primary leukemia and normal bone marrow cells.
Results: NUP98-NSD1 blasts showed selective sensitivity (measured as sDSS, Figure 1) to the mTOR inhibitor temsirolimus (sDSS 12) and the multi-kinase inhibitors dasatinib (sDSS 15) and sunitinib (sDSS 17). Following treatment with a combination of temsirolimus, dasatinib and sunitinib (TDS), a rapid decrease of the bone marrow blast cell count occurred accompanied with normalization of peripheral blood neutrophil count, but no increase in platelet count (a CRi-response). Within 30 days of treatment, a relapse occurred and repeated DSRT showed loss of sensitivity to the applied drugs (temsirolimus: sDSS1, dasatinib: sDSS 6 and sunitinib: sDSS 4) reflecting good correlation of *ex vivo* and *in vivo* sensitivity and resistance. The *NUP98-NSD1* fusion was detected in all samples, including the diagnostic sample (600_0) suggesting that this was an early initiating event in the development of the patient's disease. Exome sequencing revealed a diverse subclonal clonal architecture highlighted by *FLT3-ITD* and *WT1* mutations. After induction chemotherapy, the predominant *FLT3-ITD* subclone was no longer detectable, while subsequent therapies, including the dasatinib, sunitinib and temsirolimus combination, induced a change in the proportional sizes of different subclones containing *WT1* mutations, as well as appearance of a second *FLT3-ITD*.

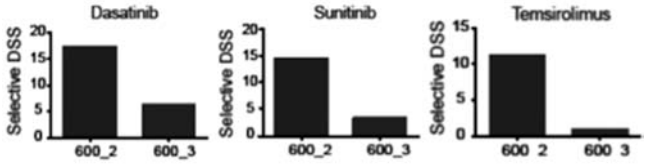

Figure 1.

Summary and Conclusions: The TDS-combination therapy may present a novel targeted therapy option for patients with *NUP98-NSD1*-driven AML and warrants further preclinical and clinical experimentation, which are ongoing in our laboratory. The comprehensive DSRT platform covering the entire cancer pharmacopeia and many emerging agents rapidly identifies individually optimized combinatorial therapies, and when coupled with deep molecular profiling provides insights into the molecular events underlying disease progression in *NUP98-NSD1*-positive and other molecularly defined subgroups of AML.

S1177

PF-114, A NOVEL SELECTIVE PAN BCR/ABL INHIBITOR FOR PHILADELPHIA CHROMOSOME-POSITIVE (PH+) LEUKEMIA, EFFECTIVELY TARGETS T315I AND THE OTHER RESISTANCE MUTANTS

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Background: Targeting BCR/ABL by ABL-directed kinase inhibitors (AKIs) induces long lasting remissions in many patients with chronic myeloid leukemia (CML), and short remissions in Ph+ acute lymphatic leukemia (ALL). Notably in advanced Ph+ leukemia resistance attributable to either kinase domain mutations in BCR/ABL or non mutational mechanisms remains the major clinical challenge. With the only exception of Ponatinib, a multitargeted kinase inhibitor, all actually approved AKIs are unable to inhibit the "gatekeeper" mutation T315I. On the other hand Ponatinib is unable to overcome non mutational resistance in advanced leukemia. This together with the fact that Ponatinib is the AKI with the broadest spectrum of kinase inhibitions reveals that there is the urgent need for further and more selective therapeutical options in treating therapy-resistant advanced Ph+ leukemia.
Aims: We wanted to develop an AKI, which should have the following qualities: i.) ability to target all known resistance mutations in BCR/ABL but mainly the T315I; ii.) a higher selectivity as compared to Ponatinib in order to reduce undesired side effects; iii.) ability to overcome also non mutational resistance in advanced Ph+ leukemia; iv.) activity not only in chronic phase CML but also advanced Ph+ leukemia, Ph+ ALL or blast crisis CML (BC-CML).
Methods: The preclinical evaluation of PF-114 was performed in direct comparison to Ponatinib on golden standard preclinical models of CML and advanced Ph+ leukemia. Advanced leukemia model were cell lines (K562, KCL22, BV173 for BC-CML, SupB15 and TOM1 for Ph+ ALL), primary patient-derived long term culture (PD-LTC) of Ph+ ALL patients and secondary BCR/ABL-induced murine ALLs for the *in vivo* studies. The effects on mutational resistance was investigated i.) on the factor dependence of Ba/F3 cells expressing BCR/ABL or its clinically most relevant resistance mutants (Y253F, E255K, T315I, F317L); ii.) *in vivo* on the transduction/transplantation model of BCR/ABL- and BCR/ABL-T315I-induced CML-like disease; iii.) on a PD-LTC of a Ph+ ALL patient harboring the T315I. As models for non mutational resistance we used PD-LTCs from Ph+ALL patients with different levels of non mutational drug resistance and the SupB15RT, a Ph+ ALL cell line rendered resistant by the exposure to increasing doses of Imatinib, which exhibits a cross resistance against all approved AKIs.
Results: PF-114 is an orally available AKI, which is more selective than Dasatinib or Ponatinib (number of kinases inhibited at 100 nM of a drug: Nilotinib - 19; PF-114 - 27; Dasatinib - 48; Ponatinib - 80). It efficiently inhibited all tested BCR/ABL mutants in cellular and biochemical assays at dosages of 10-100nM. It also suppressed growth of Ph+ PD-LTC with non mutational resistance as well as the BCR/ABL-T315I-positve PD-LTC in this dosage range. In all models the effect was independent of the presence of either the p210 or the p185 form of BCR/ABL. No effect of PF-114 was seen in PD-LTCs of Ph- ALL. Noteworthy PF-114 (50 mg/kg) prolonged significantly the survival of mice with both BCR/ABL- and BCR/ABL-T315I-driven CML-like disease as compared to Ponatinib (25 mg/kg). Like Ponatinib PF-114 was unable to overcome the non mutational resistance in SupB15RT.
Summary and Conclusions: Our work supports clinical evaluation of PF-114 as a pan BCR/ABL inhibitor for treatment not only for chronic phase CML, but also for advanced and resistant Ph+ leukemia such as Ph+ ALL or BC-CML.

S1178

UPDATED INTERIM RESULTS OF AN INTERNATIONAL, MULTICENTER, PHASE 2 STUDY OF IBRUTINIB (PCI-32765) IN RELAPSED OR REFRACTORY MANTLE CELL LYMPHOMA

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Background: Bruton's tyrosine kinase (BTK) is a central mediator of B-cell receptor (BCR) signaling essential for normal B-cell development. Ibrutinib is an oral BTK inhibitor that induces apoptosis and inhibits migration and adhesion of malignant B-cells. Mantle cell lymphoma (MCL) is an aggressive non-Hodgkin lymphoma subtype, and despite high initial responses to standard therapy, patients often relapse. Preliminary results in 51 evaluable patients demonstrated ibrutinib induced rapid nodal responses in relapsed or refractory MCL (Wang, ASH 2011). Interim results for the fully enrolled study, PCYC-1104, were reported (Wang, ASH 2012), and a longer follow-up resulted in a higher overall response rate (ORR). Updated results of this international, multicenter, phase 2 study of single agent ibrutinib in relapsed or refractory MCL will be presented.
Aims: Objectives of the study were to assess ORR, duration of response (DOR), overall survival (OS), progression free survival (PFS) and the safety and tolerability of ibrutinib as a single agent in relapsed or refractory MCL.
Methods: Relapsed or refractory MCL patients who were either bortezomib-naïve or bortezomib-exposed were enrolled. All patients had the study explained to them prior to their enrollment and voluntarily consented to participate in the study. Ibrutinib 560 mg PO QD was administered continuously until disease progression. Tumor response was assessed every 2 cycles (one cycle=28 days). The study enrolled 115 patients (65 bortezomib-naïve, 50 bortezomib-exposed); 111 patients were treated; 110 were evaluable for response. Baseline characteristics included: median age 68 yrs, median time since diagnosis 42 months, median number of prior treatments 3; bulky disease (>10 cm) 13%, prior stem cell transplant 10%, high risk MIPI 49%.
Results: Safety data is reported for 111 patients. Treatment-emergent adverse events (AEs) seen in ≥20% of patients included: diarrhea (40%), fatigue (36%), URI (23%), nausea (23%), and dyspnea (21%). Grade 3 AEs included neutropenia (13%), anemia (8%), thrombocytopenia (7%), abdominal pain (5%), diarrhea (5%), dyspnea (5%), and pneumonia (5%). Grade 4 treatment-related AEs included: neutropenia (7%), hyperuricaemia (2%), pancytopenia (1%), thrombocytopenia (1%), and sepsis (1%). One Grade 5 pneumonia was reported as treatment-related. Median time on study was 9.2 months; 47% of patients remain on therapy. Median PFS was 13.9 months and DOR has not yet been reached. Boretezomib-naive (n=63): ORR=65%; CR=21%; PR=44%. Bortezomib-exposed(n=47): ORR=72%; CR=23%; PR=49%. Total (all patients, n=110): ORR=68%; CR=22%; PR=46%. Response rates increased with longer treatment. With longer follow-up of the initially reported subset of 51 patientsdescribed at ASH 2011(median time on treatment then was 3.7 months, now 14.7 months), the CR rate increased from 16% to 39%, and the ORR increased from 69% to 75.
Summary and Conclusions: Analysis upon longer follow-up demonstrates the durability of responses and confirms the unprecedented single agent activity of ibrutinib in relapsed or refractory MCL. The treatment-emergent AEs were consistent with safety data previously reported. Pivotal studies in relapsed or refractory MCL have been initiated.

S1179

FIRST CLINICAL TRIALS EMPLOYING SLEEPING BEAUTY SYSTEM AND ARTIFICIAL ANTIGEN PRESENTING CELLS TO GENERATE T CELLS EXPRESSING CD19-SPECIFIC CHIMERIC ANTIGEN RECEPTOR

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Background: T cells can be genetically modified *ex vivo* to redirect specificity upon enforced expression of a CAR that recognizes tumor-associated antigen (TAA) independent of human leukocyte antigen.
Aims: We report a new approach to non-viral gene transfer using the *Sleeping Beauty* (SB) transposon/transposase system to stably express a 2nd generation CD19-specific CAR (designated CD19RCD28) in autologous and allogeneic T cells manufactured in compliance with current good manufacturing practice (cGMP) for Phase I/II trials.
Methods: T cells were electroporated using a Nucleofector device to synchronously introduce DNA plasmids coding for SB transposon (CD19RCD28) and hyperactive SB transposase (SB11). T cells stably expressing the CAR were retrieved over 28 days of co-culture by recursive additions of g-irradiated artificial antigen presenting cells (aAPC) in presence of soluble recombinant interleukin (IL)-2 and IL-21. The aAPC (designated clone #4) were derived from K562 cells and genetically modified to co-express the TAA CD19 as well as the co-stimulatory molecules CD86, CD137L, and a membrane-bound protein of IL-15. The dual platforms of the SB system and aAPC are illustrated in Figure 1.
Results: To date we have enrolled 11 patients with multiply relapsed ALL (n=4) or B-cell lymphoma (n=7) on three investigator initiated trials at MD Anderson Cancer Center to infuse thawed patient- and donor-derived CD19-specific T cells in the adjuvant setting after autologous (n=3), allogeneic adult (n=7) and umbilical cord (n=1) hematopoietic stem-cell transplantation (HSCT). Each clinical-grade T-cell product was subjected to a battery of in-process testing to complement release testing. Four adult patients have been treated following allogeneic HSCT (ALL, n=3; NHL, n=1), beginning at a dose of 10^6 and escalating to 10^7 modified T cells/m^2. No toxicities have been noted to date. In our

first dose cohort (10^6T cells/m^2), we could not detect sustained persistence of CAR+ T cells, and the 3 patients in this cohort relapsed. We then infused a higher dose of T cells (10^7T cells/m^2) on a compassionate basis to two of these patients with ALL: One patient relapsed, while one patient obtained a 4th complete remission with B-cell aplasia and persistent expression of the CAR+ T cells as of his last follow-up 4 months after T cell infusion.

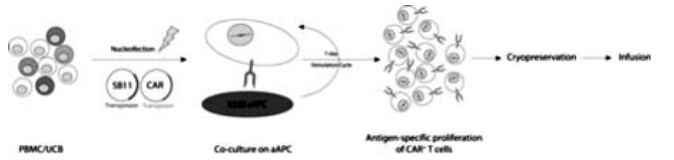

Figure 1.

Summary and Conclusions: We report the first human application of the *SB* system to genetically modify clinical-grade cells, which can be achieved at less cost compared with clinical grade virus. Our data enhance the translational appeal of electroporation of the *SB* system and aAPC-mediated propagation of T cells. Importantly, infusing CD19-specific CAR+ T cells in the adjuvant HSCT setting is feasible and safe and may provide an effective approach for maintaining remission in patients with high risk, CD19+ lymphoid malignancies.

S1180

THE BRUTON'S TYROSINE KINASE (BTK) INHIBITOR, IBRUTINIB (PCI-32765), HAS PREFERENTIAL ACTIVITY IN THE ACTIVATED B CELL-LIKE (ABC) SUBTYPE OF RELAPSED/REFRACTORY (R/R) DLBCL: INTERIM PHASE 2 RESULTS

S deVos[1,*], W Wilson[2], J Gerecitano[3], A Goy[4], V Kenkre[5], P Barr[6], K Blum[7], A Shustov[8], R Advani[9], N Fowler[10], J Vose[11], C Lih[12], M Williams[12], R Schmitz[2], Y Yang[2], S Pittaluga[2], G Wright[2], L Kunkel[13], J McGreivy[13], D Beaupre[13], S Balasubramanian[13], M Cheng[13], D Moussa[13], L Chang[13], J Buggy[13], L Staudt[2]

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Background: Diffuse large B-cell lymphoma (DLBCL) has two molecular subtypes: ABC and germinal center B cell-like (GCB). Survival in the ABC subtype is sustained by "chronic active" BCR signaling. Mutations affecting the BCR subunit CD79B occur in 21% of the ABC subtype but only 5% of GCB tumors. A second BCR-related pathway coordinated by MYD88, an adapter for Toll-like receptors, is also relevant. A constitutively active MYD88 mutant (L265P) is frequent in ABC DLBCL tumors (29%) but rare in GCB DLBCL. CD79B and MYD88 L265P mutations often coexist in ABC DLBCL tumors, suggesting oncogenic collaboration, but can also occur alone. We will present updated results of this international, multicenter, phase 2 study of single-agent ibrutinib, a first-in-class inhibitor of BTK, in R/R DLBCL.

Aims: We tested the hypothesis that ibrutinib would be more active in ABC than GCB DLBCL because of the dependence on BCR signaling. However, we also assessed the association of CD79B and MYD88 mutations with response. The primary objective was to assess the overall response rate (ORR) categorized by subtype. Response was investigator-determined using the revised IWG Criteria for NHL. Secondary objectives of the study were to assess the safety and tolerability of ibrutinib in DLBCL.

Methods: Patients received ibrutinib 560 mg PO QD until disease progression. Gene expression profiling of formalin-fixed paraffin-embedded biopsy tissues using Affymetrix arrays was used to identify the subtype; Sanger sequencing was used to identify CD79B and MYD88 mutations. Patients underwent CT and PET scans pre-treatment and every 2 cycles (one cycle=28 days). 70 patients were enrolled; median age 64 yrs (28-92); male 71%; High-Intermediate/High IPI 59%; disease ≥10 cm 23%; median prior treatments 3 (1-7); relapsed 27%, refractory 54%, unknown 19%; prior SCT 23%; median time from diagnosis 19 months (5-332). For analysis of ORR and mutation status, we also included 10 patients with ABC DLBCL treated on an expansion component of the phase I, at the same dose and schedule. All patients had the study explained to them prior to their enrollment and voluntarily consented to participate in the study.

Results: Safety data from 70 patients identified no new safety signals; 66 patients were evaluable for response. In ABC patients, responses were observed in 12/29 patients for an ORR=41% (CR 17%, PR 24%); in GCB, ORR was 5% (1/20) with no CRs; no responses were observed in the unclassifiable cases. Thus, ibrutinib showed a preferential response activity in ABC *versus* GCB DLBCL (P=0.0071, Fisher's exact test). Median overall survival (OS) was 9.76 months for the ABC subtype and 3.35 months for the GCB subtype. Responses were seen in ABC DLBCL with CD79B mutations (71%; 5/7) and wild type CD79B (34%; 10/29), suggesting ibrutinib sensitivity does not require a BCR mutation. Interestingly, 4 of 5 cases with mutations in both CD79B and MYD88 responded indicating that MYD88 status does not preclude response to ibrutinib. However, tumors with only MYD88 mutations (n=5) did not respond (P=0.0476, Fisher's exact test), suggesting a MYD88-dependent but BCR-independent pathogenesis for some ABC DLBCL tumors.

Summary and Conclusions: Ibrutinib showed a clinically meaningful ORR in R/R ABC DLBCL, but not in the GCB subtype. Results are consistent with an essential role of BCR signaling in ABC DLBCL, and future trials will be planned with this subtype.

Stem cell transplantation - Experimental

S1181

RECOMBINANT CD95-FC (APG101) PREVENTS GRAFT-VERSUS-HOST DISEASE IN MICE WITHOUT DISABLING ANTI-TUMOR CYTOTOXICITY AND T CELL FUNCTIONS

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Background: Graft-*versus*-host disease (GVHD) induced by transplant-derived T cells represents a major complication after allogeneic bone marrow transplantation (BMT). These T cells, however, also support engraftment, early T cell immunity and mediate the graft-*versus*-tumor (GVT) effect. Cytotoxic effector functions and subsequent target cell destruction by transplant-derived allogeneic T cells are predominantly mediated by the CD95/CD95L and the perforin/granzyme system.
Aims: APG101 is a novel human recombinant fusion protein, consisting of the extracellular part of CD95 and the Fc domain of an IgG1 antibody and inhibits CD95L-mediated apoptosis. APG101 was tested for its ability to prevent GVHD in an allogeneic BMT model without abrogating the GVT effect.
Methods: To analyze APG101 treatment on GVHD development, lethally irradiated B6D2F1 (H-2^{bxd}) mice were reconstituted with C57BL/6 (H-2^{b})-derived BM and spleen cells and treated with APG101 starting either one day before or 6 or 13 days after transplantation. By co-injection of either P815 mastocytomas or primary B-ALL cells into transplanted mice, influence of APG101 on GVT effect was tested. Further, effect of APG101 on donor chimerism and phenotype, function, and homing of allogeneic T cells was monitored.
Results: *In vitro*, APG101 treatment inhibited CD95L-mediated T cell apoptosis but did not affect T cell proliferation or development of alloantigen-specific cytotoxic T cells. Treatment of allogeneic BM-transplanted animals starting one day before or 6 days after transplantation efficiently prevented clinical GVHD and increased survival to nearly 100%. No inhibition of GVHD was detected when treatment started 13 days after transplantation. Most importantly, APG101 treatment did not interfere with the GVT-effect since P815 mastocytoma and primary Bcr-Abl-transformed B cell leukemias were completely eradicated by the alloantigen-specific T cells. Donor chimerism of transplanted animals or phenotype, function, and homing of alloantigen-specific T cells was unaltered by APG101 treatment.
Summary and Conclusions: Inhibiting CD95/CD95L interaction by APG101 is an effective therapeutic approach to prevent GVHD while preserving anti-tumor cytotoxicity and early T cell immunity. These data suggest that APG101 could be incorporated into protocols for GVHD prevention and treatment during BMT.

S1182

KREC QUANTIFICATION IS A SUITABLE MARKER TO MONITOR THE DELAY IN EARLY B CELL RECONSTITUTION ASSOCIATED WITH ACUTE GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: The curative effect of allogeneic hematopoietic stem cell transplantation (alloHSCT) is limited by infectious complications and graft-*versus*-host disease (GvHD). B cell dysfunction following delayed immune reconstitution substantially contributes to an increased risk for life-threatening infections after alloHSCT.
Aims: Whereas chronic GVHD is known to negatively influence the B cell lymphopoiesis, less is known about the impact of acute GvHD on early B cell reconstitution. For this, we investigated B cell subset reconstitution kinetics in adult patients diagnosed with acute leukemia. Patients gave informed consent.
Methods: We phenotypically and functionally determined different B cell subsets by flow cytometry before and within six months after alloHSCT and performed RT-PCR quantification of kappa-deleting recombination excision circles (KREC), which are non-replicative episomal plasmids generated during bone marrow B cell development.
Results: We observed a significant deficiency of CD19+ B cells already pretransplant that proceeded during the first month post transplantation (mean cells/mL blood±SEM: 10±3 pretransplant, 4±1 day14, 3±1 day28 post alloHSCT; 43±8 healthy donor). During that time period, detectable B cells exhibited a highly activated (CD95+ and CD86+) phenotype with significantly reduced expression levels of the survival receptor BAFF-R and the chemokine receptors CCR7, CXCR5 and CXCR4. The predominance of naïve and memory B cell subsets indicated the putative origin from the donor graft. Early onset of bone marrow neogenesis was seen in 52% of patients, characterized by a strong increase of transitional B cells between day 60 and 90 post alloHSCT (120±75 day90). These transitional B cells exhibited normal expression of activation markers, but reduced expression of BAFF-R and CCR7. KREC quantification revealed a highly positive and significant correlation between absolute KREC copy numbers and transitional B cells (Spearman: r=1.00, P=0.003) indicating ongoing B cell neogenesis in patients with an early recovering B cell reconstitution. In contrast, naïve and memory B cells did not significantly correlate to the KREC copy numbers. In those patients, characterized by a late recovering B cell reconstitution (48%), low transitional B cell counts between day 60 and 90 were correlated with low KREC copy numbers (r=0.94, P=0.02). Interestingly, the delay in B cell reconstitution was significantly associated with acute GvHD as well as pretransplant myeloablative conditioning (MAC) compared to patients without GvHD and non-MAC treatment (GvHD: 73% *vs* 29%, Fisher´s exact P=0.047; non-MAC: 45% *vs* 93%, P=0.021). KREC level positively correlated with high transitional B cell counts in patients without GvHD (r=1, P=0.003).
Summary and Conclusions: From our data we conclude, that early onset of B cell reconstitution in alloHSCT patients is characterized by a switch in B cell subset distribution and function, which parallels the shift from graft-derived B cells towards newly regenerating B cells within three months after transplantation. Systemic acute GvHD is significantly associated with delayed B cell reconstitution and potentially reflects the consequence of bone marrow niche destruction by alloreactive T cells and pretransplant conditioning. Furthermore, our data reveal KREC quantification as a suitable marker to monitor early B cell neogenesis after alloHSCT as KREC levels are highly comparable to transitional B cell reconstitution kinetics, allowing to estimate bone marrow output post alloHSCT in dependency on different clinical parameters such as acute GvHD.

S1183

MULTI-GENOTYPE OF MINOR HISTOCOMPATIBILITY ANTIGENS (MHAGS) TO STUDY GRAFT VERSUS HOST DISEASE (GVHD) AND GRAFT VERSUS LEUKEMIA (GVL) EFFECTS IN ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: The outcome of allogeneic stem cell transplantation (Allo-SCT) is closely related to graft *versus* host disease (GvHD) and graft *versus* leukemia (GvL) effects which, in part, are mediated by mHAgs. Twenty-six mHAgs (see Table 1) have been identified and reported to be differently and variably correlated with GVHD or GVL, but a simultaneous method to genotype a so large panel of mHAgs has never been employed.

mHAgs correlated with GvHD	mHAgs correlated with GvL	mHAgs correlated with both GvHD and GvL	mHAs with clinical significance to be determined
UGT2B17	ACC-1, ACC-2, ACC-6, C19orf48, HB-1, LB-ADIR-1, LB-LY75-1K, LB-MR1-1R, LB-MTHFD1-1Q, LB-PTK2B-1T, LRH1	HA-1, HA-2, HA-8, CD31	ACC-4, ACC-5, CTL7A7, DPH1, DRN7, HA-3, HEATR-1, P2RX7, LB-ECGF-1H, UTA2-1

Table 1.

Aims: The aim of this work has been to develop a feasible method to genotype all the 26 mHAgs described so far and to test them for their correlation with GVHD and GVL in a group of donor/recipient pairs submitted to allo-SCT.
Methods: For a multi-genotype of 23 mHAgs we used a Maldi-Tof IPlex Gold technology (3 multiplex). This assay is relatively fast and requires a small amount of DNA. For the other three mHAgs we designed other three assays: two based on capillary sequencing of PCR products (for LB-MR1-1R and LRH1) and the last on PCR alone (for UGT2B17). By these methods, we tested the 26 mHAgs in 70 donor/recipient pairs with 4-digit high resolution typing that underwent allo-SCT (sibling or MUD) because of Philadelphia positive CML (n=46) or ALL (n=24).
Results: Maldi-Tof IPlex Gold technology proved a high degree of efficiency. Out of a total of 3430 SNPs a good genotype was obtained in 3383 (98.6%). As expected, sibling pairs showed most identity of MUD pairs. In the setting of

MUD pairs, some immunogenic differences were correlated with a better disease free survival, suggesting a role in driving GvL reaction. Notably, the presence of these differences did not modify the risk and the incidence of GvHD. Furthermore, we confirmed that mHAg CD31 shows frequently an immunogenic mismatch between recipient and donor with graft *versus* host direction (6 patients/70; 8.6%) and correlates with a better disease free survival.
Summary and Conclusions: Our data generated by a multi-genotype technique confirm the role of mHAgs in addressing GvL (in some cases without GvHD) and suggest that a study of mHAgs could be perfomed before transplant in order to better investigate the role of the known and new mHAgs involved in GvHD and GvL effects. Work supported by Lions Club "Bassa Bresciana" and BCC di Pompiano e Franciacorta Founds.

S1184

ROLE OF ALPHA4BETA7 INTEGRIN AND P-SELECTIN-LIGAND EXPRESSION FOR AGVHD INDUCTION AND AS POTENTIAL PREDICTIVE MARKERS FOR INTESTINAL AGVHD

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Background: Acute graft-*versus*-host disease (aGvHD) is an immune syndrome associated with allogeneic hematopoietic stem cell transplantation (allo-HCT) that is mediated by alloreactive donor T-cells attacking the gastrointestinal tract, liver, and skin of the host. To cause aGvHD, alloreactive T-cells require the expression of appropriate homing receptors to efficiently migrate from secondary lymphoid organs as their priming sites to peripheral target tissues. Previously, we identified $\alpha4\beta7$ integrin and P-selectin-ligand expressed on peripheral blood (PB) alloreactive T-cells in a hyeracute GvHD mouse model. Therefore, we envisioned the development of a predictive test based on the homing receptor expression profile of PB T-cells to identify patients at risk of aGvHD before disease onset and for timely preventive therapeutic interventions.
Aims: To functionally test the relevance of $\alpha4\beta7$ integrin and P-selectin-ligand expression for T-cell homing into aGvHD target organs and to determine the time frame of homing receptor expression on PB T cells and its potency to predict development of aGvHD.
Methods: We employed two well-established allo-HCT mouse models [hyperacute GvHD MHC major mismatch model, C57Bl/6, H-2^b→ Balb/C, H-2^d or clinical relevant aGvHD minor histocompatibility antigen mismatch (miHAg) model, C57Bl/6, H-2^b→ Balb/B, H-2^b]. To induce aGvHD we transplanted C57Bl/6 wild type bone marrow together with T-cells isolated from C57Bl/6 mice of wild type or genetic knock-out mice [deficient for $\alpha4\beta7$ integrin (β7-ko) alone or fucosyltransferase VII (deficient for P-selectin ligand) and β7 (FucT7/β7-dko)]. Intestinal donor T-cell infiltration was assessed by immunofluorescence microscopy. PB was analyzed daily for donor T-cell distribution and homing receptor expression by multi-parameter flow cytometry. Histopathological stainings were used for aGvHD scoring.
Results: Transplanting β7-ko or FucT7/β7-dko donor T-cells resulted in significantly reduced cell numbers in the small bowel compared to recipients of WT donor T-cells (β7-ko: 2.7-fold $CD4^+H\text{-}2K^b+$ and 2.6-fold $CD8^+H\text{-}2K^b+$; FucT7/β7-dko: 3.6-fold $CD4^+H\text{-}2K^b+$ and 3.9-fold $CD8^+H\text{-}2K^b+$ on day+6 after allo-HCT) confirming the importance of these receptors for the homing of alloreactive T-cells into the intestinal tract. Histopathological aGvHD scoring confirmed less tissue damage of the gastrointestinal tract in β7-ko and FucT7/β7-dko donor T-cell recipients compared to recipients of WT T-cells. Daily PB analyses revealed in miHAg allo-HCT recipients that alloreactive donor T-cells can be readily detected 6 to 10 days before the onset of clinically apparent aGvHD symptoms. Relative values as well as absolute numbers of T-cells expressing $\alpha4\beta7$ integrin and P-selectin-ligand significantly exceeded those in syngeneic controls from day+6 on after allo-HCT indicating their potential use as predictive markers.
Summary and Conclusions: Our data demonstrate that $\alpha4\beta7$ integrin and P-selectin ligand expressing T-cells essentially contribute to the pathophysiology of intestinal aGvHD. As $\alpha4\beta7$ integrin and P-selectin ligand expressing donor T-cells can be detected in the PB up to 10 days before aGvHD onset we postulate the detection of $\alpha4\beta7$ integrin and P-selectin-ligand on alloreactive donor T-cells as promising candidates for a predicitive test to identify patients at risk before the clinical manifestation of aGvHD.

S1185

IN VITRO-GENERATED MYELOID-DERIVED SUPPRESSOR CELLS (MDSC) INHIBIT GRAFT-VERSUS-HOST DISEASE (GVHD) IN MICE WHILE MAINTAINING THE GRAFT-VERSUS TUMOR EFFECT

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Background: Allogeneic bone marrow transplantation (BMT) is a curative treatment modality for hematopoietic malignancies such as acute and chronic leukemias and lymphomas. Mature donor T cells in the allograft support engraftment, promote early T cell immunity of the recipient and mediate the graft-*versus*-leukemia (GVL) effect. However, these donor T cells are also responsible for the induction of graft-*versus*-host disease (GVHD) by attacking recipient tissue such as liver, skin, and bowel leading to significant morbidity and mortality. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of myeloid precursors suppressing T cell activation and proliferation and might therefore be a putative cellular therapy for GVHD prophylaxis.
Aims: Therefore, we tested whether *in vitro*-generated MDSCs suppress GVHD development without interfering with the GVL effect.
Methods: MDSCs were generated *in vitro* by culturing BM cells in the presence of GM-CSF. After 4 days more than 90% of the cells exhibit the $CD11b^+Gr\text{-}1^+$ MDSC phenotype. To test, whether MDSCs prevent GVHD, C57BL/6 (B6, H-2^b)-derived MDSCs were co-transplanted with allogeneic BM and spleen cells from B6 mice into either lethally irradiated B6D2F1 (H-2^{bxd}) or B6.bm1 (H-k^{bm1}) mice. By co-injecting syngeneic tumor cells together with the BM transplant, the effect of MDSC transplantation was analyzed on the GVL effect. Influence of MDSCs on T cell responses after transplantation was defined by analyzing cytokine levels, activation status and number of allogeneic T cells.
Results: In-vitro generated MDSCs efficiently suppressed alloantigen-specific T cell proliferation *in vitro*. Transplantation of 1×10^7 MDSCs together with allogeneic BM and spleen cells efficiently prevented clinical GVHD. Due to MDSCs injection survival was increased to 100% in the B6 into B6.bm1 BMT model while 70% of the animals survived in the B6 into B6D2F1 BMT model. Effect of MDSCs was dosage dependent since clinical GVHD increased when numbers of transplanted MDSCs were reduced. Histological GVHD, however, was not totally prevented by MDSCs since lymphocyte infiltrations were still detectable in liver and intestine. MDSCs homed into lymphoid organs and GVHD target organs but did not interfere with the proliferation, phenotype or cytotoxicity of allogeneic T cells. However, in the presence of MDSCs, GVHD-associated cytokines TNF-α and IFN-γ were drastically decreased in the serum of transplanted mice. Most importantly, co-transplantation of MDSCs did not interfere with the GVL effect, since syngeneic thymoma cells were still efficiently eradicated by alloantigen-specific T cells.
Summary and Conclusions: Co-transplantation of *in vitro*-generated MDSCs efficiently prevented clinical and histological GVHD while preserving the T cell-mediated tumor cytotoxicity, indicating that MDSCs might represent a cellular treatment strategy after allogeneic BMT.

Hematopoiesis

S1186

FUNCTIONAL CHARACTERISATION OF NOVEL REGULATORS OF HAEMATOPOIESIS: FROM GWAS TO FUNCTION

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Background: Platelet count and volume are independent risk factors for heart attacks and ischaemic strokes. We therefore used meta-analyses studies (GWAS) to identify genes encoding novel regulators of megakaryopoiesis and platelet formation (HaemGen Consortium, Nature,2011:480,201). For about three-quarters of the 68 genetic signals associated with platelet count and volume credible gene candidates could be inferred. Two-third of these encode proteins hitherto unknown to be implicated in megakaryopoiesis and platelet formation. We hypothesise and have preliminary evidence that these new proteins are important rate-limiting factors of megakaryopoiesis and platelet formation, making them prime targets for further functional characterisation.
Aims: To delineate the function of genes identified by a platelet GWAS by gene knockdown in a relevant model organism.
Methods: To this end, we performed a high-throughput reverse genetic screen in zebrafish using morpholino (MO) knock down approach. This was followed by in-depth functional analysis of selected genes using a wide panel of different haematopoietic markers with the main aim to further our understanding of the function of the novel regulators of blood formation.
Results: We included 16 genes in the screen to identify novel pathways essential in thrombopoiesis and haematopoiesis in general. Knock down of all but four resulted in 50–90% reduction in the number of thrombocytes in a CD41-transgenic zebrafish. To further investigate lineage-specific effects of the candidate genes we assessed the status of definitive erythropoiesis, myelopoiesis and lymphopoiesis in MO injected embryos. The information gleaned from the initial knock-down/phenotyping was used to generate heat-map of gene expression profiles and to cluster genes with similar phenotypes. Consequently, we were able to hierarchically position candidate genes on the haematopoietic tree and to assign them a potential role during haematopoietic differentiation.
Summary and Conclusions: Using the from-GWA study-to-function strategy we have not only identified a series of genes that represent novel regulators of thrombopoiesis and haematopoiesis, but this work also represents, to our knowledge, the first example of a functional genetic screening strategy that is a critical step toward obtaining biologically relevant functional data from GWA study for blood cell traits. The results of these studies are now informing our next step in exploring the relationship between rare sequence variants in platelet GWAS-genes and the count and volume of platelets.

S1187

LOW/NEGATIVE EXPRESSION OF PDGFR-ALPHA IDENTIFIES THE CANDIDATE PRIMARY STROMA STEM/PROGENITOR CELLS IN ADULT HUMAN BONE MARROW.

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Background: Human bone marrow (BM) contains a rare population of non-hematopoietic mesenchymal stem cells (MSC) that are of central importance for the hematopoietic microenvironment. We and others have shown that primary BM-MSC were highly enriched in lin-/CD45-/CD271+ cells as reflected by high CFU-F frequencies up to ca. 1 in 20. However, a more precise phenotypical definition of these rare cells is required to be able to study the exact cellular properties of these putative stem/progenitor cell population.
Aims: This study therefore aimed to identify novel and potentially better markers for the isolation and characterization of primary human MSC.
Methods: Comparative gene expression profiling was performed on human lin-/CD45- BM cells sorted based on CD271 expression. Protein expression of candidate surface marker genes was evaluated by flow cytometry. Candidate primary MSC populations as defined by the novel markers were sorted by FACS, and assayed for MSC properties *in vitro* and *in vivo* at single cell and bulk level.
Results: In total, 215 genes including cytokine, growth factor and extracelluar matrix (ECM) genes such as latent transforming growth factor (TGF)-beta binding proteins (LTBP2), lumican (LUM), connective tissue growth factor (CTGF), fibulin-1 (FBLN1), and vascular endothelial growth factor (VEGF) were uP-regulated in the lin-/CD45-/CD271+ subset compared to CD271- cells. Twenty eight of the upregulated genes correlated to surface expressed molecules including previously described MSC markers such as CD140b and CD106. The majority of the genes identified by this approach, however, represented novel MSC marker candidates, such as CD151, CD81, and CD140a. FACS analysis of the expression of the potential novel markers on lin-/CD45-/CD271+ cells revealed two staining patterns, *i.e.* marker expression was either directly correlated with CD271 expression and did thus not enable to further enrich for CFU-F (*e.g.* CD151), or the novel marker was only expressed on a fraction of the lin-/CD45-/CD271+ cells, thus potentially allowing to identify a CFU-F and a non-CFU-F containing population within the CD271+ cells. In fact, sorting based on CD140a (PDGFR alpha) expression allowed to sort a population of lin-/CD45-/CD271+/CD140a low/- cells with a CFU-F frequency of 1 in 5 (24.15±4.51 CFU-Fs per 100 plated cells, n=6). Gene expression profiling of this close-to-pure population of CFU-F showed uP-regulation of cell cycle inhibitor genes such as CDKN1A, BTG3 and DUSP3, indicating a quiescent status of the cells, which was furthermore confirmed by cell cycle analysis (>98% in G_0). In accordance with the hematopoiesis maintenance function, expression of genes encoding ECM proteins such as laminin subunit alpha-4 (LAMA4), adrenomedullin (ADM) and collagen type I alpha 1 (COL1A1) were also highly higher in CD140a- cells compared to the CD140+ population. PCR analysis moreover demonstrated high expression of ALPL, PPARg, and ACAN as well as Oct4, Sox2 and Nanog in primary lin-/CD45-/CD271+/CD140a low/neg MSC. These cells furthermore gave rise to typical cultured MSC (expression of standard surface markers, in-vitro differentiation capacity), and they formed bone, adipocytes and hematopoietic stroma when transplanted s.c. into NOD-SCID mice.
Summary and Conclusions: Low/negative expression of CD140a on lin-/CD45-/CD271+ BM cells allows identify a close to pure population of candidate stroma stem/progenitor cells. These results will enable to better characterize this important cellular component of the hematopoietic microenvironment.

S1188

HIF-1ALPHA AND HIF-2APLPHA ARE NOT ESSENTIAL FOR STEADY-STATE HEMATOPOIETIC STEM CELL MAINTENANCE

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Background: Lifelong adult hematopoiesis critically depends on rare multipotent hematopoietic stem cells (HSCs). These HSCs reside in niches within the bone marrow (BM) where they are subject to very low oxygen concentrations. Therefore hypoxia signalling is thought to play important roles in HSC maintenance. So far, the main characterised mediators of these pathways are the hypoxia-inducible factors (HIFs): HIF-1α and HIF-2α. These transcription factors have both common and different characteristics such as tissue expression, regulation/degradation mechanisms or target genes. HIF-1α is known to play a major role in the primary response to acute hypoxia whereas HIF-2α is thought to be involved in late or chronic hypoxic response. Whereas the role of HIF-1α in the maintenance of HSCs has been studied, the role of HIF-2α which could be more important due to its involvement in chronic response to hypoxia still remains to be investigated.
Aims: The aim of this study was to determine the cell-autonomous role of HIF-2α in HSC maintenance and the collective requirement for both HIF-1α and HIF-2α in HSC functions.
Methods: Tissue conditional deletion of the HIF genes in mice using a Cre-LoxP approach was used. HIF-2$\alpha^{fl/fl}$or HIF-1$\alpha^{fl/fl}$; HIF-2$\alpha^{fl/fl}$ mice were crossed to Vav1-Cre strain to entirely delete the gene(s) of interest strictly within the adult hematopoietic system. These mice were subsequently analysed phenotypically using flow cytometry. Briefly, extracted cells from BM, spleen and thymus were stained to identify HSCs, progenitors (CMP, GMP, MEP and CLP) and differentiated cells (B cells, T cells, and myeloid cells). The function of HSCs and progenitors lacking HIF-2α or both HIF-1α and HIF-2α were evaluated using standard methods such as colony-forming cell (CFC) assays and serial transplantation.
Results: The constitutive hematopoiesis-specific deletion of HIF-2α or both HIF-1α and HIF-2α in mice does not affect HSC numbers and does not perturb steady-state hematopoiesis. In addition, the ability of these cells to generate colonies remains comparable to the controls. Furthermore, using serial transplantation experiments we demonstrate that HIF-2α is dispensable for HSC maintenance. We also show that HSCs lacking both HIF-1α and HIF-2α efficiently repopulate primary recipients indicating that deletion of HIF-2α does not exacerbate the phenotype resulting from HIF-1α deficiency.
Summary and Conclusions: Therefore, we conclude that intrinsic HIF-dependent signalling is not essential for steady-state HSC functions and, unlike HIF-1α, HIF-2α is dispensable for post-transplantation HSC self-renewal. HIF-1α and HIF-2α have therefore fundamentally different functions in HSCs. Taking into consideration the role of HIF factors in some leukaemias and hematopoietic disorders, these results implicate HIFs as good targets for therapy.

S1189

REGULATION OF NORMAL AND LEUKEMIC ADULT HEMATOPOIETIC STEM CELLS BY ESTROGEN SIGNALING

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Background: Although estrogens may affect the hematopoietic microenvironment, *e.g.* through their anabolic effect on bone, little is known regarding their potential role in the regulation of hematopoietic stem cell (HSC) maintenance, proliferation and survival. A potential cause of resistance to chemotherapy in leukemia is the existence of a primitive, quiescent population of leukemia stem cells (LSC) insensitive to cytotoxic therapies; therefore, interfering with pathways that regulate HSC quiescence may represent a therapeutic advantage by sensitizing LSC to chemotherapy.
Aims: To investigate the role of estrogen signaling in the regulation of HSC, and the feasibility of targeting this pathway as anti-leukemia therapy, using a drug already approved in the clinic (tamoxifen).
Methods: We performed a detailed analysis of hematopoiesis both in adult, estrogen receptor- deficient (Esr1-/- and Esr2-/-) and in wild-type (WT) male mice treated with the synthetic selective estrogen receptor modulator (SERM) tamoxifen. For leukemogenesis experiments, we used a mouse model of acute myeloid leukemia (AML) induced by MLL-AF9, which accurately reproduces the human disease. Mice treated with tamoxifen and conventional chemotherapy (doxorubicin plus cytarabine) were compared to mice receiving chemotherapy alone.
Results: Estrogen receptors Esr1 (ERα) and Esr2 (ERβ) were detectable and differentially expressed at the protein level by distinct hematopoietic progenitors. Treatment of WT mice with tamoxifen rapidly caused apoptosis and reduced the numbers of immunophenotypically defined short-term HSC and multipotent progenitors in the bone marrow. This effect was direct as per *in vitro* experiments and was associated with impaired short-term competitive reconstitution capacity of Esr1-/- bone marrow cells. In contrast, tamoxifen treatment induced cell cycle entry and increased the numbers of immunophenotypically defined long-term HSC. This expansion, however, was not associated with increased repopulation ability in competitive transplant assays, suggesting a functional impairment of HSC. In fact, tamoxifen-treated mice showed defective hematopoietic recovery after 5-fluorouracil challenge or sublethal irradiation. Deep sequencing studies have confirmed cell cycle changes and revealed candidate genes responsible for the differential effects of tamoxifen on distinct HSC populations. Hence, SERM can decrease the number of multipotent progenitors and reduce the frequency and/or activity of a quiescent, primitive repopulating stem cell. Therefore, we studied whether SERM could be used to control leukemic HSC quiescence/survival. Tamoxifen induced apoptosis of MLL-AF9-positive leukemic blasts *in vitro* and enhanced the pro-apoptotic effect of low doses of cytarabine, suggesting that SERM might allow for reduced chemotherapy regimes. Combined tamoxifen and chemotherapy significantly attenuated leukemia relapse in a mouse model of MLL-AF9-induced AML. Although tamoxifen did not prolong overall survival in this model of aggressive, chemoresistant AML, preliminary data showed decreased peripheral infiltration in tamoxifen-treated mice.
Summary and Conclusions: Our data indicate differential roles of estrogen signaling in the regulation of primitive hematopoietic cells, particularly in the control of HSC survival and proliferation, and demonstrate that therapeutic targeting of this pathway with already available ligands could prove a useful strategy to sensitize chemotherapy-resistant leukemia to cytotoxic treatments.

S1190

THE ROLE OF OSTEOCLASTS IN THE DEVELOPING LEUKEMIC STEM CELL NICHE IN A MOUSE MODEL OF ADULT T-CELL LEUKEMIA

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Background: Adult T-cell leukemia (ATL) is a lymphoproliferative disorder caused by HTLV-I infection. Although various chemotherapies have shown significant complete remission rates, most of the treated patients undergo relapse. These data indicate the existence of leukemic stem cells (LSCs) and a specific niche that regulates stemness and protects LSCs from chemotherapy.
Aims: We previously reported that ATL-LSCs isolated from a Tax-transgenic (Tax-Tg) mouse are enriched in the $CD117^+/CD38^-/CD71^-$ fraction of the lymphoma, and LSCs have the potential to reproduce the original tumor when transplanted into a NOD/SCID mouse. However, the niche of ATL-LSCs is still unclear. This study aimed to identify the LSC niche in ATL and clarify its role as a potential therapeutic target.
Methods: To identify the ATL-LSC niche *in vivo*, we performed a homing assay. Lymphoma cells isolated from a Tax-Tg mouse were transduced with the GFP gene by a lentivirus, and then sorted GFP^+ cells (2×10^6) were transplanted intraperitoneally into a non-irradiated NOD/SCID mouse. Homing of GFP^+ cells to tissues was traced by flow cytometry (FCM) at 16 h, and3,7, 14 and 21 days post-transplantation. To assess the effect of an osteoclast inhibitor in ATL bone marrow (BM), mice were subcutaneously injected with zoledronic acid (ZOL; 0.2 mg/kg) twice weekly.
Results: GFP^+ lymphoma cells were first detected in the spleen and BM at 16 h post-transplantation. No GFP^+ lymphoma cells were detected in the thymus or lymph nodes. Interestingly, more than 60% of first colonized cells in the spleen and BM at 16 h post-transplantation were ATL-LSCs ($GFP^+/CD117^+$ cells). From day 3 to7, more than 40% of colonizing cells in the BM and spleen were ATL-LSCs. These data indicate that ATL-LSCs prefer to colonize and proliferate in the spleen and BM. To identify the specific niche of ATL-LSCs in the BM, we performed imaging analysis of ATL-LSCs. ATL-LSCs ($GFP^+/CD117^+$ and $CD38^-/CD71^-/CD117^+$ cells) were mainly localized near the BM endosteal region of the trabecular bone. ATL-LSCs were attached to reticular cells in the trabecular bone, and the number of osteoclasts was significantly increased at the trabecular region. The increased number of osteoclasts correlated with an increased serum calcium concentration and decreased trabecular bone mass. FCM analysis and an *in vitro* differentiation assay confirmed that the number of osteoclast precursors was increased in ATL BM.
To clarify the role of osteoclasts in ATL BM, we treated the ATL mouse model with ZOL. As a result, ZOL significantly reduced the number of GFP^+ ATL cells in the BM. When ZOL was coadministered with an anti-cancer drug, the number of GFP^+ ATL cells was dramatically reduced in the BM, which extended the mouse survival rate significantly, whereas the anti-cancer drug alone had no effect. These data suggest that osteoclasts may have a function in development of the LSC niche. To clarify the key signals that induce osteoclasts in ATL BM, we are now attempting to isolate ATL-LSCs, GFP^+ ATL cells, blood endothelial cells, and fibroblastic reticular cells from normal and ATL BM to compare the gene expression profiles of each niche cell type.
Summary and Conclusions: We found that the ATL-LSC niche is located at the trabecular bone region in the BM, and that osteoclasts have a role in supporting ATL cells and development of the LSC niche in a mouse model of ATL. Therefore, osteoclasts may be a potential therapeutic target of ATL.

PUBLICATION ONLY

Acute lymphoblastic leukemia - Biology

B1191

OXIDATIVE STRESS INFLUENCE HYDROXYMETHYLATION STATUS IN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS IN VITRO

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Background: T-cell acute lymphoblastic leukemia (T-ALL) accounts for about 15% and 25% of ALL in pediatric and adult cohorts, respectively, and originates from the malignant transformation of lymphocyte progenitor cells. However, the biology of T-ALL is poorly understood. Reactive oxygen species (ROS) are known to play a dual role in biological system, since they may be either harmful or beneficial to living systems. Increasingly evidence shows that oxidative stress and ROS are involved in carcinogenesis. Moreover, it is now clear that epigenetic mechanisms are as important as genetic changes in the development of cancer. Same studies suggest that oxidative DNA damage can affect patterns of DNA methylation leading to aberrant gene expression and possibly contributing to the development of malignancy.
Aims: In this work, we study the effect of hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\cdot -}$) in ALL cells, with special emphasis on the action of oxidative stress (OS) in cell death and methylation status.
Methods: CEM cells (T-ALL cell line) were treated in the absence and presence of hydrogen peroxide and menadione ($O_2^{\cdot -}$ donor) and cell viability and density were analyzed by trypan blue assay. Intracellular levels of H_2O_2, $O_2^{\cdot -}$, GSH and mitochondrial potential were determined by flow cytometry (FC), using the fluorescent probes. Cell death and were also evaluated by optical microscopy and FC using the Annexin V/Propidium Iodide staining. We also analyzed apoptotic proteins expression levels, namely BAX, BCL-2, FAS, FAS ligand and caspases, and cell cycle by FC. Global DNA methylation and hydroxymethylation were analyzed by ELISA using commercial kits.
Results: Our results show that H_2O_2 and menadione decrease cell viability in a dose and time dependent manner. In fact, we observe that IC_{50} of H_2O_2 and menadione in CEM cells is 25 mM and 7.5 mM, respectively, after 24 hours. These compounds induce cell death mainly by late apoptosis/necrosis, through the decrease in mitochondrial membrane potential and increase in caspases levels, BAX/BCL-2 and FAS/FAS ligand ratios. Besides that, these effects may also be mediated by an increase in ROS levels and a decrease in GSH. Furthermore, these compounds induce S phase arrest and an increase in 5-hydroxymethylcytosine (5hmC) levels when cells are treated with 25 μM H_2O_2. These results may be related with oxidation of 5-methylcytosine via oxidative damage, and consequently induction of global hipomethylation. In contrary, in cells treated with menadione 7.5 μM we observe a decrease in 5hmC levels.
Summary / Conclusion: In conclusion, our results suggest that besides cell death induced by higher OS levels, ROS could influence hydroxymethylation status and interfere with global DNA methylation status and gene expression levels in acute lymphoblastic leukemia cells in vitro.

B1192

EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-1, VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-2 AND FMS-LIKE TYROSINE KINASE-3 RECEPTOR IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The Fms-like tyrosine kinase3, FLT3, has been a subject of several studies as a prognostic marker in both pediatric and adult acute leukaemia. The vascular endothelial growth factor family (VEGF) members are well established as key regulators of angiogenesis processes. Studies have shown that the expression of these mediators of angiogenesis in cancer patients is associated with an adverse prognosis in different leukemias.
Aims: This work was conducted to evaluate the expression of these molecules, their relation to clinical and laboratory parameters having prognostic significance as well as their relation to survival and response to chemotherapy in paediatric ALL patients.
Methods: This work was carried out on 55 newly diagnosed childhood ALL patients who presented to the National Cancer Institute (NCI), Cairo University during the period between July 2010 and June 2011, after having a written consent. Patients were followed up till January 2012. Their age ranged from 0.33 to 17 years and the male: female ratio was 1: 0.89. They included 41 patients (74.5%) B-lineage ALL and 14 (25.5%) T-lineage.
The studied markers were evaluated by flow cytometry and results are expressed as percent expression (%) and Mean Flouresence intensity (MFI).
Results: FLT-3% and MFI were significantly higher in B-lineage than in T-ALL (P=0.004 & 0.02 respectively) and within the B-ALL; both were significantly higher in pre-B-ALL as compared to c-ALL (P=0.002 & 0.017 respectively). No statistically significant difference in VEGFR-1% and MFI as well as in VEGFR-2 % and MFI in B-ALL as compared to T-ALL. In B-ALL, both VEGFR-1% and MFI (P=0.014 and 0.006 respectively) and VEGFR-2 % and MFI (P=0.009 and 0.006 respectively) were significantly lower in patients with splenomegaly than in those without. No statistically significant difference was found between the studied parameters (VEGFR-1 % and MFI, VEGFR-2 % and MFI and FLT-3 % and MFI) and gender, lymphadenopathy, and hepatomegaly in either B- or T-ALL. In B-ALL, there was a significant positive correlation between VEGFR-1% and haemoglobin level (P=0.008, r=0.415), between VEGFR-2 % and CD10 % (P=0.015, r=0.379) and between FLT3 % and BM blast % (P=0.009, r=0.405). In T-ALL, there were significant positive correlations between FLT-3 %, VEGFR-1%, and VEGFR-2 % on one hand and CD34 (%) on the other (P=0.001, 0.041, and 0.002 respectively, r=0.826, 0.596, and 0.798 respectively). In B-ALL, there was highly significant positive correlations between VEGFR-1% and VEGFR-2% (P=<0.001, r=0.704). In T-ALL, there was significant positive correlations between FLT3 % and both VEGFR-1% and VEGFR-2% (P=0.016 &0.011 respectively, r=0.627 & 0.654 respectively) as well as a significant positive correlation between VEGFR-1% and both VEGFR-2% (P=0.002, r=0.762). None of the studied parameters had any impact on either disease-free survival (DFS) or overall survival (OS).
Summary / Conclusion: FLT-3 was significantly higher in B-lineage ALL than in T-ALL, and in pre-B- as compared to c-ALL. In the B-ALL, both VEGFR-1 and VEGFR-2 were significantly lower in patients with splenomegaly. - In the B-ALL, there was a significant positive correlation between VEGFR-1% and haemoglobin level, between VEGFR-2 % and CD10 % and between FLT3 % and BM blast %. Also, there was a highly significant positive correlation between VEGFR-1 and VEGFR-2. Angiogenic receptors could play different roles in B cells and T cells as seen by the significant positive correlation between both VEGFR-1 & VEGFR-2 and the expression of the stem cell marker CD34 in childhood T–ALL but not in B-ALL.

B1193

TNFRSF13B/TACI AND TNFRSF13C/BAFFR EXPRESSION IN ACUTE LYMPHOBLASTIC LEUKEMIA

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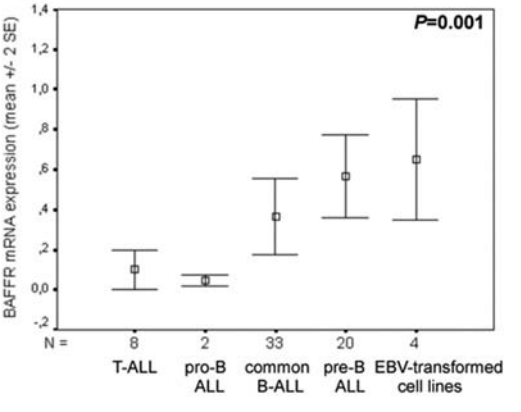
Background: BAFF is a fundamental maturation and survival factor for mature normal and malignant B-cells, and among the three receptors that mediate BAFF signaling (BAFFR, TACI and BCMA), BAFFR seems to be the key molecule. Previous studies have shown that BAFF receptors are not expressed by normal progenitor B cells, while the contribution of BAFF signaling in ALL biology is still elusive.
Aims: The aim of this study was to investigate the expression of *TNFRSF13B/TACI* and *TNFRSF13C/BAFFR* receptors in ALL patients and their associations with clinico-laboratory characteristics of the disease.



Methods: Sixty-three ALL patients (male/female: 40/23, mean age: 8.2 years, range: 9 months - 72 years), including 55 with B-ALL (pro-B:2, common: 33, pre-B: 20) and 8 with T-ALL (pre-T:2, cortical:4, mature: 2) were enrolled. All patients were assessed with complete hematologic, flow cytometric and molecular

analyses (detection of BCR-ABL, E2A-PBX1, MLL-AF4 and TEL-AML1 rearrangements) and for the majority of them (52 out of 63 patients) data from cytogenetic and FISH analyses were also available. The mRNA expression of *TNFRSF13B/TACI* and *TNFRSF13C/BAFFR* was evaluated by qRT-PCR in all cases; the relative expression of each gene is presented as a multiple of the respective gene expression in isolated peripheral blood B cells of a healthy individual. The protein expression was confirmed by flow cytometry (TACI clone 1A1, BAFFR clone 11C1) in 8 B-ALL patients. Four EBV-transformed cell lines and Jurkat cells, with high and absent BAFFR and TACI expression respectively, were utilized as positive and negative controls in both molecular and flow cytometry studies.
Results: B-ALL patients displayed a remarkable mRNA expression of *TNFRSF13C/BAFFR*, ranging from absent in pro-B ALL to a higher expression in pre-B ALL (Figure 1). Consequently, we identified a positive correlation of *TNFSRF13C/BAFFR* expression with CD20, cIgM and the presence of E2A-PBX1 rearrangement. Moreover, the majority of ALL patients displayed very low to absent *TNFRSF13B/TACI* mRNA transcripts and only 3 patients with common-B and 2 with pre-B ALL exhibited a remarkable *TNFRSF13B/TACI* expression.
Summary / Conclusion: Anti-BAFF therapeutic approaches could be useful for the management of pre-B ALL patients, as well as in a proportion of other ALL patients with an aberrant expression of the abovementioned receptors.
This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

B1194
PAIRED IMMUNOPHENOTYPE COMPARISON OF DIAGNOSIS AND RELAPSE SAMPLES IN B-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: Although great progress has been made in the treatment of acute lymphoblastic leukemia (ALL), relapse remains a major issue in the follow-up of these patients. Recent data about the emergence of subclones during haematological malignancies suggest that relapses could result from resistant cells initially in minority or from cells driven to resistance by previous treatments. Among the tools allowing for the characterization of leukemic cells, flow cytometry (FCM) is an essential approach. Increasingly used to evaluate minimal residual disease (MRD) based on the immunophenotypic features of the blasts at diagnosis, it can also allow to identify immunophenotypic shifts related to clonal evolution. Such an approach would be best studied by comparing follow-up samples from the same patients. In order to be thorough, this would however require that conditions as similar as possible are applied to both types of cells.
Aims: This work was designed to compare 1)the immunophenotypic features of B-ALL blast cells with those of normal hematogones and 2)potential immunophenotypic shifts at relapse.
Methods: FCM was performed simultaneously on thawed paired samples from 15 patients (9 children aged between 1 and 12 years old and 6 adults aged between 23 and 71 years old) with B-lineage ALL. With a three-tubes 8 colours panel comprising a backbone of CD45, CD34, CD22 and CD10, the expression of ten markers was examined and compared to that observed on normal hematogones contained in 29 bone marrow samples from healthy donors. These 10 markers were CD7, CD19, CD20, CD24, CD38, CD44, CD58, CD81, CD123 and CD304. Moreover, an additional four colours panel was used to examine the more recently described antigens CD200 and Her2Neu. The presence of leukemia associated immunophenotypes (LAIP) were defined as a difference in mean fluorescence intensity (MFI), between hematogones and blasts of at least 2 standard deviations.
Results: At diagnosis, the expression of each marker was at variance from that on hematogones for at least one patient. Antigens with the most aberrant expression were CD10, CD24 and CD81. Antigens with the least aberrant expression were Her2Neu, CD19, CD22, CD123 and CD20.
All patients retained at relapse the same global immunophenotype without any change in the EGIL classification (3 B-I, 8 B-II, 4 B-III). The expression of most markers was similar at diagnosis and relapse with at most 4 patients with modulation of a given antigen (CD10). There was no change at all for the expression of CD38 and only one patient each showed a change in the expression of CD44, CD58 or CD123. Immunophenotypic patterns associating variations of several antigens on a given population compared to hematogones, were globally stable. Only two patients showed major changes possibly associated with the emergence of a new clone.
Summary / Conclusion: This study confirms that B-ALL blast cells differ immunophenotypically from hematogones, although the latter have been reported to possibly be their normal counterpart. These data therefore comfort the interest of using LAIP in the detection of MRD in multiparameter FCM. Moreover, since molecular targets of therapeutic monoclonal antibodies do not shift sensibly, their use can also be considered at relapse.

B1195
GSTM1 GENOTYPE AS A RISK MODIFIER OF MDR1 C3435T - INDUCED RISK SUSCEPTIBILITY TO PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA
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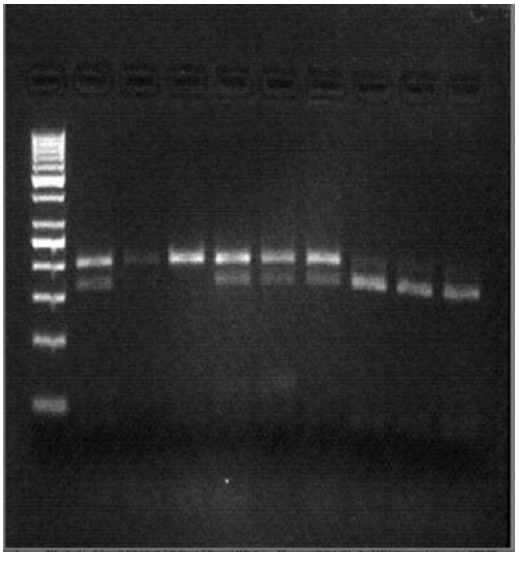
Background: *GSTM1* and *GSTT1* appear to be associated with a modest increase in the risk of acute lymphoblastic leukemia (ALL). *MDR1* C3435T polymorphism was also suggested as a risk factor for childhood ALL; individuals with TT allele have lower expression of P-gp than those with CC genotypes and hence less capable of extruding toxic substances and carcinogens.
Aims: To investigate the impact of *MDR1*gene C3435T polymorphism and both *GSTM1* and *GSTT1* polymorphisms, separately and in combination, on risk susceptibility to childhood ALL.
Methods: The study included 94 children with ALL and 314 apparently health control subjects. Multiplex-Polymerase chain reaction (PCR) was used to evaluate *GSTM1* and *GSTT1* status while PCR-restriction fragment length polymorphism (PCR-RFLP) was used for the detection of *MDR1* C3435T single nucleotide polymorphism.



Results: There was no significant effect of either *GSTM1* null or *GSTT1* null variant allele or both of them combined on susceptibility to ALL. On the other hand, *MDR1* CC, CT and TT genotype frequencies in childhood ALL patients were found to be 78.0%, 17.1% and 4.9 %, respectively vs. 91.2%, 8.8% and 0 % in the control group (P=0.016). *MDR1* gene C3435T homozygote and heterozygote have a 2.9 fold increased risk to develop ALL (OR = 2.918, 95%CI: 1.193-7.137,). There is a significant synergistic association between *GSTM1*-null allele and mutant *MDR* genotype homozygous TT or heterozygous allele CT on susceptibility to ALL with a 3.672 fold increased risk (p. value. 0.032 OR = 3.672, 95%CI: 1.059-12.733), however the presence of *GSTM1* abolished the effect of mutant *MDR1* allele on risk susceptibility to ALL (p. value. 0.193).
Summary / Conclusion: : the increased risk to develop pediatric ALL associated with *MDR1* gene C3435T homozygote and heterozygote is further potentiated by the presence of *GSTM1* null and abolished by the presence of *GSTM1*wild. Molecular genetic analysis is still required to understand genotype-genotype interaction and to clarify genotype-phenotype relation and their reflection on disease risk.

B1196
CAN WE KNOW MORE ABOUT ACUTE LEUKEMIA IN CHILDREN THROUGH EPIDEMIOLOGY?
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Background: The Acute Leukemias (AL) are responsible for 30% of all pediatric cancers. They are biologically heterogeneous with distinct morphologic, cytogenetic and molecular characteristics. Recently, the knowledge of the AL epidemiology, molecular and cytological characteristics, has gained a crescent significance. Their high mortality rate makes the collection and systematic analysis of all biographic, geographic, clinical, cytogenetic and molecular data an important step as an attempt to contribute for a better knowledge of its etiology, physiology and pathogenesis.
Aims: To contribute for the knowledge of the epidemiologic and molecular pro-

file of AL in children.
Methods: Observational, retrospective and descriptive study, in children from the Center of Portugal, aged 0-13 years, diagnosed with AL in a central Pediatric Hospital, between 1st January 1999 and 31st December 2008. Several variables were searched: biographic, geographic, familiar, clinical and laboratory presentation, morphology, imunophenotype, cytogenetics, molecular genetics, and progression of the disease. All results were analysed with a statistical approach using SPSS® version 19.0.
Results: We diagnosed 95 new cases of AL: 19% myeloblastic and 81% lymphoblastic. The global incidence was 25,7% per million children, with a uneven distribution in the different districts of the Center of Portugal. There was a male predominance. Half the children had ages between 2-5 years. The majority was born from a first or second pregnancy, with a weight percentile between 25-50 (60.1%), being the median maternal age 34 years. Seven children had Down syndrome. Recurrent infections in the first year of life occurred in 26.3% of the 91 children aged over 1 year. At diagnosis, children with Acute Lymphoblastic Leukemias (ALL) presented more bone pain (31,2%), while the hemorrhagic manifestations were predominant in the Acute Myeloblastic group (AML) (50%). No significant differences were observed when comparing hematologic parameters, except for the leukocyte count, that is higher than expected in AML (27,8% higher than $100x10^9$/L), and for the platelet count, that is lower than expected in both groups (in ALL 24,7% with platelet counts less than $20x10^9$/L, while in AML 72% presents with counts inferior to $50x10^9$/L). In ALL 67/ 77 were from B cells, predominantly the subtype B common (n=47). Hyperdiploidy was the commonest genetic alteration (25,4%), followed by the translocation *t(12;21)(p12;q22)* (15,6%). In AML the M5 subtype was the most frequent and 15 children had acute promyelocytic leukemia. At the end of this study, 15/95 children were dead mostly due to disease progression. The ALL survival rate (SR) was 85% at 5 and 75% at 10 years while in AML was equal at 5 and 10 years (75%). The presence of the t(4;11)(q21;q23), was associated with a SR of 25% at 5 years.
Summary / Conclusion: This study constitutes the first report of the molecular and epidemiological profile of AL in Central Portugal, showing its incidence, and allowing the comparison of our sample with other studies. With this study we found some districts in Portugal with a much higher AL incidence, which could be related to the presence of some types of Industries and/or environment characteristics. Even though our numbers are short, the advanced maternal age, the low birth weight and being the first son appears to be risk factors for AL. Besides that, the presence of the MLL rearrangements was associated with a worst survival rate.

B1197
CHARACTERIZATION OF MDM2/P53 AXIS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)
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Background: MDM2/p53 pathway plays an important role in the control of apoptotic and proliferation mechanisms. MDM2 binds to the N-terminus of the p53 and negatively regulates its transcriptional activity. MDM2 is over expressed in many tumors and its expression levels may be affected by several factors, including single nucleotide polymorphisms (SNP), such as the SNP309T>G (rs2279744) in the MDM2 intronic promoter P2. Conflicting evidence has linked the G-allele to enhanced cancer risk as well as early cancer diagnosis across different tumor types.
Aims: New MDM2 antagonists are now available for Phase I/II clinical development and in order to efficiently treat ALL patients (pts) with MDM2 antagonists, is important to identify *TP53* lesions and MDM2 status.
Methods: 19 patients (17 B-ALL and 2 T-ALL) visited at the Institute Seragnoli (Bologna) were analyzed for TP53 mutation screening, MDM2 SNP309T>G and MDM2 transcript levels.
Deletions and uniparental disomy (UPD) involving *TP53* were assessed by Genome-Wide Human SNP 6.0 array (Affymetrix). No 17p UPD, deletions or amplification events were detected in short arm of chromosome 17, were *TP53* is located. *TP53* mutations were thereafter investigated in all 19 samples. Three overlapping shorter amplicons covering the entire coding cDNA sequence (GenBank NM_000546.4) and the untranslated exon 1 [amplicon 1 (491 bp): exons 1-5; amplicon 2 (482 bp): exons 5-8; amplicon 3 (498 bp): exons 8-11)] and a longer amplicon (1,317 bp) starting from exon 1 and ending to exon 11 were sequenced by Sanger method. No *TP53* mutations were detected suggesting that these alterations are very rare events in ALL. In humans, a common SNP involves the substitution of an Arginine for a Proline at codon position 72 of *TP53* (rs1042522).
Results: Many studies have investigated a genetic link between this variation and cancer susceptibility, however, the results have been controversial. SNP P72R (rs1042522) was investigated in all19 adult ALL samples and it was found heterozygous in 31.6% (6/19), homozygous in 57.9% (11/19) and wild-type in 5% (1/19) of cases. Several studies have examined the prognostic value of the TP53 SNP P72R and/or MDM2 SNP309 in multiple tumors. Combined effects of p53 and MDM2 polymorphisms, for example, were described to be associated with an increased risk of developing hepatocellular carcinoma. MDM2 SNP309 was also characterized in our cohort of adult pts: 31.6% (6/19) were wild-type, 57.9% (11/19) were heterozygous and 10.5% (2/19) of cases were homozygous. The MDM2 expression was quantified using BioMark System array (Fluidigm). At the time of writing, we analyzed 15 ALL samples. To stratify our pts we used the median of 10 leukemic samples in molecular remission. The cut-off was of 3.44. Seven samples (46.7%) were higher than controls.
Summary / Conclusion: In conclusion, we did not identify any *TP53* alterations in adult ALL patients but the TP53 rs1042522 was identified in 90% of pts. The MDM2 SNP309 was identified in about 70% of cases. Moreover the 47% of pts overexpress MDM2. These data provide the rationale for further clinical investigation on using of MDM2 inhibitors.

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B1198
DETERMINATION OF CYTOTOXIC AND APOPTOTIC EFFECTS OF CAFFEIC ACID PHENETHYL ESTER AND GOSSYPOL IN COMBINATION WITH FLUDARABINE AT A MOLECULAR LEVEL IN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS
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Background: Acute lymphoblastic leukemia (ALL) is a type of cancer of white blood cells which occurs as a result of an uncontrolled and excess production of lymphoblasts. ALL is the most common type of leukemia in the period of childhood and youth (80%), and makes up 20% of all adult blood cancers. While chemotherapy is the main approach for the treatment of ALL, but the most effective treatment method has not been specified, yet. Fludarabine is a chemotherapeutic agent used in hematological cancers. Caffeic acid phenethyl ester (CAPE), a natural phenolic chemical compound, is an active component of propolis found in honeybee hives, and was demonstrated to have anti-mitotic, anti-oxidative, anti-tumoral, anti-inflammatory and immunomodulatory activities. Gossypol (2,2-bis (8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene, $C_{30}H_{30}O_8$), a natural agent obtained from the seed and root parts of the cotton, was shown to have anti-cancer properties.
Aims: The aim of this study is to determine the potential cytotoxic and apoptotic effects of CAPE, gossypol and fludarabine separately, and the effects of CAPE and gossypol in combination with fludarabine (Fludarabine + CAPE and Fludarabine + Gossypol) on Jurkat ALL cells.
Methods: Jurkat cells were propagated in RPMI-1640 growth medium containing 10% FBS and 1% penicillin-streptomycin, and was grown at 37ºC cell culture incubator containing 5% CO_2. The antiproliferative and apoptotic effects of CAPE, gossypol and fludarabine separately, and the effects of CAPE and gossypol in combination with fludarabine (Fludarabine + CAPE and Fludarabine + Gossypol) on the Jurkat cells were detected by MTT cell proliferation assay and by the changes in caspase-3 activity, respectively. The IC50 values (drug concentration where cell proliferation is inhibited at a level of 50%) of the anticancer agents were determined by MTT cell proliferation test.
Results: According to analyses of the MTT assays, at 48 and 72 hour time points, CAPE, gossypol and fludarabine were shown to significantly inhibit the proliferation of Jurkat cells in a time- and dose-dependent manner as compared to untreated control group. At different time points, the proliferation of Jurkat cells incubated with fludarabine + CAPE or fludarabine + gossypol was decreased significantly in a dose-dependent manner as compared to untreated control group and the group of Jurkat cells incubated with CAPE, gossypol and fludarabine separately. Caspase-3 enzyme activity was identified to be elevated in the groups of Jurkat cells incubated with dual combinations of fludarabine when compared to the positive control group and the groups of Jurkat cells incubated with CAPE, gossypol, and fludarabine separately. This study has shown that gossypol alone or in combination with fludarabine increases the caspase-3 enzyme activity in Jurkat ALL cells.
Summary / Conclusion: The results demonstrated that CAPE, gossypol and fludarabine separately, or CAPE and gossypol in combination with fludarabine have anti-proliferative and apoptotic effects on Jurkat cells in a time- and dose-dependent manner. If these studies are supported by *in vivo* research, herbal and natural anti-cancer agents, CAPE and gossypol, could be considered to be used in the treatment of ALL.
This study was supported by Turkish Society of Hematology.

B1199

CLINICAL SIGNIFICANCE OF LOW SENSITIVITY PROBES IN MRD ANALYSIS OF ADULT ALL PATIENTS

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Background: MRD analysis emerged as the most powerful indicator of the risk of relapse and is increasingly adopted in Acute Lymphoblastic Leukemia (ALL) patients for the risk-oriented administration of consolidation therapies. In most pediatric and adult clinical trial the required sensitivity of the probe is at least 10^{-4}. MRD evaluation is not performed with low sensitivity probes and patients are treated according to clinical risk stratification (high risk, HR or standard risk, SR).
Aims: To evaluate the ability of low sensitive probes in revealing high MRD levels and classify patients as high risk of relapse due to leukemia persistence.
Methods: The prospective Northern Italy Leukemia Group trial conducted between 2000 and 2006 enrolled a total of 280 consecutive unselected patients with Ph- ALL. In this study post-consolidation treatments were administered according to the MRD status. MRD was assessed by RQ-PCR methodology using one or two patient-specific molecular probes generated according to ESG-MRD-ALL study group guidelines. MRD was evaluated at weeks 10, 16 and 22 and results were used to categorize patients as MRD^{neg} (negative at w 22 and negative or positive $<10^{-4}$ at w16) or MRD^{pos}. SCT or intensified chemotherapy was prescribed to MRD^{pos} patients whether maintenance to MRD^{neg} patients, regardless of clinical risk class. Only probes reaching the sensitivity of 10^{-4} were used for treatment allocation; patients without a sensitive probes were treated according to clinical risk stratification .
Results: Of 280 registered patients 236 achieved complete remission (CR) and 142 completed consolidation phase. For these patients the MRD risk assignment was possible in 112 case: 58 resulted MRD^{neg} and 54 MRD^{pos} and treated accordingly. For 30 patients a molecular classification was not possible ($MRD^{u/k}$) due to lack of samples, no clonal identification or low sensitive probes (10^{-3}, 9 cases) and these patients were treated according to clinical risk class. In 8 out of 9 patients with low sensitivity probe MRD evaluation was performed on available sample collected during the consolidation phase. A positive MRD signal at 10^{-3} level or greater in at least one sample was found in 5 cases. Among these, one patient experienced an early relapse, one refused any treatment and relapsed 3 months later and one relapsed after stem cell transplantation (SCT). Interestingly, two of these patients had been previously assigned to the clinical standard risk class (one SR B and one SR T lineage ALL) while the transplanted patients was a HR B lineage ALL. Furthermore, the only two MRD positive surviving patients had been classified as clinical standard risk but received intensified chemotherapy courses, followed by SCT in one case.
Summary / Conclusion: Our analysis suggests the usefulness of MRD evaluation in acute lymphoblastic leukemia even with low sensitive probes to identify, especially in clinical standard risk group, patients with high residual disease in whom intensified treatments are needed to avoid impending relapse.

Acute lymphoblastic leukemia - Clinical

B1200

PROGNOSTIC VALUE OF DAY 15 BLAST COUNT IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH ALL-BFM 95 PROTOCOL

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Background: Children with acute lymphoblastic leukemia (ALL) treated with Berlin-Frankfurt-Münster (BFM)- 95 protocol have approximately 80% event free survival (EFS). However, 20% of patients develop relapse mainly due to poor chemotherapy response. Although respond to one week prednisone therapy has evolved as the strongest prognostic factor, recent studies suggest that blast count on Day 15 (D15) of BFM protocol may be another important prognostic factor.
Aims: The aim of this study was to evaluate the impact of blast count on D15 of ALL –BFM-95 protocol on survival.
Methods: D15 blast count in bone marrow (BM) aspirates were retrieved from patients files of 130 evaluable children treated with ALL-BFM 95 protocol at the Pediatric Hematology Unit of Gazi University Medical School. D15 BM examination was categorized based on residual blast count as M1 (<5%), M2 (5% to <25%), and M3 (≥25 %). Early death before Day 33 were excluded from analysis.
Results: Of the 130 children, 53 were girls and 77 were boys. Their age at diagnosis ranged from 6 months to 18 years (median 7 years). D15 BM was reported as M2 and M3 in 36 (27.7%) of them. Twenty-one children relapsed within a median 28 months after complete remission on day 33 of induction therapy. The relapse rates in 13 children with M2 and M3 (61.9%) on day 15 was significantly higher as compared to 8 children with M1 BM (38.1%) (P<0.05). The EFS rates for D15 M1, M2 and M3 BM were 89.4%, 66.7% and 50.0%, respectively. There was a significant differences for the probability of EFS in the 3 groups by the log rank tests (P<0.05).
Summary / Conclusion: High BM blast count (≥5%) on D15 is associated with poor survival in children treated with ALL-BFM 95 protocol. These data suggest that it can be used as a risk stratification criterion when assessment of minimal residual disease is not available.

B1201

ACUTE LYMPHOBLASTIC LEUKEMIA IN THE ELDERLY: A TOUGH ONE

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Background: Elderly patients with acute lymphoblastic leukemia (ALL) or lymphoblastic lymphoma (LBL) form a special subgroup of patients with widely different outcomes.
Aims: As higher age is usually associated with an aggressive disease course and a higher susceptibility to treatment complications, the right choice of treatment approach (intensive or palliative) is sometimes difficult. Aim of the analysis was to compare results of elderly ALL therapy using intensive regimen (Czech Leukemia Study Group protocol for ALL/LBL patients over 50 years) or palliative approach (including palliative chemotherapy).
Methods: All patients aged over 50 years diagnosed with ALL or LBL at our centre between 2000 and 2012 were included into this retrospective analysis. We described the baseline features of these patients and treatment options used. The data were analysed for response and relapse rate, incidence of complications and risk factors affecting survival.
Results: A total number of 59 patients aged 50 to 83 years (median age 61 years) at the time of diagnosis were included into this analysis; forty-five (76%) patients with B-ALL, five (8%) with T-ALL, six (10%) with LBL, and three (5%) patients with acute undifferentiated or biphenotypic leukemia. Median baseline leukocyte count was 10.2G/L, range 0.4-334. Nearly half of the patients carried an adverse cytogenetic or molecular marker; BCR/ABL or Philadelphia chromosome in 23 (39%), MLL fusion gene in 2 (3%), complex karyotype in 1 (2%) and other abnormalities in 5 (11%) patients. Minimal residual disease analysis was performed repeatedly in 24 patients. Intensive treatment protocol was used in 37 (63%) cases, 22 (37%) patients received palliative treatment. Hematopoietic stem cell transplantation was performed in only 5 (8%) patients. Most of the patients developed treatment complications. These were mild or moderate in 11 (19%) patients, severe or life-threatening in 34 (58%) patients and fatal in 11 (19%) cases. Forty-four (88%) out of 50 evaluable patients achieved a complete hematologic remission (CR); 97% in the intensive arm vs. 70% in the palliative arm. Complete molecular remission was reached in 33% patients; 50% in the intensive arm vs. 17% in the palliative arm. Twenty-four (55%) patients in CR eventually relapsed. During the follow-up period with a median of 8.7 months (range 0.2 to 153 months) fourty (68%) patients died. Most common causes of death were disease progression (40%), infection (33%) and non-infectious treatment toxicity (18%). Five-year progression-free

(PFS) and overall survival (OS) in the whole cohort were 18% and 21%, respectively. The survival was significantly lower in patients over 65 years of age or treated using a palliative approach. In patients between the ages of 60-70 treated using our intensive protocol the survival was comparable to that observed in the age group 50-60 (5-year PFS 18% and 22%, respectively; 5-year OS 26% and 21%, respectively). The survival of patients over 70 years of age was poor, with 1-year PFS and OS of 18% and zero 2-year PFS and OS.
Summary / Conclusion: Elderly ALL patients in the age between 50 and 60 years clearly profit from intensive treatment. Outcome of patients over 70 years of age is still poor, with no signs of improvement over time. According to our analysis, some patients in the age between 60 and 70 can benefit from intensive therapy using less toxic protocols. These patients are candidates for further analysis.

B1202
NELARABINE FRONT-LINE THERAPY FOR ADULT T-LYMPHOBLASTIC LEUKAEMIA/LYMPHOMA (T-LBL/ALL): PRELIMINARY RESULTS OF A SINGLE CENTRE EXPERIENCE
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Background: Precursor T cell LBL/ALL occurs most frequently in late childhood, adolescence, and young adulthood, with a 2:1 male predominance; it comprises 15 and 25 percent of childhood and adult ALL, respectively, and 2 percent of adult non-Hodgkin lymphoma. Nelarabine is an anticancer prodrug of arabinofuranosylguanine (ara-G); it inhibits DNA synthesis and leads to high molecular weight DNA fragmentation and cell death.
Aims: Nelarabine has showed relevant efficacy in phase II clinical trials, both in pediatric and in adult LBL/ALLpopulations.
Methods: We report clinical outcome results of 9 newly diagnosed and younger than 60 yearsT-ALL patients (median age 29 years, range 22-45 years, 3/6 M/F, 8 T-ALL, 1 T-LBL) treated according to pediatric-like adapted schedule. Cytogentics data and molecular biologic features will be provided on site. Induction cycle included Vincristine, daunoblastine, L-asparaginase and Prednisone. After induction, all the patients received consolidation therapy with cyclophosphamyde, L-asparaginase, Cytarabine and 6-Mercaptopurine. Subsequently all the patients receveid Nelarabine 1500 mg/sqm (days 1-3-5 every 21) for two cycles. All the patients shared the same strategy for intensification, which consisted in allogenic stem cell transplantation, if available, or additional courses of consolidation chemotherapy. Durations of complete remission (CR) and overall survival (OS) were estimated according to the Kaplan-Meier method. The CR duration was dated from start of CR to first evidence of disease recurrence.
Results: After a single induction course, 9/9 patients obtained a CR (100%). Eight patients underwent an allogenic bone marrow transplantation. Aftera median follow-up of 24 months, 7/9 patients (78%) are alive in CR. The median CR duration and OS were 13.4 and 24.4 months, respectively. Neurological toxicity of grade 3has not been reported. We did not observe grade 3-4 haematological toxicity.
Summary / Conclusion: Nelarabine is a promising drug, which induces a remarkable complete remission rate at the expense of a very low and manageable toxicity.

B1203
ACUTE MIXED LINEAGE LEUKEMIA TREATMENT OUTCOME: A 5 YEARS SINGLE INSTITUTE EXPERIENCE
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Background: Mixed-lineage acute leukemias (MLL) - or acute leukemias of ambiguous lineage- represent a heterogeneous category of rare, poorly differentiated acute leukemias that possess characteristics of both lymphoid and myeloid precursor cells.
Aims: To investigate treatment outcome of pediatric, Mixed-lineage acute leukemia's (MLL) patients diagnosed and treated at the National Cancer Institute, Cairo University during a 5 years period.
Methods: Retrospective review of patient charts with newly diagnosed histologically proven MLL, diagnosed and treated at the Pediatric Oncology Department, National cancer institute, Cairo University in the period from January 1st 2005 till end of December 2010 was performed. All patients were followed up till the end of June 2012. Clinical and laboratories data of these patients were retrospectively followed and evaluated.
Results: Twenty seven patients were included in our study. Age of patients ranged between 8 months - 17 years, median 9 years. They were 17 males and 9 females. Follow Up period ranged from 4-93 months, median of 12.3 months. Total leucocytic count (TLC) at diagnosis ranged between 1.5 to 546x$10^3/m^2$ with a median 21.4 x$10^3/m^2$. Bone marrow was suggestive of ALL in 9/27 patients (33.3%), AML in 5/27 patients (18.5%), and MLL in 13/27 patients (40.7 %). Immunophenotyping showed Myeloid+ T (M+T) cell in 37%, whereas M+B in 63%. Seven patients received ALL induction like therapy, while 20/27 patients received AML like treatment. Post induction CR rate was 63%. *Disease outcome*: 17/27 (63%) of patients died due to chemotherapy toxicity, mostly due to fungal chest infection. By the end of the study OS at 5 years was 20% with no statistical difference between the two subtypes.
Summary / Conclusion: Mixed-lineage acute leukemias carry an extremely poor prognosis in our institute. There is a strong need for implementing a standardized protocol of therapy offering an early bone marrow transplant as well as a better supportive care and further improving the infection control setup.

B1204
THE LONG TERM RESULTS OF CHILDHOOD ALL AT TWO CENTERS FROM TURKEY: 15 YEARS OF EXPERIENCE WITH ALL BFM 95 PROTOCOL
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Background: Dramatic progress in the treatment of childhood ALL has been achieved during the last two decades in western countries where the 5 year event free survival rate (EFS) has risen from 10% to 80%. However, this high cure rate has not been always occurred in every center in developing countries due to limited sources.
Aims: We evaluted the treatment results of ALL BFM 95 protocol used between 1995 and 2009 in pediatric hematology departments of Bursa Uludağ University and İzmir Dokuz Eylül University hospitals.
Methods: A retrospective analysis of 343 newly diagnosed children as ALL. M/F:200/143, mean age: 6.7±4.2;1-17.5 years) was performed. The overall (OS) and EFS according to age, sex, initial leucocyte count, chemotherapy responses on day 8th, 15th and 33rd and risk groups were analysed by Kaplan Meier survival analysis. MTX dose was not reduced and given as 5 g/m^2
Results: Median follow-up time was 6.4±4.02 years. Complete remission was achieved in 98.5% of children. The gender did not have a significant effect on EFS and OS (P>0.05). Children with good response to prednisolone on day 8th achieved significantly better OS and EFS (P=0.001). Children in the standart (95%)and the medium risk groups (83%) obtained higher EFS comparing to high risk group (56%)(P=0.001). EFS for B- and T-cell ALL were 81% and 66%, respectively. Adolescents achieved 65% of EFS. Five years EFS and OS were found 78% and 80%,respectively. Relaps rate was 15%. The median relapse time from diagnosis was 23.21±13.16 months. Death occured in 69 out of 343 patients (20%). The major causes of death were infection and relapse. None died of drug related toxicity.
Summary / Conclusion: ALL-BFM 95 protocol was applied successfully in these two centers. In developing countries in which MRD could not be performed, this protocol could be still used with high survival rates.

B1205
ZAP-70 EXPRESSION IN ACUTE LYMPHOBLASTIC LEUKEMIA:RELATION TO PATIENTS' PROGNOSIS AND CYTOGENETIC RISK GROUPS
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Background: The Zeta-associated protein 70 (ZAP-70) is normally expressed in T and natural killer cells. Expression of ZAP-70 has been associated with poor prognosis in B-chronic lymphocytic leukemia.
Aims: To assess the expression of ZAP-70 in both B and T acute lymphoblastic leukemia (ALL) in pediatrics and adults and to correlate its expression to clinical, laboratory and cytogenetic characteristics as well as to evaluate its impact on patients' outcome and survival.
Methods: This study was conducted at Ain Shams University Hospitals. It included 50 patients with ALL; 32 adults and 18 children and adolescents. Regarding the phenotype; there were 33 patients with Pre-B-ALL, 10 with mature B-ALL and 7 with T-ALL. All patients were investigated for ZAP-70 expression by flowcytometry and cytogenetic abnormalities by conventional cytogenetics and Fluorescence in situ-hybridization (FISH) technique for t(9,22), t(12,21) and 14q11 rearrangements.
Results: ZAP-70 was expressed in 48/50 (96%) of patients, with no significant difference in its expression between childhood and adulthood ALL (P>0.05). Highest expression was recorded among T-ALL (48±6%) followed by mature B-ALL (37±8%) and Pre-BALL (35±7.5%); but this did not reach a statistical significance (P>0.05). High ZAP-70 expression ($\geq$51%) was associated with very high and high risk group of patients (P=0.001), poor response to chemotherapy (either death or early relapse in 82.4% of patients), as well as low platelet count (P=0.0), low hemoglobin (P=0.001), high LDH level (P=0.031), high peripheral blood blast (PBB) (P=0.022) and short disease free survival and overall survival.
Summary / Conclusion: ZAP-70 is highly expressed in both T and B-ALL in pediatrics and adults and its high expression was associated with the worse prognosis. Thus high ZAP-70 expression carries a bad prognostic impact in ALL and may be a new suitable therapeutic target.

B1206

SERUM LEVELS OF MULTIPLE CYTOKINES AND ADHESION MOLECULES IN PATIENTS WITH NEWLY DIAGNOSED ACUTE LYMPHOBLASTIC LEUKEMIA AND IN HEALTHY SUBJECTS

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Background: Cytokines and adhesion molecules have been studied as markers of immune system activation in various diseases including hematological malignancies. Alterations in this network may have direct effect on the malignant cells or have indirect effect on leukemogenesis through altered functions of bone marrow stromal elements. The knowledge gained from multiple cytokine and adhesion molecule analysis should allow better diagnosis and disease management.
Aims: The aim of our study was to evaluate serum cytokine and adhesion molecule levels by biochip array technology in patients with acute lymphoblastic leukemia (ALL) and in healthy subjects.
Methods: Serum samples of 15 newly diagnosed ALL patients (median age 46, range 24 - 63 years, 11 males) and 15 healthy subjects (median age 41, range 25 - 58 years, 11 males) were analyzed. We evaluated serum levels of the following 22 cytokines and adhesion molecules: interleukins (IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-23), vascular endothelial growth factor (VEGF), tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma), epidermal growth factor (EGF), monocyte chemotactic protein-1 (MCP-1), E-selectin, L-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1). All biomarkers were measured by biochip array technology on Evidence Investigator analyzer (Randox). Probability values (p) < 0.01 were considered statistically significant.
Results: In newly diagnosed ALL patients, we found significant increase in serum VCAM-1 (1078.54±456.96 mcg/L vs. 328.31±88.66 mcg/L; P<0.000001), ICAM-1 (499.57±237.53 mcg/L vs. 196.69±36.06 mcg/L; P<0.0001), L-selectin (2366.33±1035.37 mcg/L vs. 1104.54±243.45 mcg/L; P<0.0001), IL-8 (34.07±28.52 ng/L vs. 4.87±3.09 ng/L; P<0.001), MCP-1 (433.99±328.59 ng/L vs. 153.25±53.60 ng/L; P<0.01). On the other hand, we found significant decrease in serum IL-3 (7.34±3.41 ng/L vs. 11.53±4.66 ng/L; P<0.01), IL-4 (1.10±1.08 ng/L vs. 3.27±2.21 ng/L; P<0.01). Serum levels of other evaluated cytokines and adhesion molecules were without significant differences.
Summary / Conclusion: Our results indicate that serum levels of some cytokines and adhesion molecules (VCAM-1, ICAM-1, L-selectin, IL-8, IL-3, IL-4, MCP-1) are significantly altered in patients with newly diagnosed ALL, reflecting activity of the disease. Whether these alterations could have a prognostic meaning for ALL is not known. Further studies in a larger number of patients and comparing cytokine and adhesion molecule levels with established prognostic markers will be essential to assess the potential role of these and additional markers in the stratification of risk in ALL patients.

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B1207

MICRO-RNA PROFILE OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Micro-RNAs are functional, non-protein coding RNA molecules and their transcriptions provided by intronor exon regions of the genome and non-protein coding regions of RNA genes. The role of micro-RNAs in acute leukemia has become the subject of research increasingly.
Aims: In this study we aimed to identify micro-RNA profiles in the childhood acute leukemia that one of hematologic malignancies.
Methods: Fourty nine patients who were diagnosed with acute leukemia and admitted to Cukurova University Faculty of Medicine Department of Pediatric Hematology between December 2010 and September 2011. Blood samples were taken twice in patient groups at diagnosis and during remission and plasma samples were stored. Blood samples were taken once in the healthy group and plasma were separated. The plasma samples were investigated by PCR analysis of micro-RNA. Acute leukemia was diagnosed by cytomorphological, immuno histochemical and flow cytometric studies. Thirtyone patients who were diagnosed with ALL and fortyseven healthy children as a control group were included to study.
Results: miR20a, miR25, miR92a, miR30c, miR106b, miR203, miR150, miR192, miR302c, miR184, miR218, miR320, miR342-3p, miR223, miR328, miR483-5p, miR376a, miR381, miR451, miR576-3p, miR548a levels were increased in newly diagnosed ALL patients when compared to healthy controls (p <0.05). The miR20b, miR342-3p and miR548a levels were found higher in healthy controls than the newly diagnosed ALL patients group (P<0.05) Healthy control groups when compared with pediatric ALL patients whose in remission; miR769-3p, miR20a, miR92a, miR16, miR27b, miR192, miR320, miR223, miR484, miR451 levels were found higher in healthy control groups than the patients. Newly diagnosed pediatric ALL patients compared with patients whose in remission; miR30c, miR106b, miR25, miR184, miR218, miR302c, miR483-5p levels were increased in newly diagnosed pediatric ALL patients than ALL patients whose in remission (P<0.05)
Summary / Conclusion: miRNAs are thought to be identified at a different level of expression in normal and pathological tissues can be determined between the miRNAs that are effective diagnosis and treatment of human cancers. We showed the microRNA profils that may play new roles treatment of acute leukemia in the futures.

B1208

IMMUNOPHENOTYPIC ANALYSIS OF T-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA. A CD5 PERSPECTIVE

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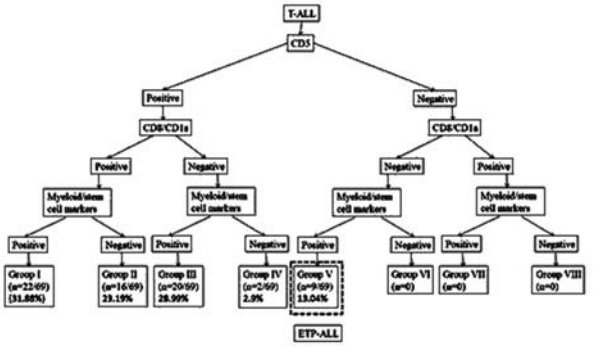
Background: T-cell markers CD5/CD1a/CD8 define early thymic precursor acute lymphoblastic leukemia (ETP-ALL). We reasoned that presence or absence of various combinations of these antigens could be used to divide non-ETP T-ALL into groups, in which to examine the expression of myeloid / stem cell (M/S) antigens and clinical features for comparison with ETP-ALL. This evaluation of non-ETP T-ALL from the perspective of ETP-ALL has not been done before may be relevant to an understanding of the spectrum of immature T-ALL of which ETP-ALL is part.
Aims: To examine expression of myeloid / stem cell (M/S) antigens and clinical features of subgroups of non-ETP T-ALL based on the defining T-cell antigens of ETP-ALL (CD5/CD1a/CD8), and compare these with ETP-ALL.
Methods: Records of 69 patients of T-ALL, immunophenotyped as part of routine diagnostic service were retrieved from the records of Laboratory Oncology, All India Institute of Medical Sciences, New Delhi. Clinical and immunophenotypic characteristics of all cases were noted. The immunophenotypic markers used were: CD3, CD45, CD5, CD8, CD1a, CD13, CD33, CD117, HLA-DR, CD34, CD65 and CD11b. The cases were classified based on expression of CD5 into two large groups: $CD5^+$ and $CD5^-$. These were further subdivided into $CD8^-/CD1a^-$ and $CD8^+/CD1a^+$ subgroups. In all these groups, presence of myeloid and/or stem cell markers were noted. Patients were thus divided into 8 groups, I to VIII, as shown in Figure 1. The different groups were compared for clinical features, remission rate, relapse rate and overall survival.
Results: $CD5^-$ was a homogenous $CD8^-/CD1a^-$, M/S antigen$^+$ ETP-ALL group (n = 9). $CD5^+$ cases were $CD8^-/CD1a^-$ pre-T ALL (n = 22) or $CD8^+/CD1a^+$ (n = 38) thymic/cortical T-ALL, with M/S antigens being significantly more frequent in the former (20/22; 90.91 %) than in the latter (22/38; 57.89%). Pro- and pre-T- ALL, which together constitute the immature T-ALL group of clinical and laboratory studies, were nearly always M/S+ (29/31; 89.9%) and had a significantly higher induction failure than cortical T-ALL. In multivariate analysis, ETP-ALL and female sex were significant predictors of event free survival (P=0.033, 0.016, respectively whereas only ETP-ALL was



Summary / Conclusion: Our results, (1) show that $CD1a^-/CD5$ pro-T ALL cases (EGIL) may all be ETP-ALL; (2) confirm pro-/ETP- and pre-T-ALL to belong to the wider spectrum of immature T-ALL on both immunophenotypic and clinical grounds; (3)indicate further studies on the T-ALL - AML interface and inclusion of CD1a and CD5 in the work-up of T-ALL; (4) confirm ETP-ALL has a poor prognosis.

B1209

ROLE OF ALLOGRAFTING IN ACUTE LYMPHOBLASTIC LEUKEMIA: A 12 YEAR EXPERIENCE

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Background: Acute lymphoblastic leukemia (ALL) is a rare disease in adults. Despite the introduction of several novelties in this field in terms of prognostic factors and new therapies, clinical outcomes remain unsatisfactory.
Aims: To evaluate the experience on 84 consecutive patients diagnosed with ALL at the Divisions of Hematology, S. Giovanni Battista Hospital, Torino, between 1999-2011.
Methods: Patients, median age 49 years (18-73), were treated according to Center guidelines or on clinical trials active at the time of diagnosis. At diagnosis, patients who presented with leukocitosis, poor-prognosis cytogenetic abnormalities, or delayed response to induction therapy were defined as high risk. High risk patients younger than 60 were considered for allografting (SCT) as part of first-line treatment. Treatments for 80% (20/25) of patients with traslocation (9;22) also included tyrosine kinase inhibitors after 2002: in 14 as part of induction, in 4 as maintenance and in 2 as salvage treatment.
Results: Overall, 25% (21/84) presented with leukocytosis and 56% (38/68, not done in 16) had poor-prognosis cytogenetic abnormalities. Complete remission (CR) was achieved in 92% of patients (77/84). After a median follow-up of 5 years (3-143 months), 43% of patients are alive, with a median overall survival (OS) and event-free survival (EFS) of 2 and1,6 years, respectively. Forty patientsreceived an allograft in first (no.=32) or second (no.=7) complete remission, orin progression (no.=1), from a HLA identical sibling (no.=21), an unrelated (no.=17) or a related other than sibling (no.=2) donor. Cumulative incidences of acute and chronic graft-versus-host disease were 47% and 39%, respectively. Median OS and EFS in patients who received SCT were 60 months and not reached, respectively, whereas in those younger than 60 who did not undergo a SCT median OS and EFS were 16 and 13 months (P=0.024 and 0.026) respectively. Stratifying patients by year of diagnosis, a trend toward improved EFS in those diagnosed after 2008 as compared to those diagnosed before 2003 (2.3 years versus 1.3) was observed, whereas OS remained superimposable. By multivariate analysis, the prognostic role of leukocytosis at diagnosis was confirmed for both OS and EFS (HR 2.48, IC 95% 1.28-4.80, P=0.007, and HR 3.72, IC 95% 1.93-7.14, P<0.001, respectively). Furthermore, in younger patients, an advantage in those who received an allograft in terms of both OS (HR 0.11 CI 95% 0.03-0.44 P=0.002) and EFS (HR 0.12 CI 95% 0.03-0.47 P=0.002) was seen.
Summary / Conclusion: SCT played a significant role in improving survival in patients with ALL, though prognosis in patients with leukocytosis or older remained poor. The trend toward an improved outcome seen in more recent years may be due to less toxic transplant procedures and the introduction of "minimal residual disease-guided treatment" and the availability of tyrosin kinase inhibitors. The combination of allografting and tyrosin kinase inhibitors, or novel monoclonal antibodies such as blinatumomab, should be evaluated in prospective control trials.

B1210

FAVORABLE OUTCOME FOR ADOLESCENTS AND YOUNG ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH EORTC 58951

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Aims: Retrospective studies have shown that adolescents and young adults with acute lymphoblastic leukemia(ALL) treated with pediatric protocols have better outcomes than similarly aged patients treated with adult protocols. We examined results and feasibility of a pediatric-based protocol (EORTC 58951) in adolescents and young adults.
Methods: From January 2006 to December 2010, 30 patients aged between 16 and 25 years (17 adolescents , 13 young adults) with newly diagnosed ALL were treated, in the department of clinical hematology of Aziza Othmana Hospital, according to the pediatric protocol EORTC 58951. The patients were 21 males (70%) and 9 females. 43.3% had a WBC> 50 Giga/l. Immunophenotyping was performed in all patients: 12 B-ALL (40%), 11 T-ALL (36.7%). All patients have had an AR2-VHR arm induction.
Results: Seven patients (23.3%) were corticoresistant. Twenty nine patients (96.7%) achieved CR. One patient failed to respond after 3 courses of chemotherapy and died as a result of disease (resistant). The MRD at D35 measured by CMF was<10^{-3} in 53.3% of cases. Twenty nine patients were evaluable. Only one patient was treated according AR1 arm, 8 patients according AR2 arm and 19 (65,5%) according VHR arm. Fifteen patients (51.7%) were eligible for allogeneic stem-cell transplantation (SCT) but, only 5 were allograft: 4 were alive and 1 died by GVH. Only one patient died by chemotherapy after the third bloc. Five patients (17.2%) relapsed during the first year of treatment. The median follow up was 50 months (4,2 years). The OS and the EFS at 5 years were 71%. Seven patients (23.3%) were corticoresistant. Twenty nine patients (96.7%) achieved CR. One patient failed to respond after 3 courses of chemotherapy and died as a result of disease (resistant). The MRD at D35 measured by CMF was<10^{-3} in 53.3% of cases. Twenty nine patients were evaluable. Only one patient was treated according AR1 arm, 8 patients according AR2 arm and 19 (65,5%) according VHR arm. Fifteen patients (51.7%) were eligible for allogeneic stem-cell transplantation (SCT) but, only 5 were allograft: 4 were alive and 1 died by GVH. Only one patient died by chemotherapy after the third bloc. Five patients (17.2%) relapsed during the first year of treatment. The median follow up was 50 months (4,2 years). The OS and the EFS at 5 years were 71%.
Summary / Conclusion: The results of this prospective pediatric based study show that response to therapy and prognostic in adolescent and young adults were better than those treated with adult protocols and tolerability of chemotherapy is acceptable. This experience of feasibility allowed us, since 2011 to treat adult aged up to 30 years with this pediatric regimen and the preliminary results of tolerability were good.

B1211

LONG-TERM RESULTS OF EORTC 58951 PROTOCOL FOR CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA IN THE SOUTH OF TUNISIA

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Background: Childhood acute lymphoblastic leukemia (ALL) is one of rare malignant disease with high rate of cure.
Aims: We report the experience of the department of clinical hematology of Sfax for the treatment of childhood ALL with the EORTC 58951 protocol
Methods: We retrospectively studied the outcome of all childhood ALL treated with the EORTC 58951 pediatric protocol. For those patients we studied the leukemia characteristics (sex ratio, white blood cell counts WBC, blast's phenotype, cytogenetic abnormalities) and response to treatment: response to prophase, remission rate, risk group stratification, treatment related mortality (induction and post induction death) and survival (overall survival OS, event free survival EFS and disease free survival DFS).
Results: From January 2000 to December 2011, 138 children were treated with the EORTC 58951 protocol. Median age was 6 years (range: 13 months to 15 years). Sex ratio M/F was 1.55. WBC counts less than 10 G/l, from 10 to 100G/L and more than 100G/L were observed respectively in 41, 44 and 15% of cases. The blast's phenotype was B in 70% and T in 30%. Cytogenetic abnormalities were noted in 43% of cases. Response to prophase was noted in 86% and complete remission in 96% of cases. The EORTC risk group stratification was Low Risk (LR), Average Risk1 (AR1), Average Aisk2 (AR2) and Very High Risk (VHR) in 6.5, 43.5, 27 and 23% respectively. TRM was 12.5%: induction rate death was 8% and post-induction rate death was 4.5%. Three patients from VHR group with familial donor underwent allograft. The relapse rate was 25% of all patients in remission; it was 0, 25, 13 and 45% for LR, AR1, AR2 and VHR group respectively. At 5 years of follow up, OS, EFS and DFS were 65, 61 and 74% respectively.
Summary / Conclusion: Childhood ALL in our institut were characterized by a poor presentation at diagnosis: male sex, leucocytosis more than 100G/L, T phenotype and bad response to prophase are frequent compared to the occident reports. TRM is higher in our study than observed in the literature (12.5% vs 6 to 8%) especially the induction death rate. EFS are acceptable in our study but still less than observed in literature (85-90%). It can be improved by reducing TRM, detecting high risk patient with MRD study especially for AR1 risk group, and doing allograft for VHR patients.

B1212

ADOLESCENT AND YOUNG ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: TUNISIAN MONOCENTRIC EXPERIENCE

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Background: Adolescent and young adult (AyA) with acute lymphoblastic leukemia (ALL) are currently treated like children with pediatric protocol allowing good results.
Aims: We report our monocentric experience of AyA ALL treated with the EORTC 58951 pediatric protocol
Methods: Between January 2000 and December 2011, AyA ALL aged from 16 to 29 years were treated with the pediatric EORTC 58951 protocol in the hematology department of Hedi Chaker Hospital Sfax Tunisia. Those patients were classified into two groups: G1; patients aged from 16 to 20 years and G2: patients aged from 21 to 29 years. Further leukemia characteristics (Sexe, White Blood cell count, Blasts phenotype, Cytogenetic results), we studied the protocol results: response to prophase, risk group stratification (average: AR1 and AR2, very high: VHR), treatment related mortality (TRM), remission rate,

relapse rate and 5 years survivals (overall OS, event free EFS and relapse free survival RFS), then we compare the two groups results.
Results: Over twelve years, 51 AyA ALL were treated with the pediatric protocol. Thirty in the G1 group and twenty one in the G2 group. Sex ratio was1,7for all patients,1,2 for G1 and 3 for G3. WBC ≥ 100G/L was note respectively in 39, 35 and 45% of all patients, G1 and G2 group. A T blast phenotype was noted in 57% of cases, 53%for G1 and 63%for G2. Cytogenetic abnormalities were noted only in 53% of cases, 56% in G1 and 50% in G2. ALL were stratified into AR1, AR2 and VHR in respectively 18, 49 and 33%. G1 and G2 stratification risk group was respectively AR1: 26 and 5%, AR2: 42 and 60%, VHR: 32 and 35%. Cortico-sensitivity was noted in 71% of cases: 74% in G1 and 65% in G2. Induction death was noted in 10% of all patients (G1:10%, G2:10%). Remission rate was 98% (G1:96%, G2:100%). Consolidation death was noted in 18% (G1:22%, G2:11%). Relapse was observed in 26% (G1:19%, G2:35%). Five years OS, EFS and RFS were respectively 49, 48 and 68%. G1 and G2 OS, EFS and RFS were respectively 53, 52, 76% and 46, 45, 57%.
Summary / Conclusion: Our AyA ALL had a poor presentation: Male, leucocytosis more than 100 G/L, T phenotype and bad response to prophase were frequent particularly in G2 group. TRM is high 28% with more consolidation death in G1 group. Even good response rate, relapse still important particularly in G2 group. OS EFS and RFS are less than those observed in literature but still acceptable in our institution regarding ALL characteristics.

B1213
EVALUATION OF SURVIVAL RATES OF OUR PATIENTS WITH CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA:SINGLE CENTER EXPERIENCE
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Background: Improvements in treatment protocols result in longer expected survival rates in children diagnosed with acute lymphoblastic leukemia.
Aims: To evaluate survival rates of our patients diagnosed with childhood acute lymphoblastic leukemia
Methods: 250 children with newly diagnosed ALL in the Pediatric Hematology Department of Istanbul Goztepe Research and Education Hospital from January 2000 to January 2010 were evaluated. All of the patients included in this study were diagnosed at our institution and all were uniformly treated on a single tretment regimen, ALL–BFM (Acute Lymphoblastic Leukemia-Berlin-Frankfurt-Munster) portocole. Data were retreived from hospital records and were analyzed retrospectively.
Results: The age of patients at diagnosis ranged between 1 and 17 years (mean age6,37±4,17years). 116 (46,4%) patients were female, 134 (53,6%) were male. The follow up time ranged between 36-156 months. When patients were stratified into risk groups based on ALL-BFM protocole 83 (33,2%) patients were in standard risk group,127 (50,8%) patients were in median risk group and 40 (16%) in high risk group. 97% of patients achieved complete remission on the 33th day of induction therapy. During this 10-year follow-up period 38 (15%) patients relapsed. 24(9,6%) patients developed bone marrow relapse and 16 of these died because of the progression of the disease. 7 (2,8%) patients developed combined bone marrow and central nervous system relapse and 6 of these died. 2 (0,8%) patients developed combined bone marrow and testicular relapse. 1 patient died. 4 (1,6%) patients developed isolated testicular relapse and all of them are still in remission. 1 (0,4%) patientdeveloped retinal relapse and he's in remission. 10 (4%) patients died during induction therapy because of infections.56 events were recorded during the follow-up period. 5-year event-free survival was 75,5±3%, 10-year event-free survival was 73,1±3,4%.
Summary / Conclusion: Improvements in treatment protocols have been associated with increased survival rates in children with acute lymphoblastic leukemia.

B1214
IMPACT OF RISK FACTORS ON LONG-TERM OUTCOME IN 125 ADULT ACUTE LYMPHOBLASTIC
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Background: Adult acute lymphoblastic leukemia (ALL) is often icurable despite intensive chemotherapy with or without hematopoietic stem cell transplantation (HSCT). Estimated overall long-term survival rate for adult ALL patients (pts) is about 20-50%.
Aims: To present our results of treatment adult ALL and impact high risk factors on overall survival (OS).
Methods: Since January 1989 till February 2013, 125 (male 87, female 38) pts with adult ALL were treated, average age was 30 years. Immunophenotype were determined in 93 (B-ALL 64, T-ALL 29) and cytogenetic/molecular analysis were succeeded in 62 pts. At the time of initial presentation: 34 pts were older then 35 years, 30 pts had high white blood cells (WBC), 5 pts had CNS involvement and 10 pts had Ph/bcr-abl+. Pts were treated with induction, consolidation and maintenance therapy under modified YU-ALL regimen for "high risk" (82), HyperCVAD (21), LALA 94 (8), etc. (14). High risk pts was defined by the presence of at least one of following factors: age > 35 years, WBC > $30x10^9$/l (B-ALL) or $100x10^9$/L (T-ALL), CNS involvement, more than 4 weeks to achieve complete remission, and finding Ph+/bcr-abl+, t(4;11)+ or t(1;19)+. Medicament prevention (without radiotherapy) of CNS disease was applied to every pts under 50 years.
Results: Complete remission (CR) was achieved in 114 pts (delayed CR in 24). Resistant to therapy were 5 pts (4.2%), 6 pts (4,8%) have died (2 early deaths, 2 before evaluation of remission, and 2 due to infective complications, 1 during II induction, 1 in CR during of intensification, respectively). Maintenance therapy (MT) was applied for 24-36 months in 69 pts. HSCT was done in 51 pts: allo in 34 pts (CR1 in 22, CR2 in8, and with partial response in 4 pts; 8/10 Ph/bcr-abl+ pts transplanted) and auto in 17 pts (CR1 11, CR2 5 and with molecular relapse 1 pts). Relapses have occurred in 75 pts (65,8%) with median time of 9 months. Frequency of relapses were in pts on MT 71% (45/63) and in HSCT pts 58,8% (30/51) or more exactly: in auto HSCT 76,5% (13/17) and allo HSCT 50,2% (17/34) respectively. The secondary allo HSCT was done in 6 pts (2 are still alive). Long.term survival without relapses had 31/114 (27.2%) pts; 10-years disease free survival in MT pts was 16.17±5%, auto HSCT pts 28.1±12%, and allo HSCT pts 33.1±11%. Uni- and multivariate analysis of impact high risk factors on OS showed significance with respect to CR achieved in 4 weeks (P=0.001), CNS involvement (P=0.01), and age (P=0.0176), while cytogenetics and WBCs were without impact on OS in this cohort of pts.
Summary / Conclusion: Results of our retrospective analysis are similar to the others and confirms that treatment of adult ALL is unsatisfactory. Further investigations of biology ALL, new targeting therapy and better control of minimal residual disease are necessary same as redefinition of traditional prognostic factors and according to them we can decide to use intensive chemotherapy with or without HSCT.

B1215
INFLUENCE OF SERUM CORTISOL LEVEL TO COLONY-FORMING EFFICIENCY OF BONE MARROW STROMAL FIBROBLASTS AT CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: It is known that application of corticosteroids promotes violation of various origin fibroblasts function, including stromal fibroblasts. Our previous data suggesting that low serum cortisol level in debut (SCL-D)) of acute lymphoblastic leukemia (ALL) at children is accompanied by the worst prognosis in comparison with patients who had standard SCL in debut of acute leukemia.
Aims: To study the colony-forming efficiency of the bone marrow stromal fibroblasts (CFE -F) at children with ALL taking into account SCL in the debute of disease (before start of the treatment) for prognosis of leukemia course
Methods: 55 children with ALL were under investigation. Prior to executing ALL protocol treatment CFE of bone marrow stromal fibroblasts were studied in vitro by A.Y. Fridenstein's method in V.S. Astakhova's modification (2000). SCL before start of ALL treatment and overall survival (OS) of the patients were taken into account.
Results: Mean value of CFE-F in children ALL debut was 9,3±2,5 for 10^5 mononuclear cells, in control group this index was 52,3±3,2 for 10^5 mononuclear cells. On that time 14/55 children with ALL had SCL-D<200nmol/l , the others 41/55 had SCL-D>200nmol/l. Low CFE-F at children with ALL correlated with SCL-D lower than 200 nmol/l (Mann-Whitney U=10) and these children also demonstrated the excretion of calcium phosphates in urine raised by 2-2,5 times and bone densitometry decreased by 20-25% from the standard. Overall survival of ALL children with low SCL-D (<200nmol/ was 34,5±2,1 months, as at the standart value of SCL-D (483,4±11,5 nmol/l) OS of ALL children was 76,1±2,2 months.
Summary / Conclusion: Children with ALL who have SCL-D less than the 200 nmol/l also demonstrate lower CFE-F, shorter overall survival and osteoporosis phenomena as compared with patients who had standard SCL-D. The results give the chance to prognosticate ALL course at children taking into account SCL-D.

B1216
EARLY T CELL PRECURSOR LEUKEMIA: INMUNOPHENOTYPE AND CLINICAL CHARACTERISTICS OF A RARE AND AGGRESSIVE ALL-T SUBTYPE. EXPERIENCE IN ONE CENTER
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Background: Currently there is special interest in the uncommon subtype of acute lymphoblastic leukemia T (ALL-T) known as early T-cell precursor leukemia (ETP). This has a particular inmunophenotype (CD1a-, CD5+dim, CD4-, CD8-,

and at least one myeloid or stem cell marker) that allows the distinction between other leukemias and confirms their intrathymic origin. However, their myeloid antigen expression could confuse them with biphenotypic leukemias. The main clinical characteristic is bad prognosis with poor response to treatment. Some genetic alterations have been related to ETP, but it is not yet identified a defined pattern; this has been analyzed mostly in children and there is few data available in adults.
Aims: Our goal is to identify ALL-T patients with ETP profile by inmunophenotype characteristics and correlate it with clinical course: overall survival (OS), event free survival (EFS), relapse free survival (RFS) and mortality rate (MR).
Methods: We reviewed data from 164 patients with ALL-T and biphenotypic leukemias diagnosis from Hospital La Fe Hospital in Valencia Spain from 1990 until January 2013. The clinical presentation was obtained from clinical records. The OS, EFS, MR were calculated using SPSS Statistic 17.0.
Results: There were identified 6 patients (3,6%) with ETP profile. Five cases expressed stem cell antigens and all of them at least one myeloid marker. Four patients (67%) met the EGIL (European Group for the Immunological Characterization of Leukemia) criteria for biphenotypic leukemia. The mean age was 24 years old (9 months to 63 years) and there was no gender predominance. Peripheral white blood count and bone marrow blast percentage average at diagnosis were $49.2x10^3$ cell/µL and 80% respectively. Two patients presented extramedular disease. No common genetic or molecular alterations were identified. The OS and EFS average were 11.8 months and 9.9 months respectively. In patients responding to induction therapy RFS was 3 months. Two patients had positive minimal residual disease >0.5% and they relapsed. All cases needed at least one reinduction and mortality rate was 83%.
Summary / Conclusion: ETP ALL-T is a rare subtype of ALL-T with specific characteristics. The outcomes are often unfavorable despite of aggressive treatment. The identification of this specific immunophenotype allow an accurate diagnosis. It would be necessary a multicenter study for the complete evaluation of the biological behavior of this entity to offer a targeted treatment.

B1217

CMV REACTIVATION IN ACUTE LYMPHOBLASTIC LEUKEMIA PH POSITIVE IN TREATMENT WITH DASATINIB

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Background: Dasatinib, a potent tirosine multikinase inhibitor targeting to a ABL and SCR ,among others, has transformed the treatment of chronic myeloid leukemia (CML) and Acute Lymphoblastic Leukemia Ph positive (Ph+ ALL). However, the information on infectious complications associated with its use is limited. Preliminary studies have reported 7% to mainly viral infections in patients with Ph + ALL who receive high doses of steroids.
Aims: To analyze the incidence of CMV reactivation during dasatinib treatment in Ph+ ALL patients
Methods: Between January 2012 and February 2013, we treated 3 Ph+ ALL patients with dasatinib and poli-chemotherapy according to PETHEMA LAL-070 protocol. In order to rule out CMV infection, we tested CMV antigenemia/PCR in patients with fever of unknown origin (FUO).
Results: We found one patient with CMV rectivation during dasatinib treatment in Ph+ ALL. An increased in number of LGL wasn´t observed. A56 year-old woman diagnosed with (Ph + ALL) started induction treatment as PETHEMA LAL-070 protocol, for over 55 years-old. The fifth day of treatment was confirmed positive by FISH (Bcr-Abl) and Imatinib therapy started at 200mg daily day (doses of imatinib were reduced to 50% for acute liver failure (ALF), DXM 10mg/m2 per 2 days a week, VCR 1mg/week, TIT weekly x 3 doses. As severe deterioration of liver function tests for probable liver infiltrative, we began polyQT with: Ciclofosfamide 20mg/m2/day for 5 days, prednisone 60 mg/m2/day for 21 days, VCR 1 mg weekly, Daunorubicine 15mg/m2 weekly. At day +8, for improvement of liver, the patient started imatinib 400mg daily, achievedat 3 weeks, Morphologic Complete Remission (MCR), Complete Cytogenetic Response (CCyR), absence of the transcript bcr/abl p210 and p 190 by real-time quantitative polymerase chain reaction análisis and persistent minimal residual disease (0.6%), which initiated maintenance therapy with mercaptopurine 50 mg/m2, methotrexate 20 mg/m^2/week and Dasatinib 70 mg/12h. In Day +21 of treatment, presented fever of 38° C, diarrhea, pancytopenia, cholestasis and cytolysis. The fever and diarrhea persisted with progressive worsening liver functions so we requested antigenemia and PCR CMV in blood, that resulted positive. The immunophenotype blood was normal without increase in number of LGL. As a result we started treatment with oral valganciclovir with clinical improvement, negativization antigenemia/PCR for CMV and progressive improvement of the Liver tests. As the diarrhea persist, we did a colonoscopy with biopsy result negative for CMV. Given the absence of fever, normalization of blood counts and improvement of Liver function, decided control consultation and increasing doses of imatinib.
Summary / Conclusion: Dasatinib presented *in vitro* Immunosuppression of T and NK cells. Some patients with leukemia treated with dasatinib, develop LGL cell expansion. Which is associated with a better therapeutic response, however, may be linked to a reactivation of CMV. The findings suggest that cytomegalovirus screening should be routinely in patients treated with dasatinib.

B1218

ACUTE LYMPHOBLASTIC LEUKEMIA OF HIGH AND INTERMEDIATE RISK. SINGLE CENTER EXPERIENCE SINCE 2000

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Background: It is generally considered that adult patients with Acute Lymphoblastic Leukemia (ALL) reached only limited results after current BFM-based chemotherapy programs, especially those with age over 50.
Aims: We wanted to learn from our single center experience in the treatment of adult patients with ALL.
Methods: Between 2000 and 2012, 56 consecutive patients were diagnosed of ALL in our center. 46 cases were considered high risk, being the rest intermediate risk. Median age at diagnosis was 40 (range: 13-89). There were 29 women and 27 men. 7% were of T lineage, being the other 93% of B lineage. Among these, most frequent type was B common (41%), followed by preB (21.4%) and proB ALL (12.5%), with the rest being biphenotypic leukemias. Burkitt/Burkitt like ALL were excluded from this analysis. At diagnosis, 31% of cases had a white cell count over $30x10^9$/L. Protocols mainly used were PETHEMA versions of 1993 and 2003 (for high risk) and 1996 (for intermediate risk).
Results: Complete remission (CR) were reached in 94% of cases. 30 out of these 52 responding patients eventually relapsed. Progression free survival (PFS) was 58% at 1 year and 42% at 2 years. Median overall survival (OS) was 5 years. 37% and 12% had OS over 5 and over 10 years, respectively. 12 of the 30 relapsed patients had OS over 5 years, with a median OS of 4.7 years. OS for high-risk patients treated with PETHEMA 1993 protocol was over 10 years in 57% of cases, while 46% for the same population treated with 2003 protocol. Intermediate ALL cases had a median OS of 5 years.
Summary / Conclusion: Despite the small numbers, and a potential positive selection bias for a referral center, we found better results for ALL patients treated with conventional chemotherapy BFM-based programs, than the ones previously published for our global national group. Of note, quite elderly ALL patients can be long term survivors.

B1219

CYTOCHROME P450 3A4(CYP3A4) GENE POLYMORPHISM AMONG EGYPTIAN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer with a cure rate of approximately 80%.Relapse occurs despite treatment stratification based on clinical criteria **.** Cytochrome P450, family3, subfamily A, polypeptide 4(CYP3A4), a member of the cytochrome P450 mixed-function oxidase system, is the most abundant hepatic and intestinal P450 enzyme and is involved in the metabolism of more than 50% of all drugs used in humans including glucocorticoids such as dexamethasone, phenobarbital, and anticancer drugs . Interindividual variation in drug metabolism is a complicating factor in pharmacotherapy.
Aims: The aim of this study was to clarify the characteristic distribution patterns of the genotype of CYP3A4*1B in Egyptian children with ALL and in healthy control, and then explore whether the polymorphism of CYP3A4 is related to vulnerability to childhood leukemia and the development of relapse , secondary malignancy and chemotherapy induced toxicity.
Methods: Patients were subjected to full history taking with special stress on age, type of chemotherapy and their doses, then complete physical examination, laboratory assessment with adequate volume of venous blood was withdrawn from each patient and control for determination of liver, kidney functions, complete blood picture and bone marrow aspirate at day15,42 and 49.
PCR-RFLP and DNA sequencing was applied to assay genotypes of CYP3A4 in 67 Egyptian ALL children and 72 healthy children, then the difference of phenotypes and genotypes of CYP3A4 was compared between these two groups.
Results: In 72 healthy children, only two children were heterozygote genotype of *CYP3A4*1B*, others were all in wild type(n=70) and non in variant type,the percentages of genotypes were as follows: 2.8% for heterozygote, 97.2% for wild type and 0.0% for the variant type respectively. While in 67 children with acute lymphoblastic leukemia, eight children had (11.8%) heterozygotic genotype of *CYP3A4*1B*, one (1.5%) ALL child was homozygote genotype and 58 (86.6%) in wild type respectively. A significantly higher prevalence of the heterozygote polymorphic variant CYP3A4-A-290G (CYP3A4*1B) gene in ALL cases, when compared with controls (11.9% vs. 2.8%,P=0.000).Eight patients with heterozygote genotype developed complete remission at day 15 bone marrow aspirate while 56patints with wild genotype developed complete remission at day 15 bone marrow aspirate and two patients with wild type developed partial remission and one patient with variant type developed partial remission(P=0.000). In wild type group ; one patient developed relapse and another one developed secondary malignancy as biphenotypic leukemia(P=0.92). We could not find any association between CYP3A41*B polymorphism and other risk factors like age at diagnosis (P=0.500), initial total

leukocyte count (P=0.695), LDH (P=0.152),gender (P=0.468), clinical risk degree (P=0.238), immunophenotyping (P=0.746) and FAB classification (P=0.298).None of the patients developed symptoms or signs of toxicity from Vincristine, Adriamycin and VP16 .
Summary / Conclusion: Mutation frequency of CYP3A4*1B in children with ALL was higher than that of in healthy control, thus it could interfere with the leukemogenesis in Egyptian children but not the risk of relapse or secondary malignancy.

B1220
THE SERUM TRAIL AND TRAIL LIGAND ON THE APOPITOTIC PATHWAY IN CHILDHOOD ACUTE LEUKEMIAS
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Background: In leukemias and malignant tumors the balance and interaction between apoptosis and cell proliferation is dysregulated. Many types of cancer cells have tumor necrosis factor related apoptosis inducing ligand (TRAIL) on their cell membran surfaces. Targeting the cell extrinsic pathway which triggers p-53 independent apoptosis offers a unique therapeutic stragety to induce apoptosis in only cancer cells thus prevents the damage of healthy cells. TRAIL induces apoptosis with its receptors TRAIL-R1, TRAIL-R2 however apoptosis can not be induceed by receptors TRAIL R-3 and TRAIL-R4. There are many trials to search the correlation between leukemia and apoptotic pathway disorders.
Aims: In this study we determined the seum levels of TRAIL and TRAIL receptors (TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4) in acute childhood leukemias at diagnose. We aimed to determine the relation between the levels of soluble TRAIL and TRAIL receptors and patient's survey, clinical parameters.
Methods: The study was performed in pediatric hematology and pediatric oncology department of Çukurova University Medical Faculty between October 2009 and July 2010. Twenty-three cases with ALL at the ages of 9 months-12 year 8 months and fourtteen cases with AML at the ages of 9 days-19 years are included in this study. The age distrubition of the control group varied 3 months-17 years consisted of twenty-one healty children with similar sex and age were included into the study. In this study we investigated serum TRAIL and TRAIL receptor levels a by using ELISA method and device elisa plate reader.
Results: The comparison of the average values of the TRAIL levels in acute leukemia patients and control group have shown that patients with leukemia have low soluble TRAIL levels (P=0.002) (P<0.05). The comparison of the average values of the TRAIL levels in ALL and AML patients have shown no difference (P>0.05). In patients with HRG of ALL have shown low soluble TRAIL levels (P=0.08) (P<0.05). In patients with CALLA- B ALL have shown low soluble TRAIL levels (P=0.04) (P<0.05). Children with acute leukemias (ALL, AML) who died during treatment have shown low levels of serum TRAIL (P=0.04) (P<0.05).The patients with AML who survived have shown high levels of serum TRAIL-R1 (P=0.03) (P<0.05). In ALL group we found high levels of TRAIL-R3 (P=0.04) (P<0.05).
Summary / Conclusion: This study indicate that children with leukemias (AML, ALL) have low levels of soluble TRAIL. In children with HRG ALL and CALLA-B ALL have low levels of soluble TRAIL. These results indicate that apoptosis may not be induced in this two groups which have poor prognosis. In ALL patients the receptor TRAIL-R3 levels were high. The low levels of sTRAIL and high levels of TRAIL-R3 decoy receeptors suggest that apoptosis can not be induced and this may be related in the pathogenesis of ALL. We found high serum TRAIL-R1 levels in AML patients who survived. As a result, soluble TRAIL and TRAIL receptors might play a role in leukomegenesis. However many investigations are needed involving determined inner cell mRNA levels and cell surface levels of TRAIL and TRAIL receptors to suggest soluble TRAIL's and TRAIL receptor's role in leukomegenesis.

B1221
LOW PLATELET COUNTS AS A PROGNOSTIC MARKER IN ALL TREATMENT IN PLACE OF MRD IN COUNTRIES WITH LIMITED FINANCIAL RESOURCES
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Background: The evaluate children treated for their ALL by BFM protocols.
Aims: The aim of this study was to improve our therapeutic risk stratification in trials which no MRD analyses were done. For this purpose, we used peripheral blood counts being cheap and non-invasive.
Methods: There were 242 newly diagnosed non B-ALL patients enrolled into this retrospective study, during January 1995 to January 2012 and if they had enough data in their documentation.
Results: The median age was6,18y ±3,75years (8 month- 17 years). The 59,4'ü % of the group were boys. (boys /girls 1,46). The mean leucocyte count at admission was 37.000/mm^3 (200-285.000/mm^3). CNS involvement was positive in %7,7 at the time of the diagnosis. T-cell immunophenotype was shown in11,8% of patients. Genotypically t(12;21), t(9;22) and t(1,19) were positive in %8, %3 and %3 of patients, respectively. The overall survival of the B-ALL patients was %813,1, while that of the T-ALL patients was %62,2 12,4 (p:0.007). The EFS of the patients who had CNS involvement was %47,6 , lower than the ones who had not(p:0,0001). The OS and EFS of the whole group was 74,83,9 and 743,1 respectively, while EFS ratios for boys 72% and girls 80.5% (p:0.728).The OS and EFS were lower as the age increase (>10 years OS and EFS were 59,98,4 and 57,4 (p:0,001)) except <1 year (OS and EFS 37,5 28,6). Being a high risk patient was significantly associated with X3 relaps and X7 exitus (OS 22,7 9,1and EFS 27,6 (p:0,0001). Clinically having paleness corralated with improved prognosis (+ paleness 88 %4 , (p:0,026) . In our cohort bone involvement was a component of B cell leukemia. Having splenomegaly, hepatomegaly, t (9,22), CNS involvement (CNS leukemia increased exitus risk X4 times), higher Hb and WBC counts at diagnosis were related with poorer treatment results. (WBC >100.000 /mm^3 OS %53,7 12,8 (p:0.028); .Hb >8g/dl and T-ALL patients' OS 44,4 13,5 (p.0,01). Although the platelet counts at diagnosis had no prognostic value, the counts at 15 and 33th day of treatment significantly associated with treatment outcome. Patients whose platelet count at 15 and 33th days were <50.000/mm^3 had higher risk for exitus (respectively X3 and X7), and had lower OS p:0,008, p:0,0001). Thrombocytopenia usually goes parallel with neutropenia and monocytopenia. The blast count at 15 and 33 day were a reliable prognostic marker as if the blasts were >%5 OS 35,8 15,9 and 0 % while patients with blasts
Summary / Conclusion: Childhood ALL treatment results have reached great improvements in the MRD era. The potential benefit of simple methods such as full blood count and clinical findings, can predict us the outcome. The most striking and important finding of our study is probably the strong association of platelet counts with the prognosis. Having a platelet count less than 50.000/mm^3 at the 15 and 33th day of treatment, was associated with high mortality(p:0,0001)and this count would be easily used just for a marker in countries with limited financial resources where MRD is not avaliable.

B1222
TREATMENT EFFICACY IN PATIENTS WITH ALL UNDER 1 YEAR ENROLLED IN A CLINICAL TRIAL MLL-BABY IS STRONGLY ASSOCIATED WITH TREATING CLINIC EXPERIENCE IN INFANTS CARE
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Background: Prognosis in patients (pts.) under 1 year with acute lymphoblastic leukemia (ALL) is determined not only by the particular *MLL*-rearrangement, but by number of working together proven risk-factors. Quality of medical care provided by the experienced staff, also plays the important role in outcome of treating babies and might be taken into account with other factors influencing on the prognosis.
Aims: To estimate treatment related outcome in two groups of infants treated with ATRA containing MLL-Baby Protocol either in well-, or less experienced and/or small clinics of Russia and Belarus. To determine main cause of treatment failures in both cohorts.
Methods: MLL-Baby Protocol intended for infants' ALL treatment is widely applied chemotherapy regimen in Russia and Belarus. 20 clinics are participating in this trial nowaday. From July 2003 to November 2012–59(71%) out of 83 newly diagnosed pts. were treated in 5 well experienced oncological and hematological departments (Group 1), the rest 24(29%) pts.–in 15 small and/or less experienced clinical wards (Group 2). MLL-Baby Protocol design has been previously introduced at the EHA Meetings 2008-2012. The chemotherapy regimens and initial characteristics of pts.: age, gender, initial WBC, CNS status, proportion and type of *MLL*-rearrangements were equal in both groups, except immunophenotype – BI-46,5% in Group 1 vs. BI-75% in Group 2 (P=0.01).
Results: 4(6,7%) out of 59 and 4(16,6%) out of 24 pts. died during remission induction (P=0,16); 54(91%) and 20(83,3%) achieved CR (P=0,27); 14(25%) and 6(25%) relapsed (P=0,9) in Groups 1 and 2 respectively. Only 1(1,6%) infant from Group 1 with T-ALL and *Sil/Tal* microdel1p did not respond to the therapy. In contrast 2(3,3%) in Group 1vs. 6(25%) in Group 2 (P=0,002) died in complete remission due to the treatment related complications. Survival estimates differ significantly – EFS are 0,62±0,06 vs. 0,28±0,09 (P=0,007); DFS are 0,68±0,06 vs. 0,34±0,11 (P=0,01) and OS are 0,66±0,06 vs. 0,42±0,1 (P=0,02) in Groups 1 and 2 respectively; while RFS: 0,71±0,06 and 0,52±0,14

(P=0,37) and cumulative incidence of relapse (CIR): 0,47±0,02 and 0,28±0,04 (P=0,33) in Group 1 and Group 2 did not achieve the statistical significance. At present time 38(64,4%) from Group 1 and 8(33,3%) pts. from Group 2 are staying in CCR (P=0,009). In univariate analysis the following parameters influenced negatively on EFS: age of pts. <6 months (P=0,0002); *MLL*-status (P=0,001); Group of treatment (P=0,007) and Day8 response (P=0,01). Multivariate Cox-regression analysis confirmed the significant negative impact on EFS: age <6 months with $HR_{2,70}$ (95% $CI_{1,21-6,04}$) P=0,01 and Group (place of treatment) – $HR_{2,64}$ (95% $CI_{1,30-5,35}$) P=0,007 (Table 1)

Summary / Conclusion: Patients from Group1, treated by well experienced team have significantly better survival rates: EFS, DFS and OS, comparing to the patients from Group 2 treated in less experienced and/or small clinics, because of the different treatment related mortality rates in complete remission. Well experienced staff in infants care and clinic reliable infrastructure significantly improved patients' outcome. Place of treatment and quality of medical care should be considered as an addtional prognostic factor. Similar RFS and cumulative incidence of the relapses in both Groups demonstrate the efficacy of MLL-Baby Protocol in the relapse prevention, despite of the treating clinic experience in infants care.

	Univariate analysis				Cox regression	
	Pts	Events	EFS (SE)	p	HR (95% CI)	p
Age				0,0002		
< 6 months	40	26	32% (7)		2,70 (1,21 – 6,04)	0,01
> 6 months	43	11	72% (7)		Reference	
Group				0,007		
Group 2	24	16	28% (9)		2,64 (1,30 – 5,35)	0,007
Group 1	59	21	62% (6)		Reference	
MLL Status				0,001		
MLL (+)	58	32	42% (6)		2,92 (0,90 – 8,42)	0,07
MLL (-)	24	4	81% (8)		Reference	
Day 8 response				0,01		
≥ 1000 blasts	12	9	20% (12)		1,25 (0,74 – 3,63)	0,58
< 1000 blasts	69	27	59% (6)		Reference	

B1223

INFECTIONS DURING INDUCTION OF REMISSION IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA AND LYMPHOBLASTIC LYMPHOMA- COMPARATIVE STUDY

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Background: Acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LL) are very similar diseases and in most treatments they are treated according to same or very similar protocols. Infection is an important complication and cause of death in children receiving highly myelosuppressive chemotherapy for treatment of ALL and LL. Many children experience one or more infectious complications during treatment.

Aims: To compare the characteristics of infective episodes in children with acute lymphoblastic leukemia and lymphoblastic lymphoma in primo induction treatment.

Methods: Objective of this study were 55 patients with ALL and 19 patients with LL aged1,1 to 15 years who were treated according to BFM-ALL-95 and BFM-NHL-95 protocol between January 2000 and December 2007 at the University Children's Hospital in Skopje. We explored the characteristics of infective episodes together with the causative pathogens, the episodes of febrile neutropenia (FN), the length of antibiotic treatments and treatments with G-CSF.

Results: Altogether during primoinduction treatment 132 and 35 infective episodes were detected in the patients with ALL and LL respectively. Regarding to the pathogens, 102 (77,30%) versus 32 (91,43%) were bacterial, 20 (15,15%) versus 1 (2,86%) were viral and 10 (7,58%) versus 2 (5,71%) were fungal in the two evaluated groups of patients. There is a slight predominance of Gram-positive bacteria in both groups: 42 (51,85%) gram (+) versus 34 (41,97%) gram(-) infective episodes in ALL group and 16 (55,17%) versus 13 (44,82%) in LL group of patients. Different microorganisms were isolated with Strept. pneumoniae, Staph. aureus, Hemoph. inf. and Klebsiella spp. accounting above 80% in both groups. Febrile neutropenia was observed in 16 (29,1%) patients in ALL group and in 8 (42,1%) in the LL group. The infections were treated with antibiotic treatment in average of 23,69±12,67 and 15,39±11,93 days in the patients with ALL and LL respectively. The number of treatments with G-CSF was 397 or about 7,22±4,79 for ALL patients and 172 or 9,56±7,66 for patients with LL.

Summary / Conclusion: Evaluation of the characteristics of infective episodes between two evaluated groups of patients with ALL and LL presented a difference just in the length of antibiotic treatment (longer in ALL patients). This difference is with statistical significance (P=0,0169). No difference in the number of infective episodes, neither in causative pathogens, the episodes of FN and the treatments with G-CSF was observed.

Acute myeloid leukemia - Biology

B1224

PROLIFERATION OF ACUTE MYELOGENOUS BLASTS AS EFFECT OF DIFFERENT CYTOKINES CAN BE USED TO IDENTIFIED PATIENT AND CYTOKINE SUBSETS ALSO BY GENE EXPRESSION PROFILES

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Background: Acute myeloid leukemia (AML) is a heterogeneous group of hematopoietic disorders characterized by bone marrow accumulation of immature leukemic cells. Uncontrolled proliferation of blasts is a hallmark of AML. Several cytokines can function as growth factors for AML blasts, and abnormalities of cytokine and growth factor signalling pathways are characteristic for AML

Aims: We investigated proliferation features of two different groups of AML patients, to further use the findings for classification of patients and linked them to distinct gene expression profiles.

Methods: AML cells were cultured in standard *in vitro* condition in the absence or presence of seven given cytokines; stem cell factor (SCF), granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), FLT3-ligand (FLT3-L), interleukin 3 (IL-3) and interleukin 1β (IL-1β). The two groups contained 40 (group I cohort) and 54 (group II cohort) patients respectively. Proliferation rates were detected after seven days by ^{3}H-incorporation assay. Results were used to classify patients by clustering models. Results were compared with microarray experiments performed using the Illumina iScan Reader.

Results: All cytokines gave increased proliferation rates of AML blasts compared to blasts cultured in the absence of cytokines (P>0.001). By an unsupervised hierarchical cluster model we identified three main patient clusters based on cytokine dependent proliferation (i) high proliferation rates both in the presence and absence of cytokines, (ii) high proliferation rates in the presence of cytokines, but low proliferation without cytokines, (iii) lower proliferation rates with or without cytokines. The high proliferation clusters were characterized by high expression of CD34 and low frequency of the *NPM1* mutations combined with lack off the *FLT3* mutation. To further explore the differences between patients with high proliferation rates (i) and patients with low proliferation rates (ii and iii). Gene expression data were obtained for 32 patients belonging to the group I cohort. Of these 32 patients, 13 belonged to the high proliferation cluster (i) and 19 to low proliferation clusters (ii). ANOVA analysis was performed to identify genes to be differently expressed between the high proliferative group and the low proliferative group. Gene ontology allowed us to identified different terms/pathways which were differentially expressed between high and low proliferative AML cells. For group II cohort we examined genes differentially expressed between the high proliferative patients and the low proliferative patients only among patients harbouring the FLT3-ITD mutations. 16 patients were identified; ten in the high proliferative group and six in the low proliferative group. We identified 10 genes up-regulated in the high proliferative group and seven genes up-regulated in the low proliferative group. The genes were classified according to GO-annotations, and it is noteworthy that three genes belonged to the ubiquitin-proteasome pathway in addition to the cytokine stimulation factor EIF4E3.

Summary / Conclusion: *In vitro* proliferation varies considerably among AML blasts, and can be used to subclassify patients. Gene expression profiles can be used to identifying genes involved in AML cell proliferation.

B1225

DIFFERENTIAL EXPRESSION OF NEW METNASE (SETMAR) TRANSCRIPT VARIANTS IN HEMATOLOGICAL CANCERS- INCREASE IN AML PATIENTS

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Background: Evolutionary, the *Metnase* gene, also known as *SETMAR* and present in several transcript variants, is regarded as a unique fusion protein consisting of SET- and Transpose domains. While it functions as a human DNA repair protein it has previously also been shown to suppress experimentally induced chromosomal translocation in mice (Wray *et al*, CanGenCyt, 2010). In addition, high expression of *Metnase* leads to resistance to Topoisomerase IIα inhibitors, especially VP-16 generally used as a chemotherapeutic drug in cancers. By qPCR targeting all known transcript variants of *Metnase* we have shown that the gene is over-expressed in acute myeloid leukemia (AML).

Aims: We hypothesized that patients without a chromosomal translocation (AML^{TNeg}) had an increased level of *Metnase* mRNA expression compared to patients harboring a chromosomal translocation (AML^{TPos}). In addition, we wished to quantify the expression of the different *Metnase* transcript variants in AML^{TNeg}, in AML^{TPos}, in mantle cell lymphoma (MCL) harboring the t(11;14) translocation, and in healthy individuals.

Methods: PCR using primers located in exon 1 and exon 3 of *Metnase* targeted cDNA from peripheral blood (PB) of diagnostic AML samples. The PCR-products were cloned into a plasmid vector, in order to separate the different transcript variants, and subsequently Sanger sequenced. Specific qPCR assays spanning exon-exon junctions were designed for each of the identified variants. All samples were normalized to the cell line Granta-519, thereby defining the *Metnase* levels in Granta-519 as 1. *Beta-2-microglobulin* and *beta-glucuronidase* were used as reference genes. Samples with Cq > 40 were defined as negative.

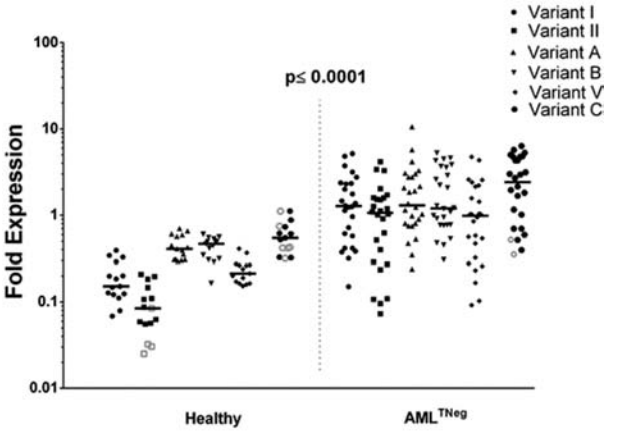

Figure 1: Expression of *Metnase* transcript variants.
Open symbols signify values with a Cq>40.

Results: Three already known (NM_006515, NM_001243723, and NR_075073, previously numbered I, II and V)) and three novel (here denominated A, B and C) transcript variants were identified. Variant I harbored the full length transcript, variant II had lacked the central part of exon2, variant A was found to have whole exon 2 spliced out, variant B harbored a deletion of the first part of exon2, variant V also had a deletion of the first part of exon 2 but was 20 base pair longer than variant B, and variant C encompassed a deletion internally in exon 2 but different from variant II. We observed a heterogeneous pattern of expression in healthy individuals and patients. Thus, while variants I, A, B and V were expressed in all selected subgroups of patients, variant II was only expressed in 11/15 healthy individuals, 26/26 AML^{TNeg}, 12/13 AML^{TPos} and 6/9 MCL patients. Finally, variant C was only expressed in 7/15 in healthy individuals, 24/26 AML^{Tneg}, 10/13 in AML^{TPos} and 2/9 MCL patients (see fig. 1 for AML^{TNeg} and AML^{TPos} comparison). All variants were significantly increased in the AML^{TNeg} group when compared to healthy individuals (P<0.0001, Figure 1), while only variant I, II, A and C were so in AML^{TPos} patients. When comparing the expression in the AML^{TNeg} and AML^{TPos} groups we found a significant higher expression in AML^{TNeg} for transcript variants A (P=0.0392) and B (P=0.0408). Interestingly, the expression pattern in MCL did not differ from that seen in healthy individuals.

Summary / Conclusion: We have identified three novel transcript variants of *Metnase* and designed specific qPCR assays targeting all six identified transcript variants of *Metnase*. All three new transcript variants can lead to non-functional proteins, if translated, and thereby non-functional as a DNA repair proteins. A significantly higher expression of *Metnase* in AML compared to healthy individuals was found. We propose that the determination of *Metnase* transcript variants expression could be of importance for the occurrence of chromosomal aberrations in AML. Further studies are needed to clarify the role of those transcript variants in hematological malignancies and to evaluate how patients with high expression of *Metnase* respond to treatment in particular Topoisomerase IIα inhibitors.

B1226

NEWLY DIAGNOSED ADULT AML AND MPAL PATIENTS FREQUENTLY SHOW CLONAL RESIDUAL HEMATOPOIESIS

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Background: The current WHO classification of myeloid neoplasms identifies three major subgroups of heterogeneous diseases, -acute myeloid leukemia (AML), myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN) - with a significant degree of overlap among them. Within AML, disease heterogeneity translates into six major subgroups each of which still contains several specific diagnostic entities, being of utmost clinical relevance because of their distinct prognosis. Among cases classified as *de novo* AML, a significant percentage of patients show AML with myelodysplasia related changes, representing a unique poor-prognosis WHO category of the disease, independently of the lineage and cytogenetic alterations of myeloid blast cells. Overall, these criteria confirm the clinical relevance of the presence of MDS-associated features in AML, for unequivocal differential diagnosis among *de novo* AML, secondary AML and AML with myelodysplastia related changes, that reflects the presence of an underlying clonal disorder of residual hematopoiesis, fact that at present, neither cytomorphology nor cytogenetics alone is sensitive enough to assess in every *de novo* AML patient.

Aims: To investigated the existence of an underlying clonal disorder of residual mature/maturing neutrophils, monocytes and NRC in the BM of *de novo* AML patients.To investigate the potential association between the molecular findings and both dysplastic features by cytomorphology and altered immunophenotypes by multiparameter flow cytometry.

Methods: iFISH studies, immunophenotypic studies, HUMARA assay (in female cases) and presence of *KIT* mutation was performed on FACS-purified cell populations from *newly-diagnosed* AML and MPAL patients

Results: The clonal nature of blast cells was demonstrated in all (59/59; 100%) *newly-diagnosed* AML and MPAL patients analyzed*,* meanwhile clonality of residual BM compartments of mature/maturing neutrophils and monocytic cells and/or NRBC was confirmed for at least one of these three cell compartments in most *newly-diagnosed* AML and MPAL cases (49/59; 83%).

No statistically significant differences were observed as regards the distribution of cases with clonal vs. non-clonal residual hematopoiesis among the different diagnostic subgroups of *newly-diagnosed* AML.

Residual BM mature/maturing granulomonocytic and NRBC showed aberrant phenotypes -similar to those recurrently described for MDS- in 52/58 cases (90%). Accordingly, immunophenotypically altered neutrophils, monocytes and NRBC were found in 51/58 (88%), 38/52 (73%) and 27/45 (60%) patients respectively.

Cases with "clonal residual hematopoiesis showed a significantly higher frequency of aberrant phenotypes among maturing neutrophils (98% vs. 50%; P<0.001), monocytic cells (83% vs. 30%; P=0.003) and NRBC (71% vs 13%; P=0.01) and a higher mean number of phenotypic alterations/case (6.9±3.0 vs. 3.7±4.2, P<0.001) as well as greater number of altered cell populations/case (2.4±0.8 vs. 1±1.2, P<0.03), than the other cases.

Summary / Conclusion: The vast majority of adults with *newly-diagnosed* AML and MPAL displays an underlying clonal hematopoiesis, residual mature/maturing granulomonocytic and/or erythroid cells displaying chromosomal alterations, which are frequently shared by the blast cells, in addition to multiple aberrant phenotypes that appears to involve most WHO 2008 diagnostic subtypes of AML and also MPAL. Whether the presence versus absence of clonal residual hematopoiesis contributes to a better prognostic stratification of *newly-diagnosed* adult AML and MPAL patients deserves further investigations.

B1227

MINIMAL RESIDUAL DISEASE AND CLEARANCE OF LEUKEMIC BLASTS IN ACUTE MYELOID LEUKEMIA: TIMING AND CUT-OFF VALUES BETWEEN MFC AND WT1

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Background: The detection of leukemia –associated immunophenotypes (LAIPs) by multiparameter flow cytometry (MFC) and the quantification of WT1-RNA levels were widely used to monitor minimal residual disease (MRD) in acute myeloid leukemia (AML). Anyway an agreement between published studies on cut-off values and timing was missing. The clearance of blasts may represent an important solution to MRD cut-offs.

Aims: AML patients in complete remission (CR) were investigated in order to establish the most predictive time point and cut off value to monitor MRD by MFC and WT1. Timing, cut-off value and impact of clearance on the outcome of patients were further evaluated.

Methods: Fresh bone marrow samples from 88 AML patients were obtained at diagnosis between January 2010 and November 2012. CR was achieved from 45 patients evaluated by MFC and WT1 to study MRD and clearance at different time points: after induction therapy (T1), after consolidation therapy (T2) before transplant (T3) and after –transplant (T4). The immunophenotypic analysis was performed using a six-color combinations in order to correctly identify LAIPs. RQ- PCR to test WT1 expression was made according to the standardized and quality-controlled method . The obtained WT1 copy numbers were normalized with respect to the ABL transcripts. Clearance of blasts was expressed as the ratio, converted to logarithmic scale, between LAIP-positive blasts and WT1 copy number at diagnosis and each time point . ROC

curves were studied to determine area under curve and optimal cut-off values at each time. A multivariate Cox regression was used to survival at different times.
Results: The assessment of MRD by MFC and WT1 at T4 predicted the recurrence better than the other time points. The more reliable cut-off values resulted 0.1% for MFC at T1, T2 and T3 and 0,055 % at T4. The cut-off values by WT1 were 90.0 at T1 and T3, 71.0 at T2 and 54.0 at T4. When the clearance was considered, the best prediction of relapse was evidenced at T4 with cut off values of 3.07 and 1.65 for MFC and WT1, respectively. Patients with values of MFC above 0.10% at T1 (n= 27 pts, 60%) had a significantly poorer expected disease free survival compared to those with lower levels (n= 18 pts, 40%; P<0.01; crude HR: 5.0; 95%CI: 1.4-17.5). This difference was preserved after adjusting for age, gender, Hb levels and stem cell transplant (P<0.01; adjusted HR: 7.7; 95%CI: 1.8-29.6). Results from MFC clearance showed that patients with log clearance at T1 equal to or below 2.81(28 pts, 62%) had a significantly poorer expected disease free survival compared to those with higher levels (17 pts, 38%; P<0.01; crude HR: 9.7; 95%CI: 2.2-43.2). This difference was preserved after adjusting for age, gender, Hb levels and stem cell transplant (P<0.01; adjusted HR: 22.6; 95%CI: 3.7-136.3). Patients undergone to stem cell transplantation showed a better disease free survival at the multivariate analysis (P=0.02; adjusted HR: 0.6; 95%CI: 0.1-0.7). At T1 no statistically significant correlations were observed between WT1 values , WT1 Log clearance values and DFS. No significant correlations between MRD and DFS were obtained at T2.
Summary / Conclusion: From our study, the most predictive evaluation of MRD was performed after the transplant while the post-induction evaluation stratified better high risk patients among pre-transplant times. Cut-off values needed to be lower after the transplant compared to previous times and the clearance of LAIP positive blasts makes an excellent prediction of the outcome.

B1228
FLOW CYTOMETRIC ASSESSMENT OF HUMAN EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1 (HENT1) EXPRESSION IN ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROMES
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Background: Transporters like human Equilibrative Nucleoside Transporter 1 (hENT1) transport nucleosides into cells. Nucleoside analogs depend on cellular hENT1 expression for entry into cells. Data on correlation of hENT1 levels with response to such chemotherapies in AML and MDS is scarce.
Aims: To examine hENT1 expression by multiparameter flow cytometry (MFC) in AML and MDS. To correlate results to morphology, cytogenetics (CG) and molecular genetics (MG).
Methods: We studied 130 AML and 94 MDS at diagnosis, 125/99 male/female, median age 66.5 (AML) and 73.3 years (MDS). CG was done in 115 AML and 84 MDS. hENT1 expression was quantified by a novel 4-color intracellular staining assay with monoclonal antibodies against hENT1, CD45, CD64 and myeloperoxidase. Median fluorescence intensities (MFI) of hENT1 were determined in myeloid progenitors (MP), granulocytes (Gra) and monocytic cells (Mo) and correlated to hENT1 MFI in lymphocytes to derive hENT1 index.
Results: Pt included 99 de novo AML, 4 t-AML, 27 s-AML; 7 FAB M0, 26 FAB M1, 43 FAB M2, 7 FAB M3, 19 FAB M4, 7 FAB M4eo, 7 FAB M5, 14 not classified; by CG (MRC): 18 favorable, 69 intermediate, 28 adverse [Grimwade, Blood 2010]. 89 were de novo MDS, 5 t-MDS; 1 RARS, 17 RCMD-RS, 37 RCMD, 3 5q- syndrome, 2 RAEB-1, 4 RAEB-2, 1 CMML, 24 not classified; by CG: 2 IPSS-R very low, 54 IPSS-R low, 7 IPSS-R intermediate, 8 IPSS-R high, 13 IPSS-R very high [Greenberg, Blood 2012]. Both in AML and MDS no correlation of index to age, gender, hemoglobin level or counts for blasts, WBC or platelets was seen. In MDS index in Mo and MP was lower than in AML (2.68 vs 3.01, P=0.008, 1.84 vs 2.65, P<0.001, respectively). AML FAB M3/t(15;17)/PML-RARA displayed higher index in MP than non-M3 AML (4.52 vs 2.55, P<0.001). FAB M2 vs M0 and M1 showed higher index in Gra (5.66 vs 3.2, P=0.035 and vs 4.46, P=0.033, respectively), in particular higher index in Mo and Gra in *RUNX1-RUNX1T1*$^+$ pt (4/69 pt, 1 M1, 3 M2; 4.32 vs 3.14, P=0.023; 8.16 vs 4.73, P=0.002, respectively) and lower index in Mo in *CBFB-MYH11*$^+$ AML (8/70 pt; 2.82 vs 3.25, P=0.007) was seen. A trend to higher index in MP in MRC favorable vs intermediate was detected (3.01 vs 2.60, P=0.076). In *FLT3*-ITD$^+$ AML index was higher in Mo and MP (18/98 pt; mean 3.64 vs 2.97, P=0.02; 3.21 vs 2.62, P=0.013, respectively), in *NPM1*mut AML higher in Mo (21/93 pt; 3.88 vs 2.88, P=0.015). MP showed lower index in *RUNX1*mut pt (10/57 pt; 2.03 vs 2.6, P<0.001). In MDS by IPSS-R, significance was reached for higher index in MP in very low risk pt compared to low, intermediate and high risk pt (4.07 vs: 1.77, P<0.001; 1.94, P=0.008; 1.76, P=0.002, respectively), and in MP and Gra in very low vs very high risk pt (4.07 vs 1.71, P=0.005; 5.86 vs 3.85, P=0.001, respectively). IPSS-R intermediate vs poor also showed higher index in Gra (5.56 vs 3.59, P=0.02). Lower index was found in Gra in both *ASXL1*mut (6/17 pt; 3.05 vs 5.72, P=0.048) and *RUNX1*mut pt (8/31 pt; 4.24 vs 5.86, P=0.056).
Summary / Conclusion: Assessment of hENT1 by MFC is feasible. In AML with good risk MG, higher hENT1 expression in MP, Gra and Mo was observed, suggesting a causal mechanism for better response to therapy and outcome. Coherently AML with poor risk MG displayed lower hENT1 in MP. Detection of higher hENT1 in *FLT3*-ITD$^+$ AML fits into the observation of their good responses to induction therapy while overall worse outcome is mostly due to early relapses. Higher index in AML than MDS may be considered causal for better response to nucleoside-based chemotherapies in AML.Data within MDS may be interpreted accordingly, low risk CG (IPSS-R1) showing higher index in MP and pt with poor risk MG presenting lower index in Gra, though the latter results have to be judged cautiously due to low pt numbers. Further analyses are warranted to explore hENT1 expression in AML and MDS more comprehensively and to correlate with outcome.

B1229
CELL CYCLE MODELING OFFERS A PLATFORM TO OPTIMIZE COMBINATION OF CYTARABINE AND FLT3 INHIBITORS IN ACUTE MYELOID LEUKEMIA
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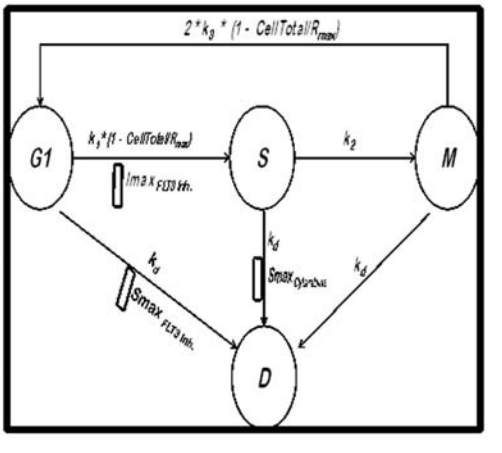
Background: Acute myeloid leukemia (AML) patients with *FLT3* mutations have poor response to cytarabine-based therapy. FLT3 inhibitors may overcome cytarabine resistance and improve treatment outcome. Depending on the sequence of administration, combining FLT3 inhibitors and cytarabine can achieve synergistic or antagonistic effects.
Aims: The goal of our work was to develop a pharmacodynamic model that mechanistically describes the effects of cytarabine and FLT3 inhibitors on cell proliferation, cell cycle distribution, and optimize combination regimens.
Methods: Three AML cell lines were exposed to varying concentrations of cytarabine and a panel of FLT3 inhibitors (AC220, PKC-412, sorafenib, and JP11646) over 96 hours. The cell lines were: HEL (negligible expression of wild-type FLT3), EOL1 (wild-type FLT3 which depends on FLT ligand for activation), MV4-11 (FLT3 with internal tandem duplication resulting in constitutively active kinase). Cell proliferation kinetics and cell cycle analysis were assessed using trypan blue and propidium iodide staining. Model fitting was performed using Pharsight Modeling Language in Phoenix. Plasma concentration profiles for cytarabine and AC220 in humans were digitized [1, 2] using computer digitalizing program (Digitizer Version 1.9). One and two compartment models were fit to cytarabine and AC220, respectively. Estimated cell cycle, drug sensitivity, and pharmacokinetic parameters were used to simulate different combination regimens to predict synergism.



Results: Experimental data and model selection criteria showed that cytarabine induced apoptosis in S-phase. FLT3 inhibitors induced apoptosis and cell cycle arrest at G1 phase (Figure 1). MV411 was most resistant to cytarabine, followed by EOL-1, with HEL cells being most sensitive reflecting the role of FLT3 status in conferring resistance to cytarabine. AC220 showed highest sensitivity among tested FLT3 inhibitors (KC50 = 0.4 nM in MV411 cells). HEL cells lacking FLT3, were resistant to all FLT3 inhibitors ($KC50\text{-}_{HEL}$ >40 fold higher than $KC50\text{-}_{MV411}$). Simulations of candidate clinical regimens predict better cell kill upon adding FLT3 inhibitors simultaneously with or immediately after cytarabine exposure. In vitro combination experiments to validate the effects of administration sequence on cell kill are ongoing.
Summary / Conclusion: Patients with FLT3 perturbations are likely to benefit from combining FLT3 inhibitors to cytarabine. Simultaneous administration of cytarabine and FLT3 inhibitors is predicted to achieve highest cell kill. Our model presents a mechanistic interpretation for cytarabine and FLT3 inhibitors effects in AML cell lines and provides a useful tool to optimize combination regimens.

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B1230
EXPRESSION OF PIM-2 GENE, BAD AND 4E-BP1 PROTEIN IS INCREASED IN PATIENTS WITH AML AND ALL AND CORRELATES WITH COMPLETE REMISSION RATE, OVERALL SURVIVAL AND APOPTOSIS RATE.
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Background: Background. *PIM-2* is a proto-oncogene that encodes for serine/threonine kinase which interacts with various signalling molecules. PIM-2 kinase suppresses apoptosis and promotes cell survival. These events are a consequence of phosphorylation of pro-apoptotic factors: 4E-BP1 translation inhibitor and BAD protein belonging to BCL-2 family. *PIM-2* is highly expressed in neoplastic tissues and in leukaemic and lymphoma cell lines which is consistent with a its role during oncogenic transformation. In particular, *PIM-2* plays an important role in bone marrow cell growth, differentiation and survival.
Aims: The aim of this study was to investigate whether the *PIM-2* (both mRNA and protein level), BAD, 4E-BP1, p-BAD p-4E-BP1 expression is altered in acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL). Patients and methods. One hundred twenty-six patients were included: 74 with AML and 52 with ALL aged 18-85 (median=48,1). Seventy-one patients reached complete remission (CR): 39 with AML and 32 with ALL. Bone marrow samples were collected at the time of diagnosis and in CR phase. Control samples were obtained from 24 healthy donors.
Methods: We analysed *PIM-2* expression by RQ-PCR analysis and proteins (PIM-2, BAD, 4E-BP1, p-BAD, p-4E-BP1) by Western blot. Moreover siRNA targeting human *PIM-2* in leukemic cell line HL-60 was used to examined influence on apoptosis rate.
Results: Median expression of *PIM-2,* both mRNA and protein level, BAD and 4E-BP1 in all leukaemic patients and in subgroups: AML and ALL was significantly higher than in controls. In AML group patients who reached CR expressed *PIM-2* at significantly lower levels than patients with primary resistance to chemotherapy (with no CR, NCR). Moreover in AML, we have found significant difference between survival in patients with *PIM-2* expression above and below median value. We revealed negative correlation between apoptosis rate of blastic cells and *PIM-2* expression in both AML and ALL patients. In leukemic cell line HL-60, the siRNA-mediated decrease of *PIM-2* expression induced the increase of cell apoptosis rate.
Summary / Conclusion: Our data indicate that *PIM-2*, BAD, 4E-BP1 expression is increased in patients with AML and ALL. In AML patients *PIM-2* expression correlates with CR, OS and apoptosis rate.

B1231
FLUVASTATIN CAN INCREASE APOPTOSIS INDUCED BY VALPROIC ACID AND ALL-TRANS-RETINOIC ACID IN FLT3-ITD-POSITIVE AML CELLS
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Background: FLT3 internal tandem duplications (ITDs) represent the most frequent molecular aberration in acute myeloid leukemia (AML) associated with an impaired prognosis. The pattern of downstream activation by this constitutively activated receptor tyrosine kinase is influenced by the localization of mutated FLT3 depending on its glycosylation status. The important process of post-translational maturation can be inhibited by fluvastatin. Furthermore, overcoming of resistance to tyrosine kinase inhibitors (TKIs) reflects a current challenge in the treatment of FLT3-ITD-positive AML.
Aims: The objective of this study was to investigate whether fluvastatin has an impact on the induction of apoptosis or on differentiation of FLT3-ITD-positive AML cells in the presence of the histone deacetylase inhibitor valproic acid (VPA) or the differentiation-inducing compound all-*trans*-retinoic acid (ATRA).
Methods: The murine Ba/F3 leukemia cell line was stably transfected with a FLT3-ITD variant resulting in IL-3-independent growth. Signal transduction after exposing cells to fluvastatin, VPA, and/or ATRA was analysed by Western blotting. Apoptosis, cell cycle analyses, and differentiation were detected by flow cytometry.
Results: In FLT3-ITD expressing Ba/F3 cells, VPA or ATRA or the combination of both compounds were not able to induce apoptosis while fluvastatin alone resulted in a slight increase of apoptotic cells compared to the DMSO control. Co-treatment with fluvastatin, VPA, and ATRA, however, demonstrates additive effects and is associated with a significant increase of apoptosis in this cell model. Interestingly, acetylation of histone H3 is much more pronounced in the presence of fluvastatin as compared with VPA alone. Besides, phosphorylation of the anti-apoptotic protein AKT (Ser473) is strongly decreased in the triple combination while VPA plus ATRA results in the highest phosphorylation level of AKT. Surprisingly, these observations do not correlate with the phosphorylation status of the p70S6 kinase.
Summary / Conclusion: Co-treatment with fluvastatin can increase the susceptibility to VPA and ATRA. We suggest that compartmentalization of FLT3-ITD by statins might improve the effect of such a therapeutic approach and could represent a promising strategy to overcome TKI resistance in FLT3-ITD-positive AML.

B1232
OVEREXPRESSION OF WILMS TUMOR GENE 1 (WT1) IN ACUTE PROMYELOCYTIC LEUKEMIA.
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Background: *WT1* gene is known to be highly expressed in the majority of acute myeloid leukemia (AML), but remains unclear its role in the development of myeloid and leukemic cells. *WT1* gene is note to be expressed in highly proliferative cells (1) and recently, an anti-apoptotic effect of *WT1* has been demonstrated in acute promyelocytic leukemia (APL)-cell lines (2). However there are only few data regarding expression levels of *WT1* in APL.
Aims: Between January 2007 and June 2011 we evaluated the *WT1* expression in 169 AML patients at diagnosis. To assess the pattern of expression in different subtypes of AMLs, we performed a quantitative polymerase chain reaction (RQ-PCR) assay on bone marrow (BM) samples using the ELN ProfileQuant Kit (Ipsogen, Marseille, France) following the European Leukaemia Net protocol. As normal expression cut-off was established 250 *WT1* copies/10^4 copies Abelson (*ABL).* According to WHO classification of myeloid neoplasm we observed: 49 (29%) AMLs with myelodysplasia–related changes, 27 (16%) AMLs with minimal differentiation, 15 (9%) AMLs with maturation, 22 (13%) Acute Myelomonocytic leukemia, 35 (21%) Acute Monocytic leukemia and 21 (12%) APL. All the APL were PML-RARalpha and t(15;17)(q22;q12) positives.
Results: There were only 9 *WT1* normal expressing patients (5%), whereas 160 (95%) AML cases presented levels of *WT1* expression higher than the established cut-off, with a median value of 6776 copies *WT1*/10^4 copies ABL (range 235-62567). Inside each FAB subtypes, the median level of *WT1* in the overexpressing group at diagnosis was: 8669 *WT1* copies/10^4 copies ABL (range 1368-30529) in M0-M1 group, 4995 copies (1008-25585) in M2 group, 30110 copies (2069-62597) in M3 group, 8111 copies (235-41148) in M4 group, 6226 copies (416-15661) in M5 group, 4530 copies (353-24205) in secondary AML (Msec) group. The level of *WT1* expression in APL was significantly higher ($P<0.01$) than in all other subtypes (figure 1). All APL patients presented an overexpression of *WT1*, differently from M4, M5 and secondary AML groups. Moreover, the majority of APL patients (81%, 17/21 pts) had more than 20000 *WT1* copies at onset. Conversely, only 9 out of 139 (6%) Non APL cases demonstrated a number of *WT1* copies higher than 20000.
Summary / Conclusion: In our cohort of AML patients, Acute Promyelocytic Leukemia group showed highest levels of *WT1* expression. In particular, we observed that all APL patients overexpressed *WT1* gene at diagnosis and the transcript levels were significantly higher in APL cases than in other FAB subtypes. It is still unclear the role of *WT1* gene expression to promote or sustain proliferation of leukemic cells, but this observation could reinforce a possible relationship between the pathway stimulated by *PML-RARα* transcript and the *WT1* anti-apoptotic and pro-proliferative mechanisms(1;2).

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B1233
ALU-REPEAT REGIONS ARE NOT HYPER- AND DEMETHYLATION TARGET POINTS .
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Background: Alu repeats contain nucleotide AGCT, a cleavage site for the Alu I restriction endonuclease. A high concentration of Alu elements in the chromosome regions which contain a lot of genes allows duplication or elimination of genome fragments located between two Alu copies, as well as chromosome rearrangements. Alu repeats can affect the composition, organization and expression of the genome. Owing to their own promoter or enhancer activity, Alu repeats may enhance transcription of the adjacent locus. Transcriptional suppression is also possible, as Alu elements may expedite nucleosome assembly on the adjacent region. In addition, Alu repeats seemed expedite methylation of neighboring loci, contributing to gene expression regulation. While methylation commonly suppresses transcription, cases are known when methylation of Alu repeats increases the transcriptional activity of the neighbor-

ing locus. Site-specific DNA methylation analysis of short-term granulocyte cultures of healthy volunteers and on the leukemic cells' DNA was performed.
Aims: Our aim was to investigate whether ALU-repeats are the targets of demethylation and hypermethylation process in acute myeloid leukemia.
Methods: The investigation was performed on the DNA of short-term ganulocyte cultures of healthy volunteers and on the DNA of leukemic cells of patients with acute myeloid leukemia (AML). Totally 22 DNA samples (12 AML and 10 of normal blood donors) obtained from mononuclear cells of peripheral blood or bone marrow were involved in the study. We used the DNA samples of untreated patients. Both peripheral blood and bone marrow samples were investigated. The methyl- sensitive (HpaII) and methyl-insensitive (MspI) restriction endonucleases were used for the digestion of the DNA-samples as well, as bisulfite sequencing, restriction analysis and gene trasfer methods were applied in the study. For the *in vivo* data confirmation the genetic construction based on pEGFP vector, containing eucariotic chromomethylase CMT3 gene, was created. It was designed under cytomegalovirus promoter. The abovementioned genetic construction was used to transform HEK293 kidney epithelium cells. After the selection analysis the stable cell lines expressing CMT3 gene were obtained.
Results: We have shown the morpho-physiological and biochemical difference of these CMT3-gene expressing cell lines. DNA restriction analysis showed hydrolysis protection from the methyl-sensitive CpHpG-endonucleases. This fact confirms transgen functional activity.
Summary / Conclusion: It was shown that the ALU-repeats are not demethylation and hypermethylation targets in acute myeloid leukemia.

B1234
CLINICAL AND BIOLOGICAL CHARACTERISTICS OF ACUTE MYELOID LEUKEMIA WITH ABERRANT EXPRESSION OF CD56+CD11B+
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Background: Immunophenotyping is a widely used diagnostic tool which, together with cytology and cytogenetics, allows for a more accurate diagnosis of acute myeloid leukemia (AML). The literature describes a group of AML with a peculiar clinical and biological characteristics, such as monocytic/monoblastic AML, whose blasts coexpressed CD56 and CD11b (CD56+CD11b+). This subtype of leukemia has been associated most often with aberrant karyotypes (mainly 11q23/MLL gene rearrangement), as well as extramedullary disease and refractoriness to treatment and, ultimately, with a worse prognosis.
Aims: In our study we analyzed all cases of AML diagnosed at our institution in the years 2002-2012, and in particular those whose blasts coexpressed CD56+CD11b+ and its possible association with extramedullary involvement, abnormal karyotype and response to treatment.
Methods: We analyzed a total of 133 cases divided into three different groups according to WHO classification: Group 1 (n = 60), not otherwise specified AML; Group 2 (n = 44), AML with myelodysplasia-related changes; and Group 3 (n = 29), AML with recurrent genetic abnormalities. Among others, analyzed variables included age, sex, cytogenetics (karyotype and FISH), immunophenotyping, especially the coexpression CD56+CD11b+; blood count at diagnosis and LDH, number of blasts in the blood and bone marrow, extramedullary involvement at diagnosis (skin, liver, spleen, lymph nodes and other locations), treatment features (complete remission after induction, number of treatments to achieve complete remission), and data on survival (disease-free survival, overall survival and the mortality rate). Statistical analysis was performed using SPSS (Chicago, IL) and significance level (p value) was established at 0.05.
Results: Ten of the 133 studied cases (7.5%) had this phenotype. Extramedullary involvement at diagnosis was detected in 50% of patients with CD56+CD11b+ coexpression, while only in 19.4% of patients who had not (P=0.032). According to groups, extramedullary involvement incidence showed no significant differences between groups 1 and2, while in group 3 the combination CD56+CD11b+ was not observed. A statistical association between this phenotype and karyotypic alterations or FISH, was not found. MLL gene rearrangement only appeared in one of 96 AML available cases, and the blasts of this unique patient were not CD56+CD11b+. Concerning overall survival, we did not find differences in these patients when compared to double CD11b and CD56 negative (P=0.105). Cases of AML CD56+/CD11b+ more frequently corresponded to those with monocytic component, both monoblastic/monocytic and myelomonocytic, clearly different to the remaining AML subtypes (p <0.001). The frequency of this phenotype was similar in monocytic/monoblastic (62.5%) and myelomonocytic (66.7%) AML (P=0.898). Extramedullary involvement was also more common in those AML with monocytic component (52.4%) than in non-monocytic AML (18.5%) (P=0.001).
Summary / Conclusion: In our experience, AML with the CD56+CD11b+ coexpression present more frequently a monocytic component (monoblastic/monocytic and myelomonocytic subtypes of AML), and also show a clear tendency to present with extramedullary involvement. By contrast, in our study it was not possible to establish an association of this phenotype with 11q23/MLL gene disorders (which has been reported by other authors), and CD56+CD11b+ coexpression did not seem to correlate with overall survival in AML patients.

B1235
REDUCED EXPRESSION OF 90 KD HEAT SHOCK PROTEIN (HSP90) IS RELATED TO PU.1 AND PML-RARA COMPLEX IN ACUTE PROMYELOCYTIC LEUKEMIA
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Background: The 90 kD Heat Shock Protein (*HSP90*) is a dimeric molecular chaperone required for conformational maturation and stabilization of numerous client proteins involved in cell cycling, receptor function, signal transduction, and apoptosis. A high expression of *HSP90* has been reported in leukemic cell lines and in patients with acute myeloid leukemia (AML) where it has been associated with poor prognosis. In acute promyelocytic leukemia (APL), the disease-specific *PML-RARA* oncoprotein deregulates the expression of various genes involved in differentiation and apoptosis. Selective targeting of *PU.1*-regulated genes by *PML/RARA* is a critical mechanism for the pathogenesis in APL. To date, no studies have investigated the potential role of *HSP90* in APL pathogenesis.
Aims: Objective of this study was to comprehensively analyze the involvement of *HSP90* in pathogenesis of APL.
Methods: *HSP90* expression levels were analyzed using Western Blot and Real Time PCR in APL patient blasts and in NB4 cells both at baseline and after treatment with ATRA and ATO, as well as in the zinc inducible, *PML/RARA* transfected PR9 cells. ChIP was used to analyse the regulatory sequence of *HSP90* alfa and beta for the presence of Pu.1 and *RARE* half (*RAREh*) sites.
Results: A significantly lower *HSP90* expression was detected in blasts from APL patients, compared to those from non-APL leukemias at both transcriptional as well as translational levels. Furthermore, in vitro treatment of NB4 cells and APL primary blasts with ATRA and ATO restored the expression of *HSP90.* Moreover, treatment of PR9 cells with zinc reduced the expression of *HSP90*. Using ChIP, we found in-vivo binding of *Pu.1* and *PML-RARA* to the *HSP90* alfa and *HSP90* beta promoter regions. *PU.1* motifs coexisted with one or more *RAREh* sites in *HSP90* promoter regions.
Summary / Conclusion: The DNA binding domain, conserved in the *PML-RARA* fusion protein*,* displaces from the gene promoter sequence a *PU.1* containing complex necessary for the activation of *HSP90* transcription, thus suppressing the expression of the protein. We demonstrate here a new peculiar feature of APL which differentiates this subset from other tumors and leukemias, usually showing increased HSP90 level. Further studies are needed to define the significance of this finding.

B1236
ACUTE LEUKAEMIA WITH MULTILINEAL DYSPLASIA - CLINICAL AND IMMUNOPHENOTYPICAL FEATURES - 5 YEARS EXPERIENCE OF A SINGLE CENTER
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Background: Non-lymphoblastic acute leukemia with multilineal dysplasia is a particular subset of acute leukemia, with a complex diagnostic and therapeutically approach.
Aims: Our study proposes to identify specific features in acute myeloid leukemia with dysplasia.
Methods: We analyzed 96 patients previously diagnosed with acute myeloid leukemia with myelodysplasia over a period of five years, compared to a control group of patients with acute myeloid leukemia without dysplastic features. Demographic characteristics (age, gender), hematological parameters, morphology and immunophenotype were assessed. Further analysis of dysplasia de novo and post myelodysplastic syndrome (MDS) was done.
Results: Dysplasia was observed more frequent in myelo-monocytic acute leukemia (53.5%), but without a statistical significance versus other FAB types. Age under 40 years was a protective factor (P=0.0005) for the development of AML with dysplasia, while age over 70 years seemed to be a risk factor, but less strong (P=0.35). Neither thrombocytopenia, nor leucopenia correlated significantly with the presence of dysplasia. In the control group, thrombocytopenia correlated significantly with low hemoglobin values at diagnosis (P=0.012), but not in the group of patients with dysplasia. This would suggest that thrombocytopenia and anemia were influenced simply by the degree of marrow infiltration in patients with leukemia, and not by the degree of dysplasia. The marrow blast number (controlled by CD34 expression) at diagnosis was lower in the group of patients with dysplasia versus control group (40.15% vs. 61.38%) (P=0.0001). This could be considered more as a hematopoiesis alteration in such patients with dysplasia. Regarding the correlations between peripheral and marrow parameters, we observed that, in patients with dysplasia, anemia (stratified by slight, medium and severe) was associated with a lower marrow blast count versus control group. This correlation enlightens that impaired erythropoiesis in patients with AML with dysplasia is more likely generated by erythrocyte dysplasia, rather than bone marrow infiltration. In the control group, throm-

bocytopenia was statistically significant associated (P=0.012) with a higher percentage of blasts. Concerning immunophenotype, we observed that expression of CD11b, known as a diferrentiation marker of myeloid precursors, was mostly associated with dysplastic AML, while expression of CD36, CD64, CD65w, was lower in patients with dysplasia, although without a strong statistical value (P>0.05). Antigens CD56, CD7, CD14 were not differently expressed in the two groups. Common antigens such as CD33, CD13, CD117, HLA-DR, CD15, have not been significantly different expressed in both patients groups. Kaplan-Meyer curves were used to compare survival in AML patients with or without dysplasia. 6 months survival was not different in the 2 groups. However, when AML developed after myelodysplastic syndrome, relative risk for death before 6 months was higher (odds ratio 2), valid only for 70% of the population studied, without statistical significance (P=0.3).
Summary / Conclusion: Dysplasia alters the clinical and immunophenotypic profile of AML, especially as an expression of hematopoiesis alteration and the prognosis is particularly worsened when AML develops after MDS.

B1237
ARE MUTATIONS IN THE IDH2 GENE SUITABLE MOLECULAR MARKERS FOR MRD MONITORING IN AML PATIENTS?
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Background: Heterozygous missense mutations in isocitrate dehydrogenase 2 gene (IDH2) have been recently reported in pacients with acute myeloid leukemia (AML), notably in cytogenetically normal AML (incidence 12,1 %). These mutations affect mainly arginine codons p.R140 and/or p.R172, both localized in exon 4. The most frequently is c.G419A (p.R140Q). Potential of detected IDH2 gene mutations for monitoring of minimal residual disease (MRD) in AML patiens is unclear and is now under investigation.
Aims: i) to verify the use of most frequently detected mutation c.G419A (p.R140Q) for MRD monitoring in AML patients, and ii) to compare these results with those obtained from routine monitoring of NPM1 mutations in AML patients, who harbor IDH2 and NPM1 mutations simultaneously.
Methods: Method of DNA-based RQ-PCR with a specific set of primers and Locked Nucleid Acid (LNA) probe were used for quantification of IDH2 c.G419A (p.R140Q) mutation in a total of 73 samples (45 bone marrow and 28 peripheral blood samples) of 9 AML patients. These samples were obtained during AML treatment at the different time points. All patients signed an informed consent before the samples were collected. Six of nine (6/9) analyzed patients harbored parallel mutation in NPM1 gene. Quantification of NPM1 mutation was performed according to previously published work. The results were reported as the normalized copy numbers (NCN) defined as the number of mutated IDH2 or NPM1 gene copies for every 10^6 cell equivalents (CE).
Results: In our cohort, 7/9 AML patients revealed concordant results for the NCN of the IDH2 c.G419A mutation and disease status. Moreover, in 5/6 analysed patients, the kinetics of IDH2 mutations was nearly identical to the kinetics of NPM1 mutations. However, in the remaining two patients, IDH2 mutation status did not correspond to clinical course of AML. Both patients revealed persistent IDH2 c.G419A positivity (10^5 – 10^6 NCN) althougt they were in hematological remission. Moreover, one of them was also in molecular remission according to NPM1. In these discrepant cases (2/9) further analyses were performed and germinal origin of IDH2 mutation were excluded.
Summary / Conclusion: Our data indicate that, in the majority of AML patients, status of IDH2 mutation correlate with the clinical course of disease. Thus mutation c.G419A in IDH2 gene seems to be suitable marker for MRD monitoring in AML patients. However, we have shown that discrepant cases exist. Therefore further studies will be necessary to identify cases where the IDH2 mutation burden does not correlate with clinical course of AML.

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B1238
ASSESSMENT OF WT1 TRANSCRIPT REDUCTION FOR PROGNOSTIC IMPLICATION IN ACUTE MYELOID LEUKEMIA PATIENTS
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Background: Wilms' tumor 1 (WT1) gene is over-expressed in most cases of acute myeloid leukemia (AML). It is one of proposed markers for minimal residual disease (MRD) monitoring.
Aims: Our study aims to estimate the utility of WT1 transcript reduction for prognostic implications in acute myeloid leukemia, including acute promyelocytic leukemia (APL).
Methods: Real time quantitative RT-PCR was made according to the ELN guidelines. WT1 transcript level was determined in BM samples of adult patients with AML and APL. We examined the samples of 122 patients with AML and 25 patients with APL at diagnosis. We also analyzed 384 specimens of AML and APL patients during follow-up. The obtained WT1 values were normalized with respect to the βGUS transcripts.
Results: The median value of WT1 levels at diagnosis was 755 (range 2.4-16283) in AML patients and 3528 (range 547-8103) in APL patients. The median value of WT1 levels of patients in complete remission was 8.5 (range 0.4-33) for AML and 11.3 (range 3.5-61) for APL . At diagnosis 89% of AML patients showed the expression of WT1 one log higher the median values in complete remission. WT1 transcript reduction after induction above 2 log showed 69% of patients in favorable subgroup with APL + AML with AML1/ETO, CBFb/MYH11, and only 25% of patients in subgroup of poor prognosis - AML with BCR/ABL, DEC/CAN, dupMLL; P=.042 (Fisher Exact test). In full group (all AML patients + APL) we assessed the correlation between the value of reduction in WT1 after induction and unfavorable event (relapse, failure in complete molecular remission after induction in APL patients) during 12 months: in subgroup with WT1 reduction above 2 log only 19% of patients had unfavorable event, whereas in subgroup with failure in WT1 reduction (under 2 log) 50% of patients had unfavorable event; P=.024 (Fisher Exact test).
Summary / Conclusion: Quantitative assessment of WT1 transcript reduction after the first cycle of chemotherapy can be used to assess treatment response. Failure in WT1 reduction is predictor of subsequent relapse risk.

B1239
ABERRANT EXPRESSION OF LYMPHOID-ASSOCIATED ANTIGENS IN ACUTE MYELOID LEUKEMIA: INCIDENCE AND CLINICOBIOLOGICAL IMPLICATIONS
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Background: A variable proportion of patients with acute myeloid leukemia (AML) express lymphoid-associated antigens (LAA). The exact incidence and clinical significance of this phenomenon remains unclear due to inconsistencies between series, likely related to methodological aspects and/or case selection biases.
Aims: We aimed at obtaining insight into the prevalence and clinical impact of LAA expression in AML by retrospectively evaluating the expression of LAA in blast cells from 343 consecutive and unselected patients with AML diagnosed in our Department between 2002 and 2012.
Methods: The patient cohort included 203 males and 140 females with a median age of 61 years (range, 10-88); 183/343 (53%) cases were above the age of 60. Within this cohort, 235 cases (68%) had de novo AML, whereas the remaining 108 cases (32%) concerned secondary AML (sAML) to either hematologic (n=94) or other non-hematologic malignancies (n=14). Patients were treated uniformly according to age with Aracytin/Idarubicin induction regimens ("3+7" or "2+5" for ages <60 or ≥60, respectively). The immunophenotype was determined by flow cytometric analysis of bone marrow aspirate and/or peripheral blood samples utilizing a primary CD45/side scatter (SSC) gating procedure with antibodies against CD7, CD13, CD19, CD33, CD4, CD10, CD34, CD117, CD64, HLA-DR, CD20, CD2, CD15,CD56, CD14, CD8, MPO, CD3, CD79a, CD22, TdT and lysozyme; a cut-off value for positivity of 20% was adopted.
Results: Overall, 184/343 cases (54%) were found to express at least one LAA. The most commonly expressed LAAs were CD4 (outside AML with monocytic differentiation), CD56, CD7, CD2, CD10 and CD79a (in 47%, 31%, 30%, 11%, 10% and 7% of LAA+ AML cases, respectively); interestingly, all CD79a-positive cases co-expressed at least one more LAA. A significant association was identified between LAA expression and cytogenetic profiles. In particular, at least one LAA was detected in 39/57 cases (68.4%) with SWOG adverse cytogenetics (including monosomal karyotype, MK), compared to 123/232 (53%) in SWOG cytogenetically favorable and intermediate risk cases (P=0.036). No other statistically significant associations were found for LAA expression (positive vs. negative) in respect to age, de novo AML (128/235 cases, 54.4%) versus sAML (56/108, 52%), complete remission (CR) rate and overall survival (OS). Associations were sought for the six more frequent LAAs. This analysis revealed significant associations (P<0.05) between: (i) CD7 or CD10 expression and adverse cytogenetics; and (ii) CD4 expression and shorter disease-free survival (DFS) and OS. Cox-multivariate analysis identified CD4 expression in addition to sAML and adverse cytogenetic profile as negative prognostic indicators (P=0.05) for DFS. Regarding OS, only sAML, advanced age and adverse cytogenetic profile retained significance.
Summary / Conclusion: Expression of LAAs is frequent in AML, among both de novo AML and sAML, and significantly associated with adverse cytogenetics. However, the precise prognostic implications of this phenomenon require further evaluation, ideally in large prospective and well-controlled studies.

B1240

AML CELLS SENSITIVE TO THE NOVEL TYROSINE KINASE INHIBITOR AKN-028 SHOW A HIGHER OVERALL TYROSINE KINASE ACTIVITY THAN MORE RESISTANT SAMPLES

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Background: AKN-028 is a novel tyrosine kinase (TK) inhibitor with preclinical activity in acute myeloid leukemia (AML), presently undergoing investigation in a phase I/II study. AKN-028 is a potent inhibitor of FMS-like kinase 3 (FLT3) and has shown in vitro activity in a wide range of AML samples, irrespective of FLT3 mutation status or quantitative FLT3 expression.
Aims: To further characterize the mechanism of actions involved in the AML cell response to treatment with AKN-028.
Methods: TK-activity profiles in AML cell lines HL60 and MV4-11 and three primary AML samples (UPN 1-3) were determined using the PamChip® tyrosine kinase peptide microarray system. For the primary AML samples, serine/threonine kinase (STK) activity profiles were generated on the PamChip® serine/threonine kinase array.
Results: Previous characterization regarding the cytotoxic properties of AKN-028 in vitro showed that the patient sample UPN1 and the cell line HL60 represent relatively resistant samples, UPN2 an intermediate variant and UPN3 and MV4-11 are relatively sensitive to AKN-028. The TK-activity profile between the cell lines resistant and sensitive to AKN-028 revealed a significant difference (p-value <0.05, Student's t-test) in basal phosphorylation level of 57 of the 141 peptide substrates tested. For primary AML samples, this pattern was even more pronounced with 73 out of the 141 peptides showing significantly higher peptide phosphorylation signals (p-value <0.05) in UPN3 as compared to the more resistant UPN1. The differences in STK-activity between the samples were in general less pronounced. The TK-activity in lysates from AML cell lines and from the three primary patients was also analyzed in the presence of AKN-028. In all samples tested, 10 µM of AKN-028 reduced peptide tyrosine phosphorylation to approximately the same level. Furthermore, the cell samples were tested against four different concentrations of AKN-028 (0-25 µM), showing a dose-dependent inhibition in all samples. STK-activity in UPN2 and UPN3 was not affected by 10 µM of AKN-028 whereas UPN1 showed slight inhibition on some peptides.
Summary / Conclusion: Cells sensitive to AKN-028 showed a higher overall TK-activity than more resistant ones. The TK-activity was inhibited by AKN-028 in a dose dependent manner in all samples tested, whereas the overall STK-activities were low in all cells. The results suggest that the difference in cytotoxic activity of AKN-028 may be due to the variation in basal overall TK-activity between cells. If confirmed in larger number of patient samples, this can indicate that TK-activity profiling could become a novel way of classifying AML, in order to predict good responders, i.e. serve as a predictive biomarker.

B1241

A NOVEL APPROACH IN THE DEFINITION OF "BLAST GATE" IN AML USING CD66 AND CD14 AS MATURATION INDICES

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Background: CD45/side scatter (SS) gating is widely used for isolating blasts by flow cytometry (FC). However, mature cells contaminate the "blast gate" (BG) or blasts are located in "mature" monocytic and granulocytic regions CD45/SS gating is thus imprecise, particularly when there are few blasts as in myelodysplastic syndromes.
Aims: Aim of this study was the application of the maturation antibodies CD66abce and CD14 for the location of immature cells on CD45/SS gating acute myeloid leukemias (AML).
Methods: A multiparameter approach encompassing CD66abce, CD14, CD45, CD10, CD1c, CD33 and CD34 was applied in order to define myeloid and monocytic immature cells, which were CD66 and CD14 negative. Thus two blastic components were discriminated:1) CD34+/CD66-/CD14- 2) CD34-/CD33+/CD66-/CD14-. Normal counterparts were estimated in 25 control marrows, representing myeloid CD34+ stem cells and immature CD34- myelomonocytic precursors. From now on we will refer to them as stem cells and myelogones. The corresponding blastic components were defines in 146 marrow samples of de novo and secondary AML, in adults and children.
Results: We used the FAB nomenclature for the simplicity of data presentation. Specifically we analyzed 14 M0, 24 M1, 22 M2, 20 M3, 22 M4, 24 M5(a or b), 6 with M6 (myeloid erythroid) and 14 AML with myelodysplasia related changes. In each category we report the percentage of the two components (mean±SD),, stem blasts (SB) and myelogone blasts (MB). 1) M0: SB 30±7,5% and MB_{6},8±4,7% 2) M1:SB 51,3±8,3% and MB 14,5±6,6% 3) M2: SB 20,8±5,4% and MB 15,4±2,5% 4) M3:SB_{5},9±3,76% and MB 71,9±8,5% 5 M4:SB 11,7±4,1 and MB 28,5±7,9% 6) M5: SB_{1},5±1% and MB 43±7,4% 7) M6 SB 14,4±4,1% and MB_{2},6±1,4%, 8) AML-mrc SB14,3±2,6% and MB12,8±3,6%. There was statistical significance in the observed differences of the BG components (P<0.05) among the various leukemia subtypes. SB component as expected prevailed in M0 and M1, while MB in M3, M4 and M5. Reference range in control marrow samples were: stem cells 0.2-1.2% and myelogones 0.5-2.5%.
Summary / Conclusion: Leukemia subtypes presented specific SB/MB patterns. The above approach could be applied in the analysis of myelodysplastic syndromes and minimal residual disease.

B1242

GLOBAL GENE ARRAY ANALYSES TO IDENTIFY BIOMARKERS ASSOCIATED WITH CLINICAL OUTCOME IN ACUTE MYELOID LEUKEMIA

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Background: Among prognostic factors associated with outcome in acute myeloid leukemia (AML) cytogenetic aberrations, although detectable in only half of the patients, have long been recognized as clinically highly important. During recent year several distinct gene mutations, preferably detected by PCR, have also been linked to treatment response and long-term prognosis. Such mutations involve e.g. the FMS-like tyrosine kinase 3 (FLT3) and nucleophosmin 1 (NPM1) genes. There is, however, still a need to discover additional disease-related molecular signatures, including aberrant gene expression patterns that may serve as novel biomarkers and/or as targets of novel therapeutic regimens in AML.
Aims: Using global genomics to identify biomarkers at the molecular level, predictive of clinical response to chemotherapy, by comparing AML patients with a poor vs. those with a better clinical outcome.
Methods: We analysed mononuclear cells obtained from 42 patients at AML diagnosis, using Affymetrix U133 Plus 2.0 and the GeneSpring GX10 software. Subsequent pathway assessments were performed through IngenuityPathway-Analyses. Patient characteristics and clinical outcome were collected. All patients entered complete remission (CR) after high-dose chemotherapy, typically consisting of an anthracyclin and cytarabine. The median CR duration was 161 (range 12-3701) days. The patients were subdivided into two groups according to their CR duration: those with "short CR duration" (<180 days, n=24) and those with "long CR duration" (> 180 days, n=18). The groups were equivalent regarding age, gender, with blood cell count, cytogenetic status and presence of secondary leukemia.
Results: Gene array analysis revealed markedly differences with 383 genes to be up-regulated and 610 genes down-regulated more than 2 fold, in pooled samples from patients with short CR vs. those with long CR duration. Among the differentially most expressed genes we identified RUNX1T1 to be 116-fold up-regulated, while ANXA1 was 58-fold down-regulated in those with short CR. These gene expression data were confirmed using real time quantitative PCR (qPCR). Thus, a strikingly higher expression of RUNX1T1 was observed in individual patient samples with short CR duration (n=10) as compared to those with long CR duration (n=10); median relative expression 0.01 vs. 0.59 (P=0.0002). The transcription factor gene RUNX1T1 is a part of one of the well-known t(8;21)(q22;q22) cytogenetic aberration, creating the RUNX1-RUNX1T1 fusion gene that translates into a protein known to block normal hematopoietic differentiation. It is observed in 5-12% of adult AML patients and is associated with a favourable clinical prognosis. Our pathway analyses identified connections between RUNX1T1 and CD34 and TCF3, all found up-regulated in short CR duration patients. We are currently validating our results by making use of publicly available gene array data from two other, independent AML cohorts (a total of approx. 200 patients). Preliminary data reveal genes mutually up-regulated in all of the three patient cohorts with poor outcome, as compared to those with better outcome.
Summary / Conclusion: By using global gene array analysis to compare AML patients with short vs. those with long CR duration, we observed striking differences in gene expression. In our material RUNX1T1 had the highest differential expression, a finding confirmed by qPCR on individual patient samples. Further assessments of interacting signaling networks and *in silico* validation of independent patient cohorts are on-going, aiming at discovering additional biomarkers predictive for AML prognosis and, ultimately, collecting information that may lead to future novel targeted therapies.

B1243

TREATMENT WITH G-CSF AND AMD3100 DIMINISH THE CLONOGENIC CAPACITY OF PRIMARY ACUTE MYELOID LEUKEMIA BLASTS IN VITRO

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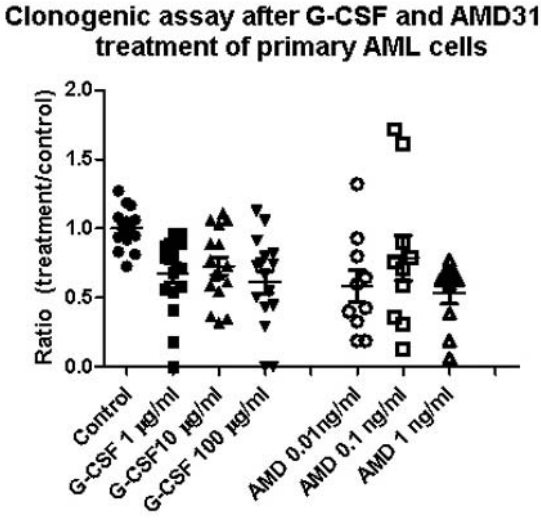
Background: The simultaneous administration of G-CSF and chemotherapy as a priming strategy has resulted in a clinical benefit in determined subsets of patients diagnosed with acute myeloid leukemia (AML) (Lowenberg et al, NEJM 2003; Pabst T, et al, Blood 2012). On the other hand, the CXCR4 antagonist AMD3100 has shown to prolong survival in AML-bearing mice (Nervi Blood 2009). However, the mechanism responsible for these anti-leukemic effects is not fully characterized. We hypothesized that these observations could be explained based on a higher sensibility of leukemic stem cells (LSCs) to these priming agents.
Aims: The main goal of this project is to study the *in vitro* effect of G-CSF and AMD3100 on LSCs and the bulk leukemic population from primary AML samples.
Methods: Primary peripheral blood cells from 10 AML patients were treated with G-CSF or AMD3100 at different doses for 72 h. Cell viability was measured by 7-AAD (eBioscience) exclusion; cell surface phenotype and volumetric cell count were obtained by flow cytometry (FACSVerse, BD) and analyzed using the FlowJo software (TriStar). For the clonogenic assays, primary AML cells were treated for 18 h with G-CSF and AMD3100 at increasing doses and cultured on MethoCult H4034 Optimum (StemCell Technologies) for 14 days.



Results: Cell viability of the bulk blast population and the more immature CD34+ leukemic population remained unaffected in the presence of G-CSF or AMD3100. G-CSF treatment upregulated CXCR4 expression in a dose-dependent fashion. A 1.44-fold increased expression was observed at the highest G-CSF dose (100 µg/ml). However, the expression of VCAM-1 and VLA-4 did not change. On the contrary, AMD3100 treatment induced a 0.71-fold reduction of CXCR4 surface expression at the highest dose (1ng/ml) (P<0.0001). Additionally, AMD3100 also induced a significant downregulation of the VLA-4 and VCAM-1 surface expression (P=0.0004 and P=0.02 respectively). AML clonogenic capacity was significantly reduced in a dose-dependent manner after treatment with G-CSF and AMD3100 (Figure 1). At the highest concentration, G-CSF (100 mg/mL) and AMD3100 (1 ng/mL) decreased the clonogenic capacity by 41% (P=0.0004) and 44% (P=0.0002), respectively.
Summary / Conclusion: The addition of G-CSF or AMD3100 impairs remarkably the clonogenic capacity of primary AML cells. These results suggest that the use of these two molecules as priming agents may contribute to eradicate the LSC population. Our findings support the design of further studies aimed to explore a potential synergistic effect of these agents in combination with standard chemotherapy against LSCs.

B1244

CLINICAL AND BIOLOGICAL CHARACTERISTICS OF IDH1 AND 2 MUTATIONS IN ACUTE MYELOID LEUKEMIA PATIENTS WITH NORMAL KARYOTYPE (NC-AML) IN SERBIA

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Background: Acquired mutations in exon 4 of both isocitrate dehydrogenase 1 and 2 genes (*IDH1* and *IDH2*) were recently reported in patients with acute myeloid leukemia (AML). Numerous studies have tried to reveale the association of these mutations with clinical features, prognosis and the outcome of the disease.
Aims: To evaluate the frequency and examine the impact of *IDH1/2* mutations on outcome, as well as their association with other molecular and clinical prognostic markers in adult *de novo* NC-AML patients.
Methods: Diagnostics bone marrow samples from 96 adult *de novo* NC-AML patients diagnosed between September 2009 and December 2012 were analyzed for *IDH1/2* mutations by DNA PCR amplification/sequencing. The patients (55 males/41 females; median age 54, range 19-78 years) were treated as follows: patients aged < 60 years- induction "3+7"; consolidation 3 cycles HIDAC; medically fit patients aged >60- 70 years-induction "3+7"/"2+5"; consolidation 3 cycles IDAC; patients aged >70 years- ara-C 20 mg s.c. 10-14 days/ 6 cycles or purinethol.
Results: *IDH* mutations were found in 21.9% of the patients. *IDH1* mutations were detected in 8 patients (7.9%, R132 (n=4), R132H (n=3), R132G (n=1)). *IDH2* mutations were detected in 13 patients (9.9%, R140Q (n=9), R140W (n=1), R140L (n=1) and R172K (n=2)). None of the patients exhibited simultaneously *IDH1* and *IDH2* mutations, and all of the mutations were in heterozygous form. They were predominantly detected among FAB M2 and M4 subgroups (M2, M4IDH mutations were not associated with *NPM1* and *FLT3* mutations or any other clinical characteristics except for the higher percentage of blasts in peripheral blood. *IDH* mutations impacted complete remission (CR) rate; only 13/21 *IDH* positive patients reached CR (P=0.044). Moreover, multivariate logistic regression analysis showed that the presence of *IDH* mutations is independent unfavorable factor for CR achievement (P=0.048). Similar finding was detected when we investigated the impact of *IDH* mutations on overall survivor (OS), i.e. IDH^+ patients exhibited shorter OS (median 4 months) compared to IDH^- patients (median 9 months)(P=0.058). The effect was more prominent in $IDH2^+$ patients (P=0.018). Nevertheless, the multivariate analysis showed that only $IDH2^+$ status was an independent prognostic factor for OS (P=0.015). *IDH+* patients were most numerous in the double negative *FLT3-ITD-/NPM1-* patients (10/21), and then in *FLT3-ITD-/NPM1+* patients (7/21). Double positive group *FLT3-ITD+/NPM1+* and single positive *FLT3-ITD+/NPM1-* group of patients had 2 *IDH+* patients each. The presence of *IDH* mutations in the half of patients (7/14) belonging to the molecular low-risk *FLT3-ITD-/NPM1+* group could explain the low OS in this group of patients of only 2 months compared to the other four groups (P=0.04).
Summary / Conclusion: This study shows that the presence of *IDH* mutations predicts both low CR rate and shorter OS in our cohort of patients. It also suggests that a caution should be addressed when assigning the patients to the molecularly low-risk $FLT3\text{-}ITD^-/NPM1^+$ group.

B1245

DOWNREGULATION OF PROGRAMMED CELL DEATH LIGAND-1 PROTEIN INHIBITS MIGRATION,ADHESION AND INVASION OF LEUKEMIA HL-60 CELLS

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Background: Programmed cell deathligand-1(PD-L1) was a newly discovered apoptosis gene, which was highly expressed in children with leukemia ,and it was considered as a predictive marker for leukemia micrometastasis.
Aims: To investigate the impacts of programmed cell death ligand-1(PD-L1) downregulation on cell abilities of migration,adhesion and invasion of leukemia HL-60 cells.
Methods: PD-L1-siRNA,control-siRNA and blank-siRNA were transfected into leukemia HL-60 cells through liposome as experiment group,negative control group and blank control group,respectively.The transfection efficiency was observed by fluorescence microscope.Forty-eight hours after transfection,reverse transcription PCR and immunocytochemistry were employed to detect the expression of PD-L1 in three groups.Cell experiments were performed to assess cell migration,adhesion and invasion respectively,when PD-L1-siRNA was transfected into HL-60 cells for 48 hours.
Results: The transfection efficiency of HL-60 cells was 90%.PD-L1 mRNA and protein expression of PD-L1-siRNA group were 0.031±0.002 and 532.3±42.5,which were significantly lower than those of control-siRNA group and blank-siRNA group(t=3.452,t=2.905,P<0.05;t=3.167,t=3.792,P<0.05).The number of migrated cells in PD-L1-siRNA group was 23.17±2.06,fewer than those of control-siRNA group and blank-siRNA group(t=2.755, P<0.05;t=3.626,P<0.05).Compared with control-siRNA group,the adhesion rate at 30and 60min were decreased by(40.97±2.25)% and(51.26±3.07)% (t=2.787,P<0.05;t=3.965,P<0.05).Moreover,the number of invaded cells in PD-L1-siRNA group was 37.65±3.12,significantly fewer than those of control-siRNA group and blank-siRNA group(t=3.378,P<0.05;t=2.613,P<0.05).
Summary / Conclusion: Down-regulation of PD-L1 expression in HL-60 cells can inhibit the abilities of migration,adhesion and invasion,which suggests that PD-L1 plays an important role in the metastatic potency of leukemia cells.

B1246

THE TRIM FAMILY UBIQUITIN LIGASE TRIM32 PROMOTES CELL DIFFERENTIATION AND SUPPRESSES CELL PROLIFERATION OF HUMAN AN APL CELL LINE VIA RETINOIC ACID RECEPTOR ALPHA-MEDIATED TRANSCRIPTION

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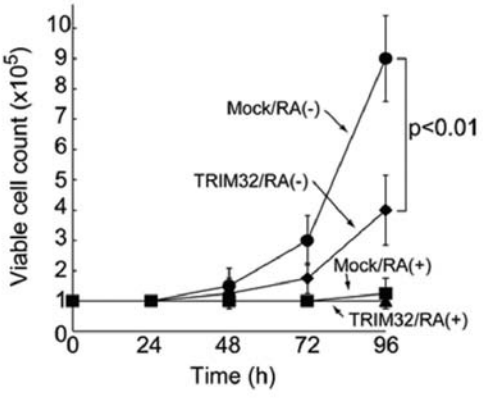
Background: Retinoids are promising for cancer therapy due to their potential effects on cell differentiation and apoptosis. Initial treatment for acute promyelogenous leukemia (APL) is now carried out by using all-trans retinoic acid (ATRA). *In vitro* studies with human APL cell lines have shown that these leukemic cell lines provided clues for achieving satisfactory therapeutic results. However, the mechanisms by which ATRA functions have not been elucidated. We recently found that TRIM32, one of the ubiquitin ligase family proteins, is a positive regulator of transcriptional activity of retinoic acid receptor alpha (RARa) in differentiation of neural cancer cell lines.
Aims: With the aim of elucidating the molecular function of TRIM32 in APL cell differentiation, we checked the protein levels of TRIM32 by immunoblot analysis in various human leukemic cell lines and we investigated whether TRIM32 influences cellular proliferation of HL60 cells by facilitating transcriptional activity of RARa. We also tested whether exogenous overexpression of TRIM32 alone promotes APL cell differentiation without ATRA, suggesting that TRIM32



Methods: To clarify the expression profiles of TRIM32 in human leukemic cell lines, we compared the protein levels of TRIM32 by immunoblot analysis in various human leukemic cell lines. Next, to verify whether TRIM32 drives RARa-mediated transcription in human leukemia and cancer cell lines, we performed a luciferase assay using a RAR promoter-driven luciferase construct (RAR-Luc) and we performed immunoblot analysis to verify whether TRIM32 stabilizes the expression level of endogenous RARa in HL60 cells without ATRA. We also examined whether similar morphological features of granulocytic differentiation were observed even in some of the ATRA-untreated HL60 cells in which TRIM32 was transiently transfected. In addition, we calculated the percentage of CD11b-positive HL60 cells in which TRIM32 was induced without ATRA treatment.
Results: Expression of TRIM32 protein was observed in leukemic cell lines and its expression level of TRIM32 was various in blast cell lines. Immunoblot analysis showed that endogenous RARa was more highly expressed in HL60 cells in which TRIM32 had been expressed than in mock cells. We also found that similar morphological features of granulocytic differentiation were seen in some of the ATRA-untreated HL60 cells in which TRIM32 was induced. The percentage of CD11b-positive cells was significantly higher in HL60 cells in which TRIM32 was induced than in mock cells (1.4±1.0% for mock versus 21.9±5.1% for TRIM32 (P<0.01)). We performed cell proliferation assay using HL60 cells after induction of TRIM32 or empty expression vectors. The effect on HL60 cells after induction of TRIM32 was characterized by significant suppression of cellular proliferation compared with that of mock cells (Figure).
Summary / Conclusion: Results of cell proliferation assay suggest that TRIM32 induced granulocytic differentiation via RARa-mediated transcriptional activity in the absence of ATRA, and proliferation of HL60 cells after induction of TRIM32 showed slow proliferation because of their disappearance of self-division potential. In conclusion, it is important to analyze TRIM32 protein that has functions to regulate transcriptional activity of RARa. Our findings might provide clues for generating of new anti cancer agents. Detection of substrates of TRIM32 and pharmacologic enhancers of RAR-associated ubiquitin ligases would be helpful for patients suffering from malignant diseases, whose cancer cells are induced to differentiate by retinoic acid-dependent RARa-mediated transcriptional activity.

B1247

EXPRESSION LEVELS OF WT1 AND NPM1 AND NOVEL NONSPECIFIC MARKERS AS TOOLS FOR EVALUATING MINIMAL RESIDUAL DISEASE IN AML

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Background: Monitoring of minimal residual disease (MRD) is an important tool in the medical management of acute myeloid leukemia (AML). Of the specific molecular markers, mutations of the nucleophosmin 1 (*NPM1*) gene represent the most frequent aberration. This makes NPM1 a favorite gene for MRD detection. Approximately one half of AML patients do not have a suitable specific molecular marker for MRD monitoring. Therefore, development of sensitive assays for quantification of nonspecific leukemia-associated antigens (LAA) should be focused on, along with the search for rare specific mutations. The Wilm's tumor gene (*WT1*) has been suggested as a possible molecular marker of MRD in AML as it is overexpressed in 80% of AML patients at diagnosis. The LAAs that we focused on were *PRAME, MSLN, ST18, XAGE1, CSPG4, CA9* and *BAALC* in genes.
Aims: To examine selected LAAs as potential tools for MRD monitoring in AML.
Methods: Established IVD CE protocols of fluorescence-based quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) and protocols based on TaqMan Gene Expression Assays® were used for quantification of selected targets.
Results: Of the 475 patients treated at the Institute of Hematology and Blood Transfusion in Prague in 2002-2012, 113 (23.8%) had a mutation in the *NPM1* gene. Of the 45 patients with *NPM1* mutation and follow-uP>4 months, 7 never reached MRD negativity while sustaining hematological remission and 12 suffered hematological relapse. MRD positivity preceded hematological relapse by a median of 3.23 months. Overexpression of WT1 in peripheral blood was detected in 199 of 211 patients (94.3%) at diagnosis. Thirty-three experienced relapse. Median time from MRD positivity to hematological relapse was 1.8 months. MRD quantities monitored by *NPM1* on mRNA level were compared to *WT1* expression in 60 AML patients with a median follow-up of 12.3 months (range 1.32–110.4). Both methods for MRD detection correlated significantly (P<0.0001). Levels of selected LAAs (*MSLN, ST18, XAGE1, CSPG4, CA9*) were evaluated in 153 AML patients at diagnosis. Overexpression of *MSLN* was demonstrated in 22.5%. However, the high expression of *MSLN* in normal peripheral blood makes this marker unsuitable for MRD monitoring. This is not the case of *ST18, XAGE1, CSPG4* and *CA9*, which were overexpressed in 50.3%, 17.9%, 45.7% and 36.4% respectively. In 12 patients (7.8%) with high expression of a particular LAA at diagnosis, it was possible to trace MRD during the course of the disease. MRD data were in concordance with the disease status. Moreover, in seven of these patients, LAA was the only MRD marker because of the low expression of both *WT1* and *NPM1*. Levels of *BAALC* and *PRAME* were monitored in 373 AML patients at diagnosis. Overexpression of *BAALC* was demonstrated in 169 patients (45.3%). In 14 patients, MRD was monitored during the course of disease and, similarly, significant correlation of *BAALC* expression levels with those of *WT1* has been found (P<0.0001). The high expression of *PRAME* was detected in 91 patients (24.4%) at diagnosis. In 5 patients, MRD was monitored during the course of disease. Its expression rose in concordance with *PML/RARA* transcripts while *WT1* expression stayed below the upper normal limit.
Summary / Conclusion: By combining methods for detecting *NPM1* mutations and elevated LAA expression, a suitable MRD marker was found for every AML patient in our cohort. Median time from MRD positivity to hematological relapse was longer by 1.4 months in the *NPM1* group compared to *WT1*.
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B1248

INCIDENCE AND CLINICAL FEATURES OF PATIENTS WITH AML1-ETO, CBFB-MYH11 AND PML-RARA TRANSCRIPTS IN THERAPY-RELATED COMPARED TO *DE NOVO* ACUTE MYELOID LEUKEMIAS IN A COHORT OF 449 BULGARIAN PATIENTS

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Background: *AML-ETO*, *CBFB-MYH11* and *PML-RARA* fusion transcripts (FT) are among the most frequent genetic abnormalities in de novo acute myeloid leukemia (dnAML) with geographic/ethnic-relatedvariationsinthe incidence. Generally, these FT are associated with a favorable prognosis. However data concerning the incidence of these aberrations in therapy-related AML (tAML) cases, and the association with clinical feature are contradictory.
Aims: To determine the incidence of *AML-ETO*, *CBFB-MYH11* and *PML-RARA* in tAML compared to dnAML and to define the main clinical and laboratory features of both groups of patients (pts).
Methods: In total 449 adult AML pts, including 403 (89.8%) dAML and 46

(10.2%) tAML, 222 males and 227 females, with a mean age of 54.3±16.9 years were diagnosed at the National Hematology Hospital – Sofia. All patients were screened for *AML1-ETO, CBFb-MYH11* and *PML-RARA* FT and *FLT3-ITD* by RT-PCR.
Results: In the whole cohort "favorable" FT were found in 74 pts (16.5%): 62 (15.4%) in dnAML and 12 (26.1%) in tAML (P=0.09). The overall incidence of the different markers was as follows: 4.9% for *AML1-ETO* (n=22), 4.9% for *CBFb-MYH11* (n=22), and 6.7% for *PML-RARA* (n=30), without significant differences in the frequency between dnAML and tAML (*AML1-ETO*: 3.7% vs. 8.7%; P=0.266; *CBFb-MYH11*: 3.7% vs. 8.7%; P=0.266, *PML-RARA*: 6.5% vs. 8.7%; P=0.53, respectively). Within the group of dnAML, pts with "favorable" FT were significantly younger compared to the remaining pts (55.9±16.2 years): *AML1-ETO*(+) 39.8±15.8 years (P=0.000); *CBFb-MYH11*(+) 44.1±15.9 years (P=0.003), *PML-RARA*(+) 44.3±18.9 years (P=0.001). In contrast, within the tAML group, only the *CBFb-MYH11*(+) pts were significantly younger (44.9±22.2 years, P=0.005) compared to negative cases (62.1±12.8 years), while a tendency for younger age was seen in *AML1-ETO*(+) pts (47.8±26.3 years; P=0.068), and no differences were detected in *PML-RARA*(+) pts (56.2±6.8 years; P=0.376). According to the FAB criteria, *AML1-ETO* were found in M1 (4); M2 (9); M2Eo (3); M4 (1); and not specified (1) in de novo AML, compared to only myeloid morphology M2 (3) and M2Eo (1) in tAML. *CBFb-MYH11* were found in M1Eo (1); M2 (2); M4 (8); and M4Eo (7) dnAML cases, vs. M2Eo (3) and M4 (1) in tAML. *PML-RARA* were found in M3 (22); and M3v (4) de novo cases, while in the tAML all pts had a classical M3 morphology. Furthermore, the molecular data revealed that within the *PML-RARA*(+) dnAML pts, 42.3% bared the Short form and 57.7% the Long form of the transcripts; while *FLT3*-ITD was detected in 7/15 (46.7%) patients. In contrast, all *PML-RARA*(+) tAML patients were positive for the Long form and only one was positive for *FLT3*-ITD. No differences in the mean age, gender, leukocyte and platelet counts, hemoglobin level, and blast cells/equivalents were observed between pts with different molecular abnormalities related to the origin of the disease (dnAML vs. trAML).
Summary / Conclusion: In this study we found a relatively high incidence of "favorable" fusion transcripts in patients with tAML similar to that, determined in dnAML. Our results further confirmed the heterogeneity of these entities, however most of the main characteristics did not differ significantly between de novo and therapy-related cases.

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B1249
UNFAVORABLE PROGNOSTIC IMPACT OF WT1 MUTATIONS IN PEDIATRIC ACUTE MYELOID LEUKEMIA IN KOREA
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Background: Acute myeloid leukemia (AML) is a clinically and biologically heterogeneous disease. Recently, about 65% of pediatric AML patients show long-term survival. The relevance and prognostic value of mutations in *FLT3, NPM1, CEBPA*, and *WT1* for novel classification and risk-group stratification have been studied mainly in adult AML and to a lesser extent in patients with pediatric AML.
Aims: The aim of this study was to show the clinical implications of these genetic variants on childhood AML in Korea.
Methods: We have evaluated the frequency and prognostic significance for the mutation status of *FLT3-ITD/TKD, NPM1, KIT, CEBPA, WT1, NRAS, BAALC* and *IDH1/2* in childhood AML (n=104). Mutations were analyzed by PCR amplification of genomic DNA. *WT1* and *BAALC* expression levels were measured using real-time quantitative PCR. All patients were diagnosed with AML at Samsung Medical Center and Chonnam National University Hwasun Hospital.
Results: The incidences of these genetic alterations were as follows: *NPM1*, 20 (19%); *FLT3-ITD*, 14 (14%); *CEBPA*, 14 (14%); *WT1*, 11 (11%); *NRAS*, 5 (2%); *KIT*, 3 (3%); *IDH1*, 2 (2%); *IDH2*, 2 (2%); high *BAALC* expression, 6/15 (40%); high *WT1* expression, 6/15 (40%). The 5-year Kaplan-Meier EFS rate was 65%: standard risk karyotype, 67% vs. high risk karyotype, 60%; chemotherapy, 73% vs. SCT, 61%. The 5-year EFS rate was not significantly different according to *FLT3-ITD, NPM1, NRAS,* and *CEBPA* mutation status. *WT1* mutated patients showed a decreased EFS rate (68% vs. 44%, *P*=.064). The 5-year EFS rates were as follows according to the level of *BAALC* and *WT1* expression: high, 50% vs. low *BAALC*, 100% (*P*=.110); high, 42% vs. low *WT1*, 100% (*P*=.059). In 19 normal karyotype patients, EFS rates of *FLT3/ITD, CEBPA* and *NPM1* mutation status were not significantly different. *WT1* mutated patients of normal cytogenetic AML patients showed a poor prognosis (78% vs. 0%, *P*=.002).
Summary / Conclusion: The most common mutation was found in *NPM1*. The *KIT*, *IDH1/2* and *FLT3/TKD* mutations were very rare. Patients with High *BAALC* and *WT1* expression levels were associated with poor prognosis, but statistically not significant. *WT1* mutated patients with normal karyotype showed a poor prognosis. The result of this study was not consistent with previous published data because of small sample size, selection bias, high rate of SCTs, and ethnic differences. However, these data suggest that some clinical implications of molecular genetic alterations in Korean children with AML. Further retrospective and prospective multicenter studies are needed to unravel how these genetic abnormalities affect the leukemogenesis, treatment and prognosis in pediatric AML.

B1250
MANAGEMENT OF FIT OLDER PATIENTS WITH AML : MAJOR IMPACT OF CYTOGENETICS
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Background: Median age of Acute Myeloid Leukemia (AML) patients at diagnosis is 67 years and represents a real clinical challenge. Major problems of olders AML remain co-morbidities, poor performance status and biological features increasing resistance to chemotherapy. However, some patients are cured with chemotherapy.
Aims: We studied prognostic factors in order to identify those patients deserving intensive treatment
Methods: We retrospectively reviewed 78 patients over 60 (60-86) years old referred to our centre for treatment. AML was classified according to WHO classification; cytogenetic data were obtained by routine karyotype, additional FISH analysis and molecular biology; fit patients (PS<2, reversible or controlled comorbidities and no geriatric syndromes) received intensive chemotherapy according to successive EORTC protocols. Several factors were assessed in univariate and multivariable analysis, in order to define their prognostic significance according to age subgroups. We also looked for factors predicting treatment related toxicity and/or death and disease related mortality. Kaplan-Meier curves were used to compare significant prognostic factors in terms of complete remission, overall survival and disease-free survival according to prognostic factors and complete remission.
Results: 64% of patients are male. 52% AML are secondary. 50% of all treated patients reached a complete remission post induction. Overall survival (OS) of patients over 60 years old is 48%; 31% and 17% at1, 2 and 5 years, with a median survival of 9 months. This is higher compared to other studies, since a majority of patients were "fit" enough to be referred to our centre. According to age, OS is significantly better (P<0.0001) for patients below 70 years than above (44% at 2y vs. 16%). In univariate analysis, whatever the age, favourable karyotype provides a better prognosis (2y OS 53% vs. 20%, P=0.007). Taking into account cytogenetic data and age, median survival of patients above 70 with favourable karyotype was very similar to population below 70 (38 and 44% at 2y).In multivariate analysis, OS in patients treated with curative intent is much better than supportive care (62% at 1y vs. 6%, P<0,0001). For patients who received intensive chemotherapy (77%), death due to uncontrolled disease remains a major concern compared to death induced by treatment related toxicity (10%). In multivariate analysis, levels of LDH (P=0.03), liver diseases and prior malignancy (P=0.01) come out as others majors significant prognostic factors.
Summary / Conclusion: In our single-centre experience of 'fit' olders AML patients, we confirm that cytogenetic data is the prognostic factor with the strongest predictive positive value in terms of outcome whatever the age in univariate analysis. Particularly for patients above 70, individual geriatric assessment combined with major prognostic factors should be refined in prospective studies to improve therapeutic decision making and limit treatment related toxicities. The survival of older AML patients remains poor, more likely due to disease resistance with aggressive biology, than because of treatment toxicity. New alternative treatment approaches using less toxic target therapies are urgently needed.

B1251
A RARE VARIANT TRANSLOCATION IN APL
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Background: Acute Promyelocytic Leukemia (APL) is characterized by the t(15;17) patognomic translocation. Although almost all APL patients have the classical balanced translocation, a small proportion have complex or simple variant translocation , such as t(4;15;17) or t(5;17;15). Some of them , such as t(11;17)(q23;q21), show resistance to ATRA treatment whereas detection of classical t(15;17) or underlying PML-RARα rearrangement is highly predictive of response to ATRA in virtually 100% of cases.
Aims: The aim of this study was to describe a rare variant translocation in APL patient. A 21 year-old woman was admitted to our hospital because of fever and

hematomas. Coagulation test revealed that INR was 1.52, normal partial thromboplastin time (PTT) and there was a slight reduction of fibrinogen (94.8 mg/dL). Peripheral blood examination showed 127x10^3/mmc white blood cells and 10x10^3/mmc platelets. Bone marrow aspirate showed 100% infiltration by abnormal promyelocytes. At immunophenotypic analysis 91.1% cells were CD9, CD13, CD33,CD38, CD45RA, MPO, CD45 positive. PML/RARα transcript of the bcr3 subtype was detected by reverse transciptase polymerase chain reaction. A diagnosis of APL high risk was made according to the above data.
Methods: Chromosome analysis was based on G-banded metaphase and the karyotype was described according to the ISCN 2009. A whole chromosome painting (WCP) probe for chromosome 6 and a dual-color, dual-fusion probe for PML/RARα were used for fluorescence in situ hybridization (FISH).
Results: The karyothype of the patient was interpreted as 46,XX,t(6;17;15)(p23;q21;q22) by conventional cytogenetic and it was elucidated by fluorescence in situ hybridization. Patient started treatment with ATRA 45mg/m^2 die and idarubicina 12mg/m^2 every other day but she died at 3rd day because of a massive brain bleeding although platelets count was maintened above 40x10^3/mmc.
Summary / Conclusion: The t(6,17;15)(p23;q21;q22) translocation found is, to our knowledge, one of a few series in wich 6p arm is involved. This case is a contribution to the study of the infrequent variants APL translocation and could provide futher insigts into biologic and prognostic information provided by cytogenetics.

B1252

DNA METHYLTRANSFERASE FAMILY GENES MUTATIONAL STATUS IN CANIS FAMILIARIS AML: A POTENTIAL MODEL FOR THE DISEASE IN PEDIATRIC PATIENTS

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Background: Acute myeloid leukemia (AML) is the most widely occurring acute leukemia in adults with a high prevalence of relapses. Genetic aberrations are often found in AML specimens and recent whole genome sequencing studies identified somatic mutations with biological and clinical importance. In addition, several studies revealed that both global DNA hypomethylation and regional hypermethylation occur in tumorigenesis. In this respect, mutations of DNA methyltransferases gained particular interest. Indeed, several studies reported a high occurrence of somatic mutations at codon R882 of DNA methyltransferase3A in adult AML[1]. *DNMT3* family genes comprise two enzymes, *DNMT3A* and *DNMT3B*, that play a role in *de novo* CpG methylation and a functional accessory protein, *DNMT3L*, that activates the two enzymes by binding to their catalytic domain[2]. Functional studies performed *in vitro* on human cells line models or using murine models have associated mutations of *DNMT3A* with aberrant methyltransferase activity and altered whole genome expression profiles. However, a direct correlation between aberrant DNA methylation and mutated *DNMT3* remains elusive. We propose the dog as a model for functional studies of human AML providing the advantage that the disease occurs spontaneously in out bred dogs.
Aims: The purpose of this study is to explore canine AML as a model of human AML. Canine AML specimens were screened for *DNMT3A*, *DNMT3B* and *DNMT3L* to provide a view on the mutational status of all members of the family of DNA methyltransferase genes.
Methods: Sanger sequencing of the C-terminal domain of *DNMT3A*, *DNMT3B*, and *DNMT3L* was achieved in 16 samples of canine AML. The majority of canine patients were large breed in their middle age (mean age 6.9±2.7 years); the diagnosis of AML was based on clinical evaluation and immunophenotype. Primers were designed to obtain DNA sequences of the C-terminal domain of *DNMT3A*, *DNMT3B* and *DNMT3L* using ABI PRISM 310 Genetic Analyzer.
Results: Diagnosis of canine AML was confirmed by morphology with the major part of specimens classified as M2-M4 with some cases of M5[3], complete blood count profiles (CBC), white blood cell counts (WBC), and by the immunophenotype profile (CD34+, CD45+, MPO+, CD14+, CD11b). Specific sequences were obtained for exon 22 of *DNMT3A* and *DNMT3B* and exon 9 of *DNMT3L*. No mutations were found in canine AML specimens at positions homologous to sites of hot spot mutations in adult human patients with AML. Since the mutation detection limit of Sanger sequencing is 20% we will also use next generation amplicon deep sequencing to detect mutations down to 1% to more thoroughly analyze mutations including the analysis of all exons of the C-terminal domain of *DNMT3A*, *DNMT3B* and *DNMT3L*.
Summary / Conclusion: In this study we investigated the dog as a study model of human AML. Acute myeloid leukemia occurs spontaneously in out-bred dogs suggesting it to be a natural model for clinical and biological studies. Sanger sequencing failed to detect mutations in the terminal exons of *DNMT3A*, *DNMT3B*, and *DNMT3L*which may indicate that like childhood AML also canine AML has no mutations in the family of DNA-methyltransferase genes[4]. Along this line canine AML in spite of middle age of patients seems to resemble pediatric AML.

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B1253

ANALYSIS OF ACTIVITY OF MATRIX METALLOPROTEINASES MMP-2 AND MMP9 IN BONE MARROW PLASMA OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Matrix metalloproteinases (MMPs) are of greit interest for oncohematologists because of their role in neoangiogenesis, including such processes as growing, invasion, metastazing of tumor cells. However the concept of clinical importance of MMP is still not stated.
Aims: To investigate the prognostic significance of MMPs in patients with acute myeloid leukemia (AML).
Methods: The MMP-2 and MMP-9 activities ratio (MMP-2/MMP-9) was investigated in BM plasma of 53 patients with de novo AML. During the collection of BM 33 patients were diagnosed with complete remission (CR) and 20 patients without CR. The method of zymografy in polyacrylamide gel containing gelatine as a substrate was used.
Results: MMP-2/MMP-9 ratio was approximately 1.00 (0.91+/- 0.06; from 0.42 to 1.77) in the BM samples of 30 patients in CR (91.0%) and 10 patients without CR (50.0%). The median overall survival (OS) of these patients was 27 months. At the same time the MMP-2/MMP-9 ratio was more than 3 times higher (3.34+/-0.34); from 1.80 to 5.50 (P<0.001) in 13 patients. In the group with high MMP-2/MMP-9 ratio, 10 patients had resistant variant of AML. Their median OS was only 7 months; P<0.001. Other 32 patients were in CR. The natural history of AML in 3 patients with CR and high MMP-2/MMP-9 ratio was poor. In 1 patient MMP-2/MMP-9 ratio was increased before relapse at 3 months and other 2 patients have got the sign of minimal redsidual desease.
Summary / Conclusion: The conclusion is that high MMP-2/MMP-9 ratio >/- 1.80 may be associated with unfavorable prognosis ii patients with AML./

B1254

CORRELATIONS AMONG CFU-GM GROWTH PATTERNS AND ACUTE LEUKEMIA WHO-2008 ENTITIES

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Background: Acute leukemias (AL) represent a very heterogeneous and very large group of diseases that were divided into several subgroups (categories) defined by similar characteristics of their individual entities in the World Health Organization (WHO) Classification in 2008. Different growth patterns of granulocyte-macrophage colony forming cells (CFU-GM) from diagnostic bone marrows from patients with various types of AL have been described, especially depending on the stimulating factors used and the type of AL.
Aims: To correlate the CFU-GM growth patterns of diagnostic bone marrow (BM) cells from 103 patients with AL classified according to the WHO-AL categories, entities and prognosis.
Methods: CFU-GM were assayed in triplicate in 1 ml agar cultures containing 2x10^5 nucleated BM cells, Iscove's modified Dulbecco's medium, 20 % fetal calf serum, and 5 % of the human bladder carcinoma cell line 5637 conditioned medium as a source of cytokines. After 7 days of incubation the number of colonies (consisting of 40 or more cells) and clusters (3-39 cells) were counted. Informed consent was obtained from all patients.
Results: BM from 42 healthy BM donors produced 20-150 colonies and 20-150 clusters per 10e5 seeded BM nucleated cells. Low growth of colonies and high growth of clusters (L-H growth pattern) was found in 8/10 acute myeloid leukemia (AML) M3, 3/3 AML M2 with t(8;21) and 1/1 AML M4Eo case. Low growth of colonies and low growth of clusters (L-L growth pattern) exhibited BM cells of all 4 cases with near-tetraploid AML with myelodysplasia related changes (AML/MDRC). BM from other 36 AML/MDRC cases showed 13x L-L, 9x L-H, 5x L-N (low-normal), 1x N-H, and 8x H-H growth patterns of colonies and clusters. In 22 AML without MDRC the following growth patterns of CFU-GM colonies and clusters were found: 11x L-L, 6x L-H, 3x L-N, 3x N-H. All BM from 28 patients with various types of acute lymphoid leukemia (ALL) exhibited low growth of CFU-GM colonies and clusters (L-L growth pattern).
Summary / Conclusion: Several categories of AL seem to be associated with one type of CFU-GM growth pattern that is typical for each category. Diagnostic bone marrows from patients with AML M3 and AML M2 with t(8;21) exhibited the CFU-GM growth pattern with low growth of colonies and high growth of clusters (L-H). Diagnostic BM from patients with near-tetraploid AML/MDRC

exhibited the pattern with low growth of colonies and low growth of clusters (L-L). Low growth of CFU-GM colonies and clusters was found in diagnostic bone marrows from all patients with ALL studied. A special finding was a group of 8 patients with the CFU-GM growth pattern with high colonies and high clusters (H-H) who all were from the AML/MDRC WHO category only and had a very bad prognosis even in comparison to the other nonH-H AML/MDRC. Three of these 8 patients died early within 2-11 days since diagnosis, none of the 5 remaining cases reached complete remission after 3/7 induction treatment and none was a long-term survivor.

B1255

CLINICAL SIGNIFICANCE OF COMMON LEUKEMIA GENE MUTATIONS IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Acute promyelocytic leukemia (APL) is an extremely unique subtype of acute myeloid leukemia (AML). It is a more malignant form of acute leukemia with a severe bleeding tendency and high risk of DIC(Disseminated Intravascular Coagulation). Chemotherapy (retinoic acid , arsenic trioxide and anthracycline) was the front-line treatment of APL increasing the complete remission (CR) rate to 80%~90% in newly diagnosed APL patients. So far there has not been massive primary specimens of acute promyelocytic leukemia (APL) to verify leukemia-related gene mutation synergically involved in the pathogenesis of APL and research its relevance to clinical manifestations, prognosis and genetics.
Aims: Explore whether multiple common gene mutations of leukemia synergistically involved in acute promyelocytic leukemia (APL) pathogenesis, and to explore their relevance to clinical features, cytogenetic and molecular risk stratification.
Methods: 84 new diagnosed APL patients from February 2005 to Ocotber 2010 were collected, the gene mutations of bone marrow mononuclear cells and clinical features of mutation-positive patients were analyzed by genomic DNA-PCR and sequencing.
Results: Median age of our patients was 34 years (range, 8 to 79 years) and 44 patients (52.4%) were male and 40 (47.6%) were female. We demonstrate the presence of 66 cryptic genomic aberrations in 84 patients (78.6%) consisting of mutations (51 patients), in which the mutations with the highest incidence were found as *FLT3-ITD*, reaching 27.4%(23/84). Next, there were 12 cases *WT1* mutations, 9 for *FLT3-TKD*, 7 for *TET2*, 5 for *N-RAS*, 4 for *ASXL1*, 2 for *EZH2* mutation and 1 positive in *MLL-PTD*, *IDH1* and *CBL* respectively. No mutation was found in other *JAK1*, *DNMT3A*, *c-Kit*, *NPM1*, *IDH2*, *RUNX1* and *JAK2 (V617F)* common leukemia-related genes. Combined analysis with clinical data demonstrated that the patients with *FLT3-ITD* mutation compared to those without, had higher white blood cell counts(24.3×10^9/L vs 7.2×10^9/L;P=0.004); with the patients with *N-RAS* mutation showed lower platelet counts (21.5×10^9/L vs 70.0×10^9/L;P=0.012). Overall survival of these patients were obviously shorten with OS of wild-type. The difference between mutant and wild-type above all had statistical significance(for FLT3-ITD, 39.1 months vs 71.7 months; P=0.010; for N-RAS, 24.79 months vs 68.02 months; P=0.028). There were no significant correlations between age, sex, WBC and mutations in *WT1*, *FLT3-TKD*, *TET2*, *ASXL1*, *EZH2*, *MLL-PTD*, *IDH1* or *CBL*. The difference between APL with pure t(15;17) and additional abnormal karyotype did not show a significant different OS (28.94 months vs 67.10 months; P=0.576) or a mutation incidence of leukemia-related genes (6/13 vs 45/71; P=0.219)
Summary / Conclusion: The FLT3-ITD mutation is recurring genetic change in APL, together with N-RAS mutation indicates poor prognosis. Additional abnormal karyotype does not associate with prognosis and mutation incidence of APL.

Acute myeloid leukemia - Clinical

B1256

TREATMENT OF ACUTE PROMYELOCYTIC LEUKEMIA DURING PREGNANCY

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Background: Acute promyelocytic leukemia (APL) is a favorable subtype of AML with 80-85% probability of 5-years survival on ATRA containing regimens.
Aims: Though APL in pregnant women is a very rare event we tried to evaluate the efficacy of treatment in this cohort of pts in the era of ATRA.
Methods: From Jan 1998 till Feb 2013 the Russian Acute Leukemia study group has treated 9 de novo and 1 relapsed APL in pregnant women. In all cases diagnosis was proved by cytogenetic and/or molecular approaches. The median age was 25 (19-37) years. According to the M. Sanz risk group stratification no one woman was in the favourable risk group, 5 were in the intermediate, and 4 - in the poor. APL was diagnosed in Ist trimester in 1 pt (8 weeks of gestation), in whom pregnancy finalised by spontaneous abortion, in IInd – in 4 pts (19, 20, 21, 24 weeks) and in IIIrd trimester - in 5 pts (28, 30, 32, 36, 38 weeks).
Results: Antenatal fetal mortality at 38th weeks of gestation was registered in 1 pt, followed by stimulated delivery before the APL diagnosis. The pt died within 7 days of ATRA plus chemotherapy (CT) due to septic shock and intracranial haemorrhage. 1 woman delivered by cesarean section after placental abruption and antenatal fetal mortality at the 24th week of pregnancy, complicated by uterine bleeding. She was diagnosed APL 2 months later. Now she is receiving AIDA-induction therapy. 1 woman delivered at 36 weeks of gestation before treatment. 2 pts (1- after delivery and 1 - after medical abortion at 1st trimester) were treated by 7+3 (Dauno=180mg/m^2) with ARTA. 6 pts were treated during pregnancy (5-AIDA, 1-7+3+ATRA). The CT was started at 19, 20, 21, 28 and 32nd weeks of gestation. Delivery was planned for all 6 pts at 34-35 weeks of gestation. 2 of 6 pts delivered by their own and 4 – by caesarean section. CT duration while pregnancy constituted 4-21 weeks (1-4 courses). CR was achieved after 1st course CT in all pts, in 5 of 6 – before delivery. In 1 pt the cesarean section was performed at 27 day of induction due to deterioration of hepatic function and fetus-damage symptoms. Differentiation syndrome was diagnosed in 2 of 10 pts. All pts had infectious complications during induction. CR was obtained in 8 of 9 pts (1 early death). 1 pt now is in neutropenia after induction. 1 pt died in CR after the 2nd consolidation due to Ps.aeruginosa sepsis and pulmonary aspergillosis. Late relapses occurred in 3 of 7 pts (in 2 - due to treatment refusal after induction/consolidation). 1 pt refused consolidation but is still in CR for 7 months. Only 5 pts are alive in CR now (1,5,7, 42 and 127 months). 3 y-OS and DFS constituted 30,5% and 34,3%. 3-y DFS in pts who did not refuse the treatment constituted 50%. 7 children were born at the median gestation`s time 36 weeks (34-40). All are alive (4 months -14 years) and healthy.
Summary / Conclusion: Our data demonstrate that APL in pregnant women is characterised by intermediate and high risk disease, most cases occurred in the 2nd and 3rd trimester, CR rate is high, but long term overall results are not so optimistic. We met with psychological problems resulted in 3 treatment refusals. All born children are well, without evidence of any abnormalities; cardiotoxicity in 5 treated with AIDA newborns is not registered so far. Pregnancy may be considered as an adverse prognostic factor in APL.

B1257

IMPACT OF MRD STATUS PRIOR TO HSCT IN PEDIATRIC AML

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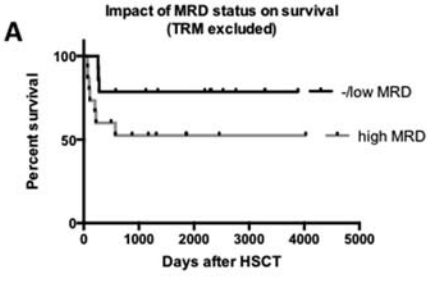
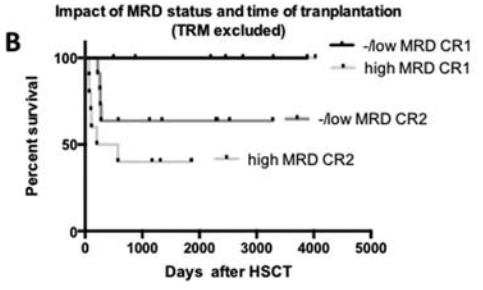
Background: Despite great improvements in treatment protocols, relapse still occurs in 30-40% of all pediatric AML patients, being the leading cause of death with an overall survival (OS) of relapsed AML of only 35%. In an attempt to

improve the OS, attention has been focused on the concept of Minimal Residual Disease (MRD), the ability to detect and quantify small trace amount of residual leukemic cells in patients who have otherwise achieved morphologic clinical remission (CR). The hope is that MRD monitoring can guide preemptive interventions and thereby prevent progression from molecular to hematologic relapse and ultimately improve the course and outcome of childhood AML.
Aims: In this retrospective study we aimed to determine if there is a correlation between the MRD level prior to the allogeneic HSCT and the disease outcome in pediatric AML patients.
Methods: For the MRD assessment we used quantitative reverse transcriptase polymerase chain reaction (RT-qPCR).
The studied cohort consisted of 38 children with a confirmed AML diagnosis comprising one of the following aberrations: *t(8;21), inv(16), t(9;11), FLT3-ITD* or *NPM1* and who had undergone allogeneic HSCT transplantation. Exclusion criteria were Down syndrome and secondary AML. The median age at initial diagnosis was 9.1 years (range 0 – 18 years) and the female:male ratio 0.93.
Results: In the study cohort MRD levels directly prior to HSCT were undetectable in a third (32%, n=12) of the patients, five of these patients died, of which four were due to treatment related mortality (TRM). In the remaining 26 patients, MRD was detectable just before the transplantation. Among those, 18 patients (47% of the entire cohort) exhibited high levels, defined as MRD ≥0.01%. The group with high MRD had a death rate of 56% (4 due to TRM) while the group with low MRD had a mortality rate of 50% (4 of8, 1 caused by TRM). By grouping the non-detectable and low MRD level patients into one group (-/low MRD) and comparing them to the patients from the high group (+ MRD), a trend towards a favourable outcome of the -/low MRD patients in regards to OS was seen, albeit non-significant (P=0.08) (Figure 1A). Looking at the time of HSCT during the disease course we saw a clear superior outcome for patients transplanted in first remission (CR1) compared to second remission (CR2) (P=0.01). Likewise for the donor-type, matched sibling (MSD) vs. matched unrelated donors (MUD) showed a preference for MSD with P=0.009. When testing the timing of the transplantation in combination with the MRD status we again saw that patients transplanted in the first remission had an excellent outcome (fig. B). In cases where HSCT were performed in the second remission the OS considerably worsened, mostly for the patients with high MRD levels prior to HSCT (Figure 1B). We did not observe significant differences for overall survival in the favourable- (*t(8;21)*, *inv(16)*, *NPM1*) and poor-risk- (*FLT3-ITD*, *t(9;11)*) groups. This may be explained by the aberrations *FLT3-ITD* and t(8;21), as the latter exhibited a very poor prognosis with 9 death (4 due to TRM) out of 15 within the first 3 years after HSCT and a good profile for *FLT3-ITD* positive patients where only 1 out of 7 included in the analysis died. However, these data should be interpreted with some caution, as the cohort size is relatively small and the study is therefore with low statistic power. In addition, treatment of the patients has not been standardized within the study cohort.





Summary / Conclusion: In this retrospective multinational study we have examined the impact of Minimal Residual Disease (MRD) levels just prior to hematopoietic stem cell transplantations and found that there seems to be some influence on the outcome when a certain MRD level is present at the time of HSCT. Whether additional therapy to reduce the tumor burden is beneficial before proceeding with HSCTs is still to be tested and verified in future clinical trials.

B1258
IMPACT OF CD200 EXPRESSION IN ACUTE MYELOID LEUKEMIA
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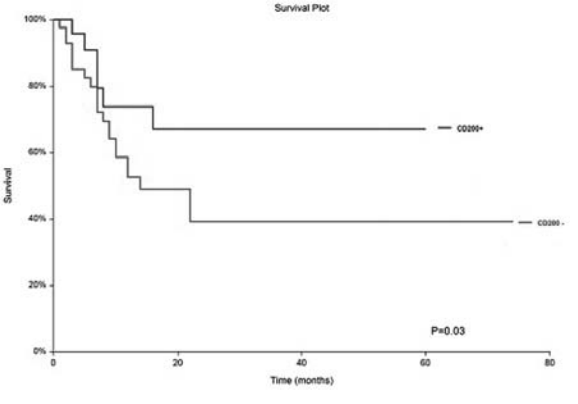
Background: CD200 has been recently included among factors by which tumor cells can escape immune control. CD200 is a transmembrane protein structurally related to costimulatory molecules CD80 and CD86, widely expressed by thymocytes, activated T cells, B cells, dendritic cell, endothelial cells and neurons. The interaction of CD200 with its receptor inhibits immune system, thus facilitating tumor progression. An overexpression of CD200 has been associated with poor prognosis in many solid tumor, in CLL and in multiple myeloma. Moreover aberrant expression of the protein has been associated with negative outcome also in a small cohort of patients with acute myeloid leukemia (AML).
Aims: In the present work we retrospectively evaluated the incidence of expression and the impact on response to therapy in a series of 114 adults with AML.
Methods: One hundred fourteen patients with non-promyelocytic AML treated at our Institutions between 2007 and 2011 were included in this analysis. Blast cells immunophenotype and CD200 expression were evaluated by multiparametric flow cytometry.
Results: CD200 was expressed in 45/114 (39.5%) cases, with a median MFI of 11.8 (range: 3.8-88). No differences in CD200 expression rate were observed according to type of leukemia (de novo vs secondary), WBC count at diagnosis, or between cases with myeloid (M0-M2) or monocytic (M4-M5) morphology. However, higher incidence of CD200 expression was observed in CD34 positive patients compared to CD34 negative ones (35/44, 79% vs 23/69, 33%, P<0.0001). CD200 expression was strongly associated also to favorable cytogenetics (8/9, 89%) compared to patients with intermediate or unfavorable karyotype (29/87, 33%) (P=0.002). Moreover, a lower incidence of FLT3-ITD mutations was detected in CD200 positive cases (3/30, 10%) compared to those lacking CD200 expression (17/53, 32%) (P=0.032). One hundred and three patients received a three-drugs fludarabine-based induction course and was evaluable for response to therapy. Complete remission (CR) was achieved in 27/42 (64%) CD200 positive and in 45/61 (74%) CD200 negative patients (P=0.38), but a higher relapse rate was observed in the CD200 negative group (21/45 vs 6/27, P=0.046). Consequently, 3-years disease-free survival was 37% in CD200 negative patients and 67% in CD200 positive ones (P=0.03) [Figure 1].



Summary / Conclusion: Our data suggest a positive impact of CD200 expression on blasts of AML patients, in term of a lower incidence of relapse. This finding is in apparent contrast with what has been previously reported by Tonks et al. (Leukemia 2007). Reasons for this discrepancy could be the different way in detection of CD200 expression (gene expression and flow cytometry) and the high prevalence, in our CD200+ patients, of favorable features such as good-risk cytogenetics and FLT3 negativity. Moreover, we used an induction therapy based on fludarabine, that is known to significantly reduce the number of CD4+ lymphocytes, probably impacting also on the CD4+/CD25+ regulatory T cells, that suppress the anti-leukemia response, so neglecting the adverse effect of CD200 expression on AML blasts.

B1259
ANTI-TUMOR VACCINATION IN COMBINATION WITH MODIFIED DONOR LYMPHOCYTE INFUSION FOR AN AML RESISTANT PATIENT WITH EARLY EXTRAMEDULLAR RELAPSES
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Background: Extramedullary relapse after allogeneic hematopoietic stem cell transplantation for AML is associated with poor prognosis as a contributor to post-transplant mortality. Due to the lack of efficacious treatment strategies, there is a need for novel approaches to manage extramedullary relapse after stem cell transplantation. PRAME is a tumor antigen that highly expressed in AML blasts and other malignancies but not in normal tissues. Moreover PRAME can elicit immune response in cancer patients.
Aims: To circumvent extramedullary relapse in AML patient by means of infusions of donor lymphocytes activated by autologous tumor cell lysate and recombinant PRAME protein as well as by consequent recombinant PRAME vaccination.
Methods: A 45 years-old male with AML M4-type had been treated with a cycle of chemotherapy following by allo-HSCT from sibling donor. 120 day after allo-HSCT the patient developed relapse with multiple extramedullary lesions in skin (leukemids) and viscera. The patient was treated with courses of chemotherapy and DLI. Subsequently 3 ordinary DLI were carry out (30, 45, 65 day after relapse) and molecular remission was achieved. The 2nd relapse was detected at 100 day after 1st relapse in developing multiple extramedullary lesions in skin (leukemids), viscera and central nervous system whereas bone marrow sample showed complete donor hematopoiesis. Chemo- and radiotherapy were carried out as well as surgical dissection of the lesions. After obtaining Informed Consent patient AML blasts were separated from leukemid-sampled cells and lysed by freezing-melting in liquid nitrogen. Donor lymphocytes ($8*10^6$/ml) were co-incubated *in vitro* in RPMI media with leukemid blasts lysate and recombinant PRAME protein (8 mcg/mL) in the presence IL-2 (100Uts) for 24h. Treated lymphocytes were used for donor lymphocyte infusion as well. Recombinant PRAME antigen was administered subcutaneously at the dose 100 mcg with 300 mcg aluminum hydroxide adjuvant twice with 3-weeks interval.
Results: RQ-PCR analysis of PRAME gene from skin biopsy-sampled cells revealed high level of expression. A bone marrow biopsy revealed complete morphological remission without evidence of minimal residual disease for all the time later. PRAME-injection was performed at the same day as DLI with stimulated lymphocytes (105 and 127 day after 1st relapse). Local inflammatory response at the injection site was observed. The extensive anti-relapse therapy was kept on. After first modified DLI the skin lesions disappeared. At parallel the stimulated donor lymphocytes were analyzed for cytotoxic effect *in vitro* in the presence auto-serum of the patient. Their ability to inhibit the growth of PRAME-expressing tumor cells (K562 line) was 2 to 5-fold better than non-stimulated donor lymphocytes. Overall survival at the time of writing is 14 months after immunotherapy.
Summary / Conclusion: Tumor specific immunotherapy in combination with allo-HSCT may be a potent curative method for AML patients with multiple extramedullary relapses.

B1260

MYELOID MORPHOLOGY AND THE NUMBER OF CO-EXPRESSED LYMPHOID-ASSOCIATED ANTIGENS CORRELATE WITH POORER CLINICAL BEHAVIOUR IN CBF-POSITIVE ACUTE MYELOID LEUKAEMIAS

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Background: Acute myeloid leukaemias (AML) with t(8;21) or inv(16)/t(16;16) are associated with rearrangements of the …core binding factor" (CBF) genes CBFA and CBFB, leading to disturbances in the transcription of genes involved in myeloid differentiation. Leukaemias defined as CBF-AML are recognized as nosological entities in the WHO classification and generally characterized by good prognosis after intensive therapy. However, heterogeneity in regard to blast cells features is still being reported.
Aims: To analyse the main morphological and immunophenotypic features of the blast cell population in AML patients (pts) with *AML1-ETO* and *CBFb-MYH11* fusion transcripts and to assess the clinical behavior in regard to overall survival (OS).
Methods: In total, 53 patients (32 male:21 female) were diagnosed with CBF-AML for a 10-years period, at a mean age of 37 years (range 6-79). *AML1-ETO* or *CBFb-MYH11* mRNA was detected by RT-PCR, complementing morphology and immunophenotyping by a panel of antibodies allowing for determining lineage affiliation, maturation and aberrant antigen expression. Follow up for a mean of 27 months (2-120) were available for 29 adult pts in whom induction therapy was completed at the National Haematology Hospital.
Results: The *AML1-ETO(+)* AML (n=26) showed variable morphology, corresponding to M1-M2-M2Eo-M4-M4Eo FAB subtypes. Characteristic morphology was observed in only part of the cases. In 23% of the pts pathological eosinophils (Eo) were increased (M2Eo, M4Eo). In addition to myeloid-associated markers, *AML1-ETO(+)* AMLs were characterized by a high incidence of CD34(91%), CD117(100%) and aberrant co-expression of CD56(69%) and/or CD19(53%) and CD4(23%). The morphological spectrum of *CBFb-MYH11(+)* AMLs (n=29) showed similar variations according to the FAB criteria. However, proliferation of Eo was found in 81% of the pts, bearing pathological basophilic granules in all cases. *CBFb-MYH11(+)* AMLs were also characterized by frequent CD34/CD117(100%) and aberrant CD56(31%) and/or CD2, CD4; CD7; CD19. Compared to *AML1-ETO*(+) p*ts, CBFb-MYH11(+)* AMLs had higher % of peripheral blood blasts (38% vs 46%, P=0,036), white blood cells (16G/L vs 32G/L, P=0,000) and platelets (18G/L vs 62G/L, P=0,036), while the analysis did not reveal any differences in terms of age, % bone marrow blasts, hemoglobin, LDH, DFS and OS. CR achievement was a major factor for better prognosis with 73% OS and a median not reached. Regardless of the type of transcripts the myeloid morphology was associated with a lower OS (22%, median - 8 mo) vs 77% with a median not reached in the M4 group (P=0.01) while abnormal Eo had no impact. The analysis confirmed the unfavorable value of CD56: median OS not reached in CD56(-) cases vs 17 mo in CD56(+) (P=0.02). Furthermore, the overall number of lymphoid-associated markers contributed to the poorer survival. Median OS was not reached for the time of the follow up in the negative cases, and was 31 mo in the presence of 1 marker and 8 mo if more than 1 marker was co-expressed.
Summary / Conclusion: The present study contributes to the heterogeneity of CBF-AML in regard to morphology and immunophenotype. Lymphoid-associated markers might provide additional prognostic information and are recommended in the diagnostic panel. An intergrated approach would allow for a risk-oriented therapies and optimized outcomes.
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B1261

DECREASED SERUM RETINOL-BINDING PROTEIN IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Retinol-binding protein (RBP) is a single polypeptide chain protein that is classified under prototypical lipocalin due to its similarity to other lipid binding proteins. The ternary complex transthyretin-RBP-retinol thus serves to transport retinol from the storage site liver in the circulation and to deliver it to target tissues. The plasma membrane RBP receptor (STRA6) transports vitamin A from its blood carrier RBP into cells. RBP also maintains the free level of retinol in the plasma. Additionally, RBP secretion was positively regulated by retinol. In previous studies, RBP was evaluated as nutritional indice for children with acute myeloid leukemia (AML). However, there is no data available assessing the RBP level in adult and elderly patients with AML.
Aims: To assess the RBP level in AML patients of all age groups and to explore the potential relationships between serum RBP and clinical characteristics (*e.g.* white blood cell count, molecular markers) of AML patients.
Methods: In this study, 208 patients with *de novo* AML, 88 patients with benign hematological diseases, and 168 healthy subjects were enrolled. Peripheral blood sample of patients before treatment and healthy subjects were collected, and then were determined RBP and prealbumin concentrations by Automatic Chemistry Analyzer. Cytogenetics and molecular mutations were determined in patients with AML. Patients gave informed consent prior to enrolment in the study. Statistical analysis was performed with the software SPSS 17.0.
Results: AML patients (35.58±15.42 mg/L) had lower RBP concentration compared with benign hematopathy (42.69±15.62 mg/L) (u = -3.662, P<0.001) and healthy control (57.12±29.23 mg/L) (u = -10.187, P<0.001). AML patients of non-APL (32.62±12.94 mg/L) exhibited lower RBP concentration compared with APL patients (41.17±18.07 mg/L) (u = -3.161, P=0.002). A negative association could be observed between serum RBP level and peripheral white blood cell count (r = - 0.179, P=0.01), which was more evident in patients with M4 (r = - 0.499, P=0.036) and M5 (r = - 0.412, P=0.029). Elderly AML patients (≥ 60 years) had highest RBP level compared with children (< 16 years) (t = 2.284, P=0.049) and adult patients (16 ~ 59 years) (t = 2.572, P=0.011). Patients carry *FLT3-ITD* mutation [27.8 (21.35, 35.15) mg/L] exhibited lower RBP concentration than those without [35.75 (27.63, 43.55) mg/L] (u = -2.173, P=0.030).
Summary / Conclusion: These phenomena may suggest decreased level of retinol in patients with AML, which might be associated with excessive consumption of RBP in the proliferation of leukemic cells.

B1262

PERCENTAGE AND/OR ABSOLUTE LYMPHOCYTE COUNT AT DIAGNOSIS: PROGNOSTIC IMPACT IN ACUTE MYELOID LEUKEMIA (AML)

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Background: White blood cell (WBC) count at diagnosis is considered an

important prognostic factor for both acute lymphoblastic (ALL) and acute myeloid leukemia (AML). Absolute lymphocyte count (ALC) after induction treatment has been related to prognosis in acute leukemias, whereas it remains of importance after hematopoetic stem cell transplantation (HSCT) as a marker of immune reconstitution.

Aims: ALC at diagnosis has been studied as a predictor of survival for non-Hodgkin lymphoma and other cancers. There is little evidence regarding the prognostic significance of ALC and lymphocyte percentage (Ly%) at AML diagnosis.

Methods: We retrospectively studied ALC in 266 AML patients with median age 50 years (range: 14-75), male/female: 155/111, de novo/secondary: 197/69. Cytogenetic analysis was available in 250/266 cases and was favorable/intermediate/poor prognosis in 31/164/55 respectively. Complete remission (CR) was accomplished in 183/266 (68.8%). Median ALC at diagnosis was 3.515 x 10^9/μL. Low ALC (<1x10^3/μL) at diagnosis was observed in 44/266 (16.5%) patients. This patient group correlated with low CR rate (21/44, ie 47.7%, P=0.001), low WBC count, neutropenia and secondary AML, in comparison to patients with an ALC count >1x10^3/μL. A trend to shorter disease free survival (DFS) was observed only at the subgroup of intermediate risk cytogenetics (P=0.07). Patients with relative lymphopenia (Ly% ≤20%) were 125/266 (47%) and presented with elevated WBC count, LDH, blast count and neutrophil count at diagnosis and had the same trend for a shorter DFS (P=0.07) in comparison to patients with ≥ 20% lymphocytes at diagnosis.

Results: Combining the above findings, a group of 147 patients presenting with relative or absolute lymphopenia could be identified. These patients had a lower CR rate (64% vs 74.8%, P=0.05). Outcome was significantly worse in this group compared with non lymphopenic patients: 5-year DFS was 24% vs 41% (P=0.01) and 5-year OS was 31% vs 46% (P=0.015). Lymphopenia did not influence survival when studied seperately in the 3 cytogenetics risk groups. In multivariate analysis lymphopenia defined as ALC < 11x10^3/μL was an independent prognostic factor affecting OS (P=0.016) but not DFS. Other independent prognostic factors for OS were karyotype, age, CR achievement post induction therapy (P<0.001) as well as LDH levels at diagnosis (P=0.005).

Summary / Conclusion: In conclusion, patient's lymphocytes at diagnosis may have some assistive role in AML and the prognostic significance of lymphopenia in this setting needs further investigation.

B1263

CD56 EXPRESSION IN ACUTE PROMYELOCYTIC LEUKEMIA – A PREDICTIVE INDICATOR OF RELAPSE?

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Background: Acute Promyelocytic Leukemia (APL, M3 in FAB classification) is a subtype of acute myeloid leukemia characterized by a unique chromosome translocation t(15;17). It is usually associated with a good prognosis despite coagulopathy often present at clinical presentation. Leukemic cell expression of CD56 at the time of diagnosis has been associated with a worse outcome, registering a shorter disease-free survival (DFS) as well as a lower overall survival (OS).

Aims: To characterize the adult patient population diagnosed with APL followed at our institution; assess the promyelocytic cell phenotype at diagnosis and verify if the presence of the CD56 antigen confers a worse prognosis. It is intended to compare the two groups (negative and positive CD56) with regard to the clinical presentation, response to therapy, relapse, DFS and OS.

Methods: We conducted a retrospective analysis of all APL cases diagnosed between 1999 and 2012. Thirty nine patients who had an evaluation for CD56 expression (> 20% positivity by flow cytometry) were selected and comparison of the two groups (negative and positive CD56) was made. Data analysis was performed using the SPSS v20.

Results: Median age of the thirty nine patients with APL was 42 years old (range 17-78 years), fifty-nine percent of the patients were women. CD56 expression was considered positive at diagnosis in twelve cases (30.8%); twenty-seven (69.2%) weren´t associated with the antigen presentation on blast cells. Those subgroups were similar in median age at diagnosis and gender (35.5 years and 58.3% female gender if CD56+, and 43 years and 59.3% female patients if CD56-). Comparing CD56+ patients with those who didn´t express that antigen, one can found (despite no statistical significance) that CD56+ patients have a more aggressive clinical and laboratorial presentation: hemorrhagic presentation (100% versus 81.5%); intravascular disseminated coagulation (33.3% versus 11.3%); median leukocyte count of 10.1x10^9/L (range, 1 to 102) versus 4.4x10^9/L (range, 0.4 to 40.8); 50% of all patients CD56+ had an hiperleukocytary presentation, only 16% in CD56- subgroup; and a median fibrinogen level of 120mg/dL (range, 62 to 209) versus 141,5 mg/dL (range, 36 to 372). CD56 expression is associated with a higher risk stratification (P=0,018) - 50% of the patient are at a high risk at diagnosis versus 14.8% in CD56- subgroup. Patients were treated with ATRA, idarrubicine, mitoxantrone and cytarabine with similar response in the two subgroups (complete remission 83.3% if CD56+ and 85.2% if CD56-). In the APL CD56+ subgroup there is a higher relapse rate 41.7% versus 7.4% in CD56- subgroup. Despite the difference in median time to relapse (16 months if CD56+ and 37 months if CD56-) we couldn't find statistical significance for disease free survival (Kaplan-Mayer). However, the relative risk for relapse is 5.63 (1.27-25.01, confidence interval 95%) in the CD56 positive population. In our population there were no differences in overall survival (median follow-up time subgroup CD56+ 39 months, range 10-115; subgroup CD56- 47 months, range 1-125).

Summary / Conclusion: Despite the limitations of our retrospective study we demonstrated a significant relative risk for relapse in patients with APL and positive CD56. This antigen is probably a prognostic marker that in association with other indicators can provide a stratification risk.

B1264

TREATMENT WITH AZACITIDINE INCREASES OVERALL SURVIVAL, REDUCES INFECTIONS AND HOSPITALIZATIONS IN ELDERLY PATIENTS WITH ACUTE LEUKEMIA COMPARED WITH INTENSIVE CHEMOTHERAPY: A MONOCENTRIC STUDY

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Background: Survival in older patients(>65 years old) with acute leukemia is shorter than young patients.The inferior outcome reflects the major frequency of higher risk karyotypes,history of antecedent hematologic disorder, comorbidity that limit delivery /clearance chemotherapy

Aims: We reviewed the baseline characteristics and outcome after therapy of 40 consecutive patient aged 65 years and older with acute leukaemia admitted and treated in our institution from 2009 to 2013. 20 patients with performance status 2 or3, or some older patients that did not accepted intensive chemotherapy were treated with azacitidine(Aza group),while 20 patients with performance status 1 or 2 younger than 75 years were treated with intensive chemotherapy(IC group)

Methods: Azacitidine was administered subcutaneously (75mg/m2/d) for 7 days of every 28-day cycle until progression,while IC consisted of 1 induction course with mitoxantrone, cytarabine and etoposide followed by 2 course with idarubicine, cytarabine and etoposide (in patients in complete remission).In aza group(7 female and 13 male)the median age was 76.5(range 65-83),median white blood cells was 3.3 x 10^9(1.1-48),median hb was 7.85(6.2-12.2),median platelets 70 x 10^9(17-245),median bone marrow blasts counts was 47%(21-90%).Karyotype risk stratification was normal in 11(55%),intermediate in 2(10%) and high risk in 7 (30%).The median number of cycle was 10 (range 1-29).The fever infections that requiring IV antiobiotics were in 7 patients(35%),but only in 2(10%)were septic and 2(10%) bronchitis that needed hospitalization and 5/7(71%)of infected patients not reduced rate and time of next cycle,while 1(5%)patient presented an Early Death(ED,within two months after diagnosis).15 patients in Aza group achieved response to treatment in fact overall response rate (ORR) was 75%(complete remission 45%+ partial remission 30%)with a median duration of 9 months (range1-29),the median overall survival (OS) was 12 months (range 2-29).The transfusion independence was in 5(25%) patients, reduced in 9(45%) and stable in 6(30%).In IC group(10female and 10 male)the median age was 70.5(range 65-86),median white blood cell was 5.3 x 10^9 (1.0-450),median hb was 8.05g/dl(4.1-13.7),median platelets 33 x 10^9μl (7-148),median bone marrow blasts counts was 65% (range 35-90%).Karyotype risk stratification was normal in 15 (80%), and high risk in 5 (20%).The median number of cycle was 1.5 (range 1-3).The fever infections 3-4 grade that requiring IV antimicrobics were in 15 patients(75%) and in 9 (45%)were observed ED.9 patients in IC group achieved response to treatment:overall response rate (ORR) was 45%(complete remission 35%+ partial remission 10%)with a median duration of 3.5 months (range1-11),the median overall survival (OS) was 1.5 months(range 2days-19 months).

Results: Treatment with azacitidine in older patients with acute leukaemia prolongs overall survival compared to the chemotherapy group (P<0.01), expecially in patients with worse performance status(P<0.001),reduces ED (p.0.008) for lower toxicity and lower incidence/severity infections and improve overall response (p0.66)compared with intensive chemotherapy.In Aza group the platelet recovery (>100 x 10^9) after three months of therapy is predictive of complete response to the therapy (P<0.0005).

Summary / Conclusion: Older patients with AML and more comorbidities may benefit from less intensive approaches as azacitidine.Prospective and randomized clinical trials are necessary to confirm the effect of Azacitidine on larger number of elderly AML.

B1265

AMBULATORY CARE OF PATIENTS RECEIVING CONSOLIDATION CHEMOTHERAPY FOR ACUTE MYELOID LEUKAEMIA : A FEASIBLE AND SAFE OPTION FOR SELECTED PATIENTS

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ment of Haematology, East Kent Hospital Trust, Ashford, United Kingdom

Background: Conventional management of patients undergoing induction chemotherapy for acute myeloid leukaemia (AML) involves prolonged inpatient stays during treatment and the following myelosuppressive period. However whether consolidation chemotherapy for patients in remission requires a prolonged inpatient stay, is more contentious.
Aims: To prospectively assess the safety and suitabililty of the ambulatory care model for AML patients in remission after first induction undergoing consolidation chemotherapy.
Methods: We prospectively audited 23 patients deemed sufficiently fit to undergo consolidation chemotherapy as outpatients or to be monitored on an ambulatory basis following inpatient chemotherapy. All patients were in remission following induction chemotherapy and were managed through a single site BCSH Level 2b inpatient and day unit facility with 24-hr access to specialist nurse advice. All ambulatory patients received prophylactic levofloxacin and posaconazole as per local protocol.
Results: The median age was 51 years (range 20-78 years) and the mean distance of the patient's home from the unit was 14.2 miles (range 0.9 – 26.9). Twenty three patients received a total of 61 cycles of chemotherapy of which 16 were given purely on an outpatient basis. The average length of neutropenia during the first consolidation cycle was 13.7 days (range 0-48 days) and 10 patients required admission during the second course (average length of stay 3.7 days) for febrile neutropenia. No patients required organ support. Eleven patients received a third course, with 5 as inpatients. The mean duration of neutropenia was 17.8 days (range 4-46). There were 9 admissions during the third cycle (average LOS 8.7 days), and 2 required ITU admission. There were no fatalities. Four patients received a fourth cycle.
Summary / Conclusion: These data suggests that for AML patients in remission, the ambulatory care model for early discharge following chemotherapy and/or outpatient delivery of consolidation chemotherapy is safe. The second consolidation cycle was associated with more admissions and number of ITU stays, reflecting cumulative toxicity and myelosuppression. We had no deaths throughout the study period and the approach was well received by patients.

B1266
FLAG-IDA: A HIGHLY EFFECTIVE SALVAGE REGIMEN IN PRIMARY REFRACTORY ACUTE MYELOID LEUKEMIA
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Background: The prognosis for patients with primary refractory acute myeloid leukemia (AML) is poor. Achievement of complete remission (CR) in patients with this disease is a prerequisite for subsequent treatment with allogeneic stem cell transplantation. The FLAG-Ida protocol has shown the most success compared to other salvage therapy regimens preceding transplantation.
Aims: An investigation to find the most efficient salvage protocol for achieving CR of long duration and a high rate of overall survival (OS) in primary refractory AML.
Methods: The single-center study involved 53 patients with primary refractory AML who had failed to achieve CR after first-line standard-dose remission-induction therapy. During the period January 2008–December 2012, the subjects had received one cycle of the 3+7 protocol: ARA-C, days (D) 1–7 and daunorubicin D 1–3. After that FLAG-IDA (Fludarabin 30mg/m^2 D1-5; ARA-C 2000mg/m^2D1-5; Idarubicina 8mg/m^2 D1-3, G-CSF 300mg/m^2) or Mitoxantrone+Vepesid (Mitoxantron 10mg/m^2 D1-5 and Vepesid 100mg/ m^2 D1-5) or the HiDAC regimen (ARA-C 2x3g/m^2 D1,3,5) were given as salvage therapy. We determined the rate of achieving CR, duration of CR and OS.
Results: The median age of the patients was 53 years, range 19-70 years. Among them 16 (32.2%) received the FLAG-IDA regimen, 22 (41.5%) Mitoxantrone+Vepesid and 15 (28.3%) HiDAC. The rate of CR was 62.5% for patients treated with the FLAG-IDAregimen, which was significantly greater than for the other two protocols, (22.7% for Mitoxantrone+Vepesid and 13.3% for HiDAC;P=0.006). An age greater than 55 years was the only factor predictive for a significantly lower response rate (P=0.05). The duration of CR in subjects who receivedthe FLAG-IDA protocol was 4 months. This was significantly longer than for those given the other two regimens (2 months for both; P=0.022). Moreover, OS was significantly longer for patients treated by the Flag-Ida regimen (6 months) than for those receiving the other two regimens (4 months each; P=0.004). Three of the ten responders underwent successful bone marrow transplantations. The median remission duration for the remaining seven patients was 5 months, and the median survival time was 7 months.
Summary / Conclusion: The FLAG-IDA regimen showed significantly higher efficiency in comparison to the other two regimens in our group of patients with primary refractory AML. These data confirm that the FLAG-IDA regimen has high antileukemic activity in refractory AML.

B1267
TREATMENT OUTCOME OF PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA (APL) - A SINGLE CENTRE EXPERIENCE
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Background: Acute promyelocytic leukemia (APL) is a unique subtype of acute myeloid leukemia (AML) with distinct hematopathologic, cytogenetic, molecular and clinical features. The prognosis of APL has transformed from the worst among AML as it used to be, to currently the best ever since the emergence of all-trans-retinoic acid (ATRA). Patients in our centre have been routinely induced using ATRA in combination with anthracycline-based chemotherapy as first line regimen since 1994.
Aims: In this study, we evaluated the outcome of adult APL patients who received modified AIDA protocol in our institution from January 1994 till December 2012. The treatment protocol consisted of remission induction using co-administration of ATRA and Idarubicin followed by 3 courses of Idarubicin consolidation, and 18 months of maintenance treatment with 6-Mercatopurine/ Methotrexare/pulse of ATRA.
Methods: All records of the patients were analyzed in a non-randomized retrospective study on 1st January 2013. Overall survival was estimated by the Kaplan-Meier curve.
Results: A total of 114 patients were diagnosed as APL for the last 18 years which represented 12.98% of all AML cases. Most of the patients were young with the median age of diagnosis at 29 years (range 12 to 76) and predominantly female (male 39.47% and female 60.53%). The ethnic composition of our cohort was Malays 48.25 %, Chinese 35.96 %, Indian 10.53 % and others 5.26 %. Majority of our patients were at high risk of relapse on presentation as most of them fell into the high risk group 47.06% (intermediate risk group 44.78% and low risk group 8.95% respectively). Five patients died right after hospitalization before treatment initiated due to bleeding complication mainly CNS hemorrhage (60%). The remaining 109 patients proceeded with ATRA-Idarubicin chemotherapy with an induction death of 2.75% (2/3 of mortality due to intracranial hemorrhage and 1/3 attributed from sepsis) while during consolidation we observed a mortality rate of 1%. There was only 2 patients (1.8%) complicated with ATRA syndrome. Our patients achieved an overall complete remission (CR) rate of 90.48%. The cumulative relapse rate and mortality were 28.71% and 25.44% respectively over the 228 months observation period. 24 out of 29 relapsed patients managed to be salvaged with mainly Arsenic Trioxide (ATO)-based (66.67%) and achieved second CR of 75%. The event-free survival (EFS) and overall survival (OS) of the cohort will be presented later.
Summary / Conclusion: A modified AIDA protocol for adult APL leads to improved treatment outcomes, with limited ATRA syndrome-associated toxicity in our centre despite majority of our cohort are of high risk patients. Relapsed cases can be salvaged with ATO-based therapy. Early detection and active management of bleeding complications remained the most important measure that may help in reducing mortality.

B1268
TREATMENT OF MOLECULAR RELAPSE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA
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Background: Evidence for beneficial effect of minimal residual disease (MRD) monitoring and early intervention (=pre-emptive therapy) in non-APL acute myeloid leukemia (AML) pts. are very limited.
Aims: To assess usefulness, efficacy and toxicity of various treatment regiments in the therapy of molecular relapse in non-APL AML patients.
Methods: We have performed a retrospective analysis of molecular relapses in AML pts. with molecular target and its treatments. RQ-PCR was used for the MRD monitoring. Molecular relapse was defined as confirmed reappearance of the fusion transcript or mutated gene detection or its 10-fold increase in pts. with persistent positivity and corresponding bone marrow cytology, immunophenotype and cytogenetic analysis remained negative.
Results: In the study period 1/1/2003 – 1/2/2013 we have treated 38 molecular relapses in 21 pts.. Median follow up was 35,5 months (range 12-121 months). The median time from the end of initial AML therapy to the first molecular relapse was 5 months (range1,0-29,0 months) –4,0 months (range1,0-14,0 months) for RUNX1/RUNXT1 positive pts.; 2,1 months (range2,0-14,0 months) for CBFB/MYH11 positive pts.; 13,5 months (range5,0-29,0 months) for patients with MLL fusion abnormalities;4,0 months (range2,0-4,0 months) for patients with NPM1 mutation. The frequency of treatment regimens and their efficacy in the first molecular relapse (n=21) was as follows: conventional chemotherapy ("5+2" like regimens) (CHT) – 19,0% (n=4), clofarabine (CLO) – 42,8% (n=9), gentuzumab ozogamicine (GO) – 14,3% (n=3), immunomodulation after allogeneic hematopoietic stem cell transplantation (IMMUNO) – 19,0% (n=4), and low dose ARA-C (LD-ARAC) –4,8% (n=1). The overall response rate (RR = CMoR + PMoR) to pre-emptive therapy of the first molecular relapses was

66,6 % (14/21) (47,6% CMoR+ 19,0% PMoR) - CLO – 77,8%RR (55,6% CMoR + 22,2% PMoR); CHT – 100% RR (50% CMoR + 50% PMoR); IMMUNO – 50% RR (CMoR); GO - 33.3% RR (CMoR); LD-ARAC - 0% RR.
In the follow up period 69,2% (n=9) of responding pts. revealed the second molecular relapse with median7,0 months (range1,0-19,0 months). Moreover, in 15,9% (n=2) hematological relapse occurred without foregoing molecular relapse with median3,5 months (range3,0-4,0 months). The second molecular relapse was pre-emptively treated in 9 patient with RR 66,6% (n=6) (CR+PR). However, again in 4/6 (66,6%) of successfully treated pts. with the second molecular relapse, subsequent molecular relapses occurred with median 9,5 months (range4,0 – 16 months) and were again treated with RR 25% (n=1).
At any time during treatment 61,9% of patients underwent allogeneic HSCT.
Overall, only 3 pre-emptively treated patients did not revealed any further hematological or molecular relapse during the observational period (2/3 pts. underwent allogeneic HSCT)
6/21 (28,6%) of patients with molecular relapse survived during the study period (all with initial or subsequent molecular response) with OS 68 months, compare to OS of 17 months in 15/21 (71,4%) of pts that expired during the follow up period.
Summary / Conclusion: Our study has shown feasibility of MRD monitoring and pre-emptive strategy using molecular targets in non-APL AML pts. Different pre-emptive treatment strategies led to response in 66,6% of molecular relapses with the highest frequency of CMoR when CLO was used. Even pre-emptive therapy enables us to obtain time for preparing allogeneic HSCT (performed in 2/3 of patients), majority of patients relapsed again in follow up period. However, our data showed that this approach (even in limited number of patients with initial or subsequent response) could provide prolongation of overall survival.

B1269

CYTARABINE PLUS IDARUBICIN AS INDUCTION THERAPY FOR UNTREATED ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA. A SINGLE CENTER RETROSPECTIVE ANALYSIS OF 141 PATIENTS.

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Background: The chemotherapy regimens containing cytarabine and an atracycline, traditionally known as "7+3", remain the most effective induction therapy for untreated adult patients with non-promyelocytic acute myeloid leukaemia (AML).
Aims: Present the response rates, overall survival (OS) and relapse free survival (RFS) of adult patients with non-promyelocytic AML, diagnosed and treated at single haematology center.
Methods: We performed a retrospective analysis of the clinical data of patients with non-promyelocytic AML, diagnosed between January 1995 and December 2010 and treated with the "7+3" induction containing cytarabine (200mg/m2/d as a 7-days continuous infusion) plus idarrubicin (12mg/m2/d on the first 3 days).
Results: We identified 141 patients with a median age at diagnosis of 46 years (interval 15 to 67), including 43 (31%) with more than 55 years. According to de World Health Organization (WHO) classification, 73 patients (52%) had AML NOS, 33 (23%) AML with recurrent cytogenetic abnormalities, 31 (22%) AML with myelodisplasia-related changes, 3 (2%) therapy-related AML and 1 (1%) myeloid sarcoma. The SWOG/ECOG cytogenetic risk was accessed in 135 patients. Sixty-nine (51%) were classified as intermediate risk, 25 (19%) as favorable risk, 22 (16%) as adverse risk and 13 (14%) as unknown risk cytogenetic abnormalities. After induction, 101 patients (71.6%) achieved a complete remission (CR), 5 (3.5%) a complete remission with incomplete hematological recovery (RCi) and 35 (24,8%) were resistant. Ninety-eight of the 101 patients who achieved a CR were consolidated with cycles of high dose cytarabine, followed in 15 patients by allogeneic hematopoietic stem cell transplantation (AlloHSCT) in first CR. A 28 months OS was registered, with a minimum of 18 days and a maximum of 141 months, associated with a 5 and 10 years survival probability of 43 and 34%, respectively. Fifty-two (49%) of the patients that respond to the induction treatment registered disease relapse, with a median time to relapse of 48 months, interval between 18 days to 141 months, with a 5 and 10 years RFS probability of 60 and 32%, respectively. For the group of patients with an age at diagnosis inferior to 55 years, the OS was 51 months, which was significantly higher when compared to the group with more than 55 years that registered an OS of 16 months (P=0.034). The stratification of patients by cytogenetic risk also provided an OS significantly different between favourable, intermediate and adverse risk groups, 91, 37 and 10 months, respectively, P=0.013.
Summary / Conclusion: In accordance to results published by other groups, in our department AML remains a disease associated with a poor OS, which is worse in patients with more than 55 years of age and with cytogenetic abnormalities associated with an adverse outcome.

B1270

EARLY DETECTION OF SERUM GALACTOMANNAN ANTIGEN IN HEMATOLOGIC MALIGNANCIES: IMPACT OF A LABORATORY ASSAY IN ASPERGILLUS INFECTION MORTALITY RATE

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Background:
The incidence of deep fungal infections in patients with malignancies has increased dramatically over the past decades**.** Aspergillus spp. represent the main cause of these infections. The serum galactomannan(GM) antigen detection is a relatively recent test and it is recognized as an indirect mycological criteria for diagnosis of invasive aspergillosis. Mortality rates of up to 60% have been reported with invasive *Aspergillus*infections in high-risk hematology patients,which makes early diagnosis of said infection an important challenge.
Aims: 1.-Evaluate the diagnostic potential of the determination of GMinserumin patients with hematological malignancies and fever and /or respiratory symptoms. 2.- Set mortality group diagnosed with invasive aspergillosis as EORTC criteria.
Methods: We have evaluated 70 patients between 2008 and 2012, supplying 89 episodes of fever syndrome, 39 in men and 50 in women, with an average age of 49 (range 14-83 years). Hematologic malignancies in these patients were: 42 acute myeloid leukemia, 11 acute lymphoid leukemia, 7 Hodgkin lymphoma, 7 non Hodgkin lymphoma, 5 chronic lymphocytic leukemia, 4 multiple myeloma, 3 aplastic anemia, 3 acute mixed leukemia, 6 myelodysplastic syndrome and 1 chronic myeloid leukemia. An early GM determination (ELISA)was performed for persistent fever syndrome, despite antibiotic treatment, mainly with an initial respiratory focus (56 episodes). After a positive result, a chest high resolution CT was requested followed by fibrobronchoscopy depending on real clinical suspicion and the results. The diagnosis of invasive aspergillosis was made using EORTC criteria (possible, probable and proved).
Results: The patients suffering from fever and respiratory symptoms were 58 (65%), presenting a positive GM rate of 67% (39 cases). In these cases, a probable or proved aspergillosis diagnosis was confirmed in 25 cases (64%). In the group of patients with fever without respiratory symptoms (31 episodes), 18 positive GM were detected (58%), corresponding with suggestive aspergillosis CT in 7 cases (39%). The test was more sensitive when patients have respiratory symptoms with stadistically significance (P=0,05). Amongst the probable or proved aspergillosis diagnostics (32), we find 9 related deaths, which indicates a related mortality rate of 28%.
Summary / Conclusion: An early GM determination is a profitable test in patients with hematological malignancies and persistent fever, especially if they present respiratory associated symptoms. The implementation of this diagnostic test has enabled us to set up an early treatment, reducing the associated mortality rate compared with previous published results.

B1271

SECONDARY ACUTE MYELOID LEUKEMIA – FIRST LINE TREATMENT WITH CYTARABINE, DAUNORUBICIN AND CYCLOSPORINE, A PORTUGUESE HOSPITAL'S EXPERIENCE

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Background: Secondary acute myeloid leukemia (s-AML) includes AML with myelodysplasia-related changes and therapy-related AML, whenever AML occurs after a preceding disease treated with chemotherapy, irradiation, or both. In the last years, the incidence of s-AML has been increasing, accounting for 10-30% of AML cases**.** The incidence is age-dependent, with s-AML constituting a larger part of AML in the elderly than in the young. The first line treatment offered to these patients is not consensual, and the combination ofan antraciclinwithcytarabineand cyclosporine isone of the optionsto consider.
Aims: To characterize the group of patients treated with cytarabine, daunorubicin and cyclosporine (SWOG 9126 Protocol) as first line treatment of patients diagnosed with s-AML. To present the results referents to the overall survival (OS), disease free survival (DFS) and response rates of this goup.
Methods: We analyzed the clinical data of all patients admitted in an Onco-hematology center, diagnosed with s-AML in the period of January1, 1998, to December 31, 2010, treated with SWOG 9126 Protocol as first line induction scheme. Survival curveswere obtainedbyKaplan-Meier methodand differences wereassessedby thelog-rank testconsideringsignificantdifferenceswithP<0,05.
Results: In the considered period of time, we identified 46 patients diagnosed with s-AML. The median age was 56 years (24-76), 52,2% of which were men. Around 80% of all patients had an ECOG performance status of 0 or 1. According to the World Health Organization (WHO) 2008 classification, 26 patients (56,5%) had multilineage dysplasia AML, 16 patients (34,8%) had therapy-related AML and 4 patients (8,7%) were classified as having AML not otherwise specified. Regarding SWOG's cytogenetic risk classification, patients were divided as follows: 52,2% in the intermediate group, 32,6% in the unfavorable group and4,3% in the favorable group (10,8% unknown). After treatment 32

patients (69,6%) achieved complete remission (CR). Treatment toxicity with this regimen was acceptable in all patients. Eleven patients underwent allogeneic bone marrow transplantation as postremission therapy, 9 of related donor and 2 of an unrelated donor. The median OSwas 12,1 months (0,5-81,2) and the median DFS was 24,9 months (3,2-81,2). We obtained a 3 year's survival rate of 20%. The analysis of OS according to the cytogenetic risk classification did not show statistically significant differences.
Summary / Conclusion: The use of SWOG 9126 protocol as first line treatment of patients with s-AML led to similar CR rates as those reported in current literature. Although the majority achieved CR after induction therapy, the high recurrence rate of this disease reflects on the poor prognosis of these patients.

B1272
CD117 EXPRESSION IS ASSOCIATED WITH IMPROVED EVENT AND OVERALL SURVIVAL IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA TREATED WITH AIDA BASED REGIMEN
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Background: Flow Cytometry immunophenotyping is important tool for the diagnosis of APL. The antibodies in our routine panel included CD34, CD117, HLA-DR, CD2, CD11b, CD11c, CD13, CD14, CD16, CD33 and CD56.
Aims: Our purpose to analyze the prognostic impact of immunophenotyping in patients with APL treated with an AIDA based regimen.
Results: We have prospectively analyzed the prognostic impact of antigenic markers, assessed by flow cytometry, in a series of 51 newly consecutive genetically confirmed APL between August 2004 and December 2010 treated with the Spanish PETHEMA LPA99 trial in the Department of Hematology of Aziza Othmana University Hospital, Tunis, Tunisia. One patient died from CNS bleeding before treatment and 50 patients received induction therapy according to the PETHEMA LPA99 trial. Median age was 30 yr (range 4-71), M/F was 0.64, median WBC was $4.4x10^9$/L (range 0.6 - 123). Additional cytogenetic abnormalities were seen in 17 patients (39.5%). Median body mass index (BMI) was 23.5 kg/m^2 (range 13- 40). The immunophenotyping profile of our patients is summarized in the table below:

Immunophenotyping	n (%)
CD33+	48 (94.1)
CD13+	49 (96)
CD117+	29 (67.8)
CD34-	45 (80.3)
HLADR-	47 (92.1)
CD2+	9 (17.6)
CD56+	4 (7.8)

We found a significant correlation between the expression of CD117 and BMI<30 kg/m^2 (P=0.045). The expression of CD2 was signifigantly more frequent in microgranular (61.5% vs 3%, P<0.0001) than in hypergranular form, and it is notably correlated with leukocytosis (P=0.037). Expression of CD117 was noted as an independent factor of OS (89.3% vs 61.9%) P=0.033 and EFS (89.3 % vs 52.4%) P=0.008.
Summary / Conclusion: We found the expression of CD117 to be significantly associated with better EFS and OS in patients with APL.

B1273
THREE CORE-BINDING FACTOR ACUTE MYELOID LEUKEMIAS (CBF-AML) IN A SINGLE PATIENT
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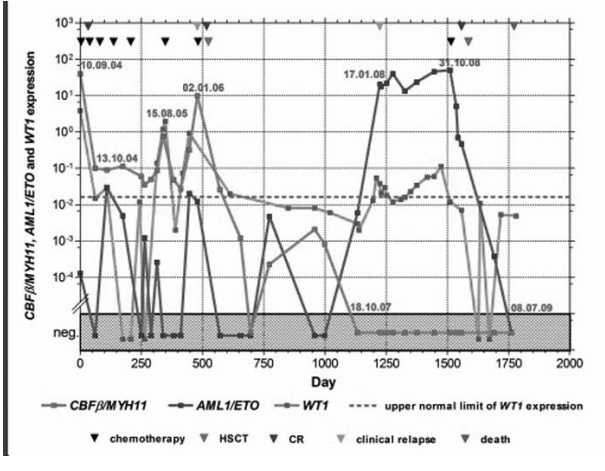
Background: CBF-AMLs carrying fusion genes *AML1/ETO* or *CBFβ/MYH11* predict a relatively favourable outcome and are classified as a low risk AML group. Hardly ever these two aberrations appear simultaneously in a single patient.
Aims: To report a male patient with AML carrying both the *AML1/ETO* and *CBFβ/MYH11* fusion genes at diagnosis, who developed a donor-derived AML with *AML1/ETO* translocation following hematopoietic stem cell transplant (HSCT).
Methods: At diagnosis, bone marrow expression of *AML1/ETO* and *CBFβ/MYH11* fusion genes was screened by qualitative RT-PCR. Quantitative real-time RT-PCR was performed to monitor MRD (the two given fusions in bone marrow and *WT1* gene expression in peripheral blood). To determine the origin of the *AML1/ETO*+ clone after the HSCT, real-time allelic discrimination assay for an HLA polymorphism was employed.
Results: Case Report: In a 30-year-old male with WBC 256.1 G/l, AML FAB M1 type of AML was diagnosed on 10.09.04, CNS infiltration was also suspected. *CBFβ/MYH11* fusion associated with an exon 8 *C-KIT* frameshift mutation was detected. Screening for *FLT3*/ITD, *FLT3*/TKD, *K-RAS*, *N-RAS* and *JAK2* mutations was negative. After the classical "3+7" idarubicin + cytarabine induction therapy (+ intrathecal methotrexate), the patient reached complete remission (CR) with a nearly 3 logdecrease of *CBFβ/MYH11* transcript and *WT1* on the borderline of normal expression on 13.10.04 (Figure 1). Consecutively, he received 4 consolidation cycles of high-dose cytarabine, which was followed by another slight decrease (but not negativity) of *CBFβ/MYH11* expression. *WT1* expression was within normal levels (01.06.05). However, both *CBFβ/MYH11* and *WT1* positivity grew again considerably and on 15.08.05 another "3+7" therapy was given.Despite it,the patient faced overt clinical relapse (with CNS infiltration on top of it) on 02.01.06. He was rescued by the "HAM" protocol (mitoxanthrone + high dose cytarabine, with additional intrathecal methotrexate). He achieved second CR and underwent myeloablative HSCT from a 7 out of 10 HLA-matched unrelated donor on 16.02.06. Only on 18.10.07 he reached stable molecular remission as judged by *CBFβ/MYH11* expression. Nevertheless, during the next two months, the *WT1* expression grew above normal and the patient relapsed again on 17.01.08,while *CBFβ/MYH11* expression remained negative. A new molecular screening was performed and the *AML1/ETO* transcript was detected (Fig. 1). Therefore, all previously taken samples were tested for the presence of *AML1/ETO* and a very low expression was found in the diagnostic sample using real-time RT-PCR. The following samples oscillated around the sensitivity threshold of the RQ-PCR method (10^{-6}). Although *AML1/ETO* was present already at diagnosis, donor-derived *AML1/ETO* clone was confirmed by allelic discrimination of the donor/recipient HLA polymorphisms, as well as by the chimerism analysis. The patient received induction treatment by "3+7" and underwent second HSCT from another donor with 3 mismatches on 13.01.09 after a non-myeloablative conditioning. On 08.07.09 he reached molecular remission of *AML1/ETO* as well as *WT1*. However, this time he suffered grade II acute GvHD and succumbed due to CMV pneumonia and acute respiratory distress syndrome on 18.07.09.



Summary / Conclusion: We deem this is the first report of multiple CBF-AMLs in a single patient. The patient may have had some unrecognized predisposition in his environment or in his hematopoietic milieu. Developing of the donor cell leukemia is a quite rare but serious complication of the HSCT.

B1274
HDAC AND AMSACRINE FOR TREATMENT OF REFRACTORY AND RELAPSE ACUTE MYELOID LEUKEMIA: A SINGLE CENTRE RETROSPECTIVE STUDY
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Background: Patients with relapse and refractory acute myeloid leukemia (AML) had poor prognosis and there is a lack of data supporting an established standard chemotherapy. The end point of a salvage treatment is to induce high remission rate without severe toxicity to allow secondary allogeneic stem cell transplantation.
Aims: We performed a retrospective study in 56 patients treated in our center from October 2010 to January 2012 with the aim to investigate: overall response rate (ORR) defined by complete response (CR) and complete response with

incomplete blood count recovery (CRi), toxicity, and outcomes of the combination of HDAC and amsacrine.
Methods: This salvage chemotherapy consisted on the association of HDAC 3 g/m²/12h (5-8 infusions) and amsacrine 150 mg/m2/day for 5 days or 100 mg/m2/day for 3 days.
Results: Median age was 57 years (range 25-69), with 23 patients (41%) ≥ 60 years. Among the 56 patients, 27 (48%) were in primary induction failure (PIF) and 29 (52%) were in first relapse (16 patient with duration of CR1 < 12 months). Among the whole cohort, one patient had a favourable cytogenetic risk, 35 (62%) had an intermediate cytogenetic risk and 20 (35%) an adverse cytogenetic risk. Among the 27 PIF and the 29 relapse, cytogenetic risk was favourable, intermediate and adverse for respectively 0/15/12 and 1/20/8 patients. Twelve patients had secondary AML. Among the whole cohort, ORR was 43% (24 patients), respectively 52% (13 CR and 1 CRi) and 34% (6 CR and 4 CRi) for PIF and relapse AML. None of tested variables: age < 60 years, good/intermediate cytogenetic risk, relapse AML and *de novo* AML was associated with improved ORR. Eighteen patients underwent an allogeneic stem cell transplant in ORR. After a median follow-up of 17 months (range 2-96), median overall survival (OS) at three years was 11.5±6%. Leukemia free survival (LFS) at 1 year for the 24 patients in ORR was 51%, with a 59% 1 year-LFS for the 18 patients allografted and 25% 1 year-LFS for the 6 patients exclusively treated by chemotherapy. Median duration of neutropenia after HDAC-amsacrine was 17 days (6-25), 44/56 patients had febrile neutropenia, with 27 bacterial bloodstream infections, one disseminated fusariosis and 6 possible pulmonary aspergillosis.
Summary / Conclusion: Our results suggest that HDAC-amsacrine regimen is an efficient salvage combination for patients with refractory or relapse AML. This regimen could represent a bridge to allogeneic transplant, which remains the only curative option in this setting.

B1275
CD96 AS A LEUKEMIA STEM CELL-SPECIFIC MARKER AND A FACTOR FOR PROGNOSIS EVALUATION IN LEUKEMIA
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Background: The cancer stem cell hypothesis holds that cancers are composed of a subset of cells that the unique ability to transplant disease as well as self- renew. Hematopoietic stem cell (HSC) has the ability of self-renewal, therefore, the clonal progression of preleukemias likely occurs in a succession of HSC subclones until augmented or poorly regulated self-renewal pathways are activated, leading to the emergence of final stage leukemic stem cells (LSCs) usually at the level of a downstream progenitor.Acute leukemia by chemotherapy remains elusive for most patients because of the leukemia stem cells (LSCs), the self-renewing component of the leukemia.
Aims: Find the relationship between CD96 and leukemia, especially for acute myeloid leukemia.

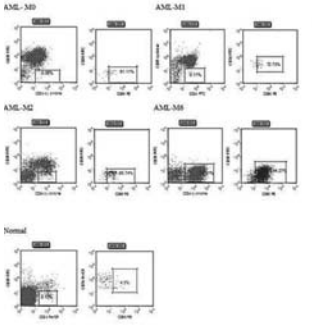

Table1. Compare the mean of $CD34^+CD38^-$ and CD96 between leukemia patients and control

Group	N (134)	Mean of $CD34^+CD38^-$%	p-value	Mean of $CD96^+$ in $CD34^+CD38^-$%	p-value
Control	15	0.10±0.16		7.77±13.86	0.644
AML	87	---	---	---	---
M0	7	3.03±4.93	0.028	44.21±31.58	0.001
M1	10	4.45±8.83	0.066	46.36±40.80	0.002
M2	47	8.39±15.55	0.045	26.07±34.47	0.051
M3	4	6.70±10.15	0.014	22.79±44.38	0.252
M4	10	7.15±10.84	0.018	20.04±22.20	0.101
M5	5	4.65±8.54	0.042	20.52±36.93	0.241
M6	3	2.77±4.30	0.014	53.70±22.77	0.000
M7	1	11.14	0.000	0.59	0.624
ALL	15	24.48±20.14	0.000	11.34±26.13	0.644
MAL	3	0.04±0.05	0.504	58.98±51.33	0.002
MDS	14	1.13±1.93	0.050	17.02±24.47	0.217

Methods: We investigated CD96 expression in 119 leukemia patients by flow cytometry, which were obtained after informed consent at diagnosis and after chemotherapeutic treatment.
Results: It was found that CD96 expressed highly in the group of CD34+CD38- cells in AML, especially in M0, M1, M2and M6 non complete remission cases. At the same time, we also observed that CD96 was high in the group of CD34+CD38- cells in MAL.
Summary / Conclusion: The results showed that CD96 was frequently expressed in LSC population, which could be as a marker of AML-LSC and a candidate therapeutic target. In addition, CD96 was related to the prognosis of AML patients, which might suggest CD96 to be an important factor to evaluate the prognosis of leukemia patients.

B1276
STUDY OF THE GM-CSF GENE EXPRESSION AND ANTI GM-CSF AUTOANTIBODIES IN RELATION TO DISEASE BEHAVIOR AND OUTCOME IN AML/MDS CASES
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Background: Blood cells under a variety of conditions produce cytokines which regulate their proliferation, communication and functioning. While, Growth and progression of many of malignant cells are mediated by alterations in the microenvironment often caused by an aberrant expression of growth factors and receptors. Granulocyte macrophage-colony stimulating factor (GM-CSF) is an autocrine and a paracrine cytokine. It stimulates growth, differentiation and function of normal and leukemic myeloid progenitors. Antibodies to GM-CSF, while uncommon and of unknown significance are implicated in the pathogenesis of few diseases. e.g.; idiopathic pulmonary alveolar proteinosis (PAP) where they inhibit GM-CSF mediated endocytosis of surfactant in alveoli. also, they have been detected in 0.3% of healthy donors (HD), Neonatal cord blood, Patients with autoimmune diseases and Exogenous recombinant human GM-CSF.
Aims: We aimed to Study of GM-CSF *mRNA* gene expression, its protein and anti GM-CSF autoantibodies in Acute Myeloid leukemia/Myelodysplastic syndromes AML/MDS patients and their correlation to disease behavior and therapy outcome.
Methods: We examined GM-CSF *mRNA* gene expression by real time PCR (RT-PCR) and GM-CSF protein by ELISA methods in sera of 50 AML/MDS. Also, we examined anti GM-CSF antibodies by ELISA methods in sera of 42 cases out of the 50 AML/MDS cases. all results were compared to 20 healthy blood donors (HD). also, we correlated results with disease behavior and response to therapy. All cases were of Egyptian origin.
Results: There was significant decrease in GM-CSF *mRNA* gene expression (P value 0.008), Increase in GM-CSF concentration (P value 0.001) and increase in anti GM-CSF antibody titer (P value 0.003) in AML/MDS patients in comparison to HD, significant negative correlation between titer of GM-CSF and initial PB blast count (P value 0.011) and significant negative correlation between GM-CSF concentration and response to therapy (P value 0.016). we found no segnificant correlation between GM-CSF mRNA gene expression and other variables especially anti GM-CSF antibodies.
Summary / Conclusion: Any alteration in GM-CSF gene expression Could have an implication in leukemiogenesis. Also anti GM-CSF could have the same role. GM-CSF concentration could be used to predict outcome of therapy, but anti GM-CSF couldn't be used to predict the outcome. Normal GM-CSF gene expression & GM-CSF is necessary for normal hematopoiesis & cellular maturation. The origin of the immunity to GM-CSF and the consequences of this immunity in leukemia patients are not clear.

B1277
NO MODIFIED ANTI-INFLAMMATORY AND ANTIVIRAL COMPOUNDS AGAINST ACUTE MYELOIDE LEUKEMIA; FROM CELL LINE TO PATIENT BLOOD
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[1]Clinical Center Dr Dragisa Misovic, University of Belgrade, [2]Department of Immunology, Institute for Biological Research "Sinisa Stankovic", Belgrade University, Belgrade, Serbia, [3]Onconox Aps, Copenhagen, Denmark, [4]Laboratory of Medicinal Chemistry, North Shore Long Island Jewish Health System, New York, United States, [5]Department of Biomedical Science, University of Catania, Catania, Italy

Background: Antiinflamatory compound VGX1027 acquired antitumoral features after the addition of NO (GIT27-NO) while anticancer potential of antiviral drug Saqunavir became stronger and highly selective against transformed cells (Saq-NO). Despite the basically same approach in chemical intervention, first mention compound acts as NO donating drug with specific feature to release NO in contact with the cell membrane while the last is qualitatively novel drug with essentially different intracellular response in comparison to original compound.
Aims: This study is created with an idea to explore the effects of both compounds on HL60 cell line as a model of acute myeloid leukemia and define the reproducibility of their efficiency on peripheral mononuclear cells isolated from blood samples of untreated AML patients.
Methods: Cell viability was assessed by acid phosphatase test while the cell cycle distribution was analyzed by cytofluorimetric analysis of on propidium-iodide (PI) stained cells. Apoptosis was detected using Annexin-FITC / PI double staining while the activity of caspases was confirmed by Apostat staining. For detection of intracellular nitric oxide (NO) DAF-FM diacetate was used. Reactive oxygen (ROS) and nitrogen (RNS) species were determined with DHR 123 staining.
Results: Both drugs modified by NO exaggerated strong cytotoxic activity

against HL60 cells. Reduced viability was accompanied with enhanced apoptosis confirmed in early and late phase of this process. Mechanistically, the dying signal involved the activation of caspases upon the treatment with both, GIT27-NO and Saq-NO. However, cytocidal activity of GIT27-NO correlated with intensive oxidative stress confirmed by specific staining of reactive oxygen and nitrogen species. In parallel, treatment with Saq-NO didn't promote significant enhancement of free radical production, indicating more sophisticated intracellular pattern of its action. Effects of GIT27-NO after 48h of exposure of PMC isolated from patients with AML remarkably varying from good to completely inefficient. Oppositely, sensitivity of the same cultures to Saq-NO was comparable with HL60 and independent from the blood source.
Summary / Conclusion: Both NO modified drugs exerted strong anticancer potential against HL-60 cells. Low reproducibility of patients MNC response to GIT 27-NO is possibly related to different capacity of individual clones to fight with oxidative stress triggered by this drug. On the contrary, the ROS independent cytocidal signal delivered by Saq-NO probably targeted important pathway involved in malignant cell proliferation, independent from individual variation.

B1278

RESULTS OF >60 AML UNTREATED PATIENTS THERAPY IN DEPENDENCY OF QUALIFICATION TO RISK AND INDUCTION INTENSITY GROUPS. RETROSPECTIVE STUDY.

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Background: The aim of this study was assessment of therapy results In acute myeloid leukemia (AML) patients in age >60 treated according to PALG stratification for AML >60 patients.
Aims:
The primary aims were comparison of overall survival (OS) and complete remission rate (CR) in whole group of AML >60 patients in dependency of qualification to risk group and a way of treatment. The secondary aims were comparison of OS and CR rates in younger older (61 – 70) AML patients.
Methods: Patients with AML >60 after diagnosis were qualified into the risk groups according to PALG stratification for AML >60. In group I patients were receiving induction chemotherapy DAC (daunorubicin, cytarabine, cladribine) or DA (daunorubicin, cytarabine), in group II patients were receiving induction chemotherapy cytarabine/thioguanine, and in group III they were receiving chemotherapy with low doses of cytarabine. Patients with group I, who reached CR after first or second induction course were receiving consolidation with mitoxantrone and cytarabine, and next they were qualified to alloHSCT or autoHSCT, or maintenance phase. Patients with group II and III independently of results were receiving successive cycles of chemotherapy like in first course every 4 weeks to 2 years. W period 02.2009-12.2012 123 untreated patients with AML, age 73 (61-89), men 50% were included to this study. Thirty three patients were qualified to group1, 47 to II and 43 to III. After preliminary analysis, because of similar early and late outcomes of the therapy in group II and III, those groups of patients were joint for next analysis.
Results: In comparison of therapy outcomes in group I and in joint group II and III of patients with AML >60 significantly higher CR rate in group I vs. II/III 47% vs. 2% (P<0,001) was confirmed. And significantly better OS after 2 years 11% vs. 7% (P=0,006) was noted
However, in comparison of those parameters In subgroup of age 61-70 despite of significantly higher CR in group I vs. group II/III 46% vs. 7% (P=0,01) we did not obtain a benefit in OS after 2 years 12% vs. 17% (P=NS) respectively.
Summary / Conclusion: In conclusion we must state, that in AML >60 proper qualification to favorable risk group (group I) and as a consequence more intensive induction chemotherapy is a main predictor of better early outcomes (CR rate), but overall survival do not depend of risk group and kind of induction therapy, but only it depends of age of patients, what can be cause by too low intensive postremission approach in younger and fit patients, who reached CR after induction chemotherapy

B1279

TRANSFUSION IRON INTAKE IN TRANSPLANT ELIGIBLE PATIENTS WITH DE NOVO ACUTE MYELOID LEUKEMIA: RETROSPECTIVE ANALYSIS OF IRON LOAD AT THE END OF CHEMOTHERAPY PROGRAM AND BEFORE TRANSPLANTATION

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Background: Iron overload is an important adverse prognostic factor for patients (pts) undergoing hematopoietic stem cell transplantation (HSCT): it increases the risk of infections, veno-occlusive disease and hepatic dysfunction. Elevated pretransplant ferritin levels have been reported to increase the risk of non relapse mortality following HSCT and might influence the risk of acute and chronic GVHD. The majority of data derives from studies regarding pts with thalassemia and myelodysplastic syndromes, with established iron chelation strategies in these diseases. On the contrary, the extent of transfusion iron overload in transplant eligible *de novo* acute myeloid leukemia (AML) and the need of iron chelation therapy (ICT) remains debated.
Aims: To evaluate transfusional iron intake in potentially transplant eligible pts with *de novo* AML treated with conventional chemotherapy regimens, focusing on pts who underwent HSCT or completed consolidation chemotherapy courses.
Methods: We retrospectively analysed 44 potentially transplant eligible AML pts (20 males, 22 females) from March 2009 to February 2013. All pts completed their treatment program: 23 of them were treated with induction and consolidation chemotherapy followed by HSCT, while 21 pts received only chemotherapy. Iron intake until HSCT or until the end of consolidation chemotherapy, expressed in mg of iron, was calculated as total amount of red blood cells (RBCs) transfused X 1.08. Ferritin levels at the end of the treatment was also evaluated.
Results: Standard induction treatment (cytarabine 100 mg/sqm twice daily for 7 days and idarubicin 12 mg/sqm daily for 3 days) was administred in 40 pts, while 4 pts received high dose induction chemotherapy with cytarabine 2g/sqm twice daily (day1,2,8, 9) and idarubicin 18mg/sqm daily (day3, 10). 11 of the 23 pts underwent transplantation in second complete remission after reinduction chemotherapy. Mean transfusional iron intake was 0.65 mg/kg per day, among a mean treatment period of 6.6 months. Mean ferritin level at the end of the treatment or before transplant conditioning regimen was 2951 µg/L (median 2123 µg/L).
Summary / Conclusion: This retrospective evaluation confirms a relevant transfusion iron load in *de novo* AML in a short time interval; of note, patients with β-thalassemia major or other refractory anemias receiving 2-4 units of blood per month have a transfusion iron intake of 0.3-0.6 mg/kg per day. It is known that serum ferritin is sensitive but not specific for iron overload and is a poor predictor of body iron burden, and maybe it is even less specific in AML as a reliable marker in monitoring iron loading. Moreover, the optimal ICT in this setting of pts is still to be defined: subcutaneous deferoxamine infusion is inconvenient and troublesome due to thrombocytopenia and neutropenia, while oral deferasirox administred in proven iron overloaded pts may not be sufficiently rapid in his action if we consider the short time available before transplantation. Considering our data regarding daily iron intake, it could be interesting to investigate the role of early low dose deferasirox administration at the beginning of chemotherapy treatment program, in order to prevent transfusion overload and his prognostic impact at transplantation.

B1280

RED BLOOD CELL ENCAPSULATING ASPARAGINASE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA UNFIT FOR INTENSIVE CHEMOTHERAPY: A PROSPECTIVE PHASE IIB STUDY

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Background: L-asparaginase (ASNase) holds a key role in chemotherapy for Acute Lymphoblastic Leukemia (ALL) in children and young adults. In elderly patients, its efficacy is counterbalanced by its toxicity, which impairs its use and creates an unmet need. To reduce toxicity, a new product, GRASPA, encapsulates L-asparaginase in Red Blood Cells (RBC). A current study in ALL patients (GRASPALL-GRAAL SA2 2008) shows an improved therapeutic index for elderly patients.
In adults, Capizzi (1988) reported a significant benefit of ASNase associated with high-dose cytarabine treatment (HiDAC) in Acute Myeloid Leukemia (AML). Indeed, there was an overall statistically superior complete remission rate for HiDAC/ASNase (40%) vs HiDAC (24%) and an overall survival benefit for patients treated with HiDAC/ASNase (19.6 weeks vs 15.9 weeks). The benefit of ASNase in different AML was also reported by Petti (1989), Horikoshi (2009) and Rubnitz, (2009). Furthermore our preclinical results also showed that an AML cell line (HL-60) and blast cells from the bone marrow of AML patients were sensitive to ASNase in vitro.
The main issue is that, up to now, the toxicity of ASNase for elderly had prevented its use in this fragile population that represents the majority of AML patients.
Aims: Considering the promising results of ASNase for AML treatment and the better safety profile offered by RBC encapsulating ASNase (GRASPA®), a multicenter, randomized, controlled IIb trial is evaluating the answer to the fragile patients unmet need Efficacy and tolerability of GRASPA® plus low-dose cytarabine will be evaluated versus low-dose cytarabine alone in treatment of AML patients over 65 year-old, unfit for intensive chemotherapy.
Methods: One hundred and twenty-three patients (65-85 year-old) newly diagnosed for AML or diagnosed for post myelodysplastic syndrome in the 6 months before enrollment are planned for inclusion in the study. Included patients must be unfit for intensive chemotherapy, with WHO performance status ≤2 an estimated life expectancy ≥3 months. Patients with M3 AML or with AML involving chromosome 16 abnormalities are not eligible for inclusion. Written informed consent will be obtained for included patients.
A 2:1 randomization will be respected (82 patients treated with GRASPA® plus low-dose cytarabine and 41 patients treated with low-dose cytarabine alone). In the experimental group, every 28 days, patients will receive one intravenous

infusion of GRASPA® (100 IU/kg) after 10 consecutive days of subcutaneous low-dose cytarabine (20mg twice daily) versus low-dose cytarabine alone in the control group.
Each patient will be followed for 24 months. Progression-free survival (PFS, time elapsed between treatment initiation and disease progression/death) will be evaluated as a primary endpoint. A 75% improvement in the median FPS is assumed in the experimental group vs control group. Percentages of remission (complete and partial), survival (event-free and overall), patient quality of life, general safety, pharmacodynamic/pharmacokinetic and immunogenicity of GRASPA® will be also evaluated.
Results: The HL-60 AML cell line was found equally sensitive to ASNase than the MOLT-4 ALL cell line in vitro. Blasts isolated from the bone marrow of 5/6 AML patients were highly sensitivity to ASNase. The only subject tested with no leukemia was resistant.
Summary / Conclusion: Given its efficacy/tolerability profile, L asparaginase encapsulated in red blood cell, GRASPA® should be the solution for elderly patients in AML. Final results of this clinical study are expected mid 2017.

B1281
MAINTENANCE THERAPY WITH CEPLENE® (HISTAMINE DIHYDROCHLORIDE) AND PROLEUKIN S® (IL-2) LEADS TO ACTIVATION OF NK CELLS BUT ALSO EXPANSION OF T REGULATORY CELLS IN AML
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Background: The prognosis of acute myeloid leukemia (AML), particularly when associated with adverse chromosomal or molecular aberrations, is poor due to a high relapse rate after induction chemotherapy. Postremission therapy for elimination of minimal residual disease (MRD) remains a major challenge in AML. For patients that are not eligible for allogeneic stem cell transplantation, there is no approved cytotoxic maintenance therapy after consolidation. Novel therapeutic strategies to prevent relapse are therefore intensively investigated.
Aims: In 2008, the EMA approved an alternative immunomodulating maintenance treatment consisting of repetitive administrations of Interleukin-2 (IL-2) and histamine dihydrochloride (HDC). However, limited data is available about the immunological effects of this therapy in AML patients.
Methods: We analyzed peripheral blood from AML patients receiving Ceplene / IL-2 for changes in inflammatory markers including C-reactive protein (CRP), IL-6, soluble IL-2 receptor and leukocyte counts. Immune cell subsets were monitored by flow cytometry. Changes in activation and function of NK cells were determined by upregulation of CD69 expression and IFN-gamma production. In addition, we surveyed the MRD level during the course of the treatment by molecular markers and immunophenotyping.
Results: We found that the inflammatory markers temporarily increased during the course of treatment. On a cellular level, different NK cell subsets were expanded and activated during the treatment. Besides, the immunotherapy resulted in a slight increase in T regulatory (Treg) numbers, consistent with recent studies showing Treg expansion by low-dose IL-2 application. Noticeably, one of the patients achieved an MRD conversion with no detection of the CBFb-MYH11 transcript after two cycles of Ceplene / IL-2.
Summary / Conclusion: Ongoing studies in a larger patient cohort will help to better understand the immunological effect of IL-2/HDC in the setting of AML maintenance therapy.

B1282
THE PROGNOSTIC IMPACT OF ABERRANT ANTIGEN EXPRESSION IN ACUTE LEUKEMIAS
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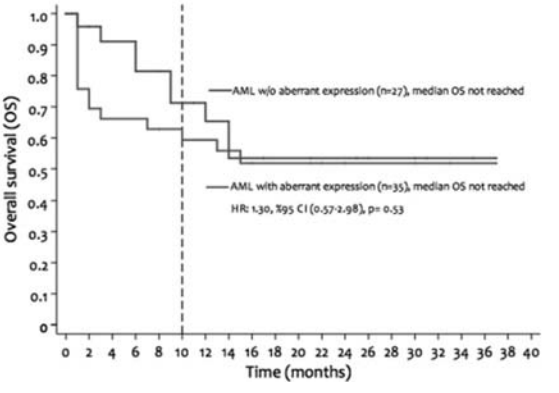
Background: Multiparametric immunophenotyping analysis allows the identification of aberrant antigen expression in acute leukemias.The clinical and prognostic impact of this phenomenon remains controversial.
Aims: This retrospective study was designed to investigate the impact of aberrant antigen expression on the response to remission induction therapy and overall survival in patients with acute leukemia.
Methods: We have analyzed the multiparametric flow cytometric (Becton Dikinson, San Jose, Calif., USA) data of 90 de novo acute leukemia adult patients (M/F:56/34, age(min-max):18-90 year) between 2008 and 2011. Aberrant phenotypes was considered to be positive when the percentage of positive blast cells was equal or greater than 20% for surface antigens and equal or greater than 10% for cytoplasmic antigens.
Results: In this study 62 (69%), 18 (20%), 5 (5,5%), and 5 (5,5%) out of all patients diagnosed with AML, ALL (16 B-ALL, 2 T-ALL), AUL, and BAL, retrospectively. The patients with AUL and BAL were not included into the analysis. Aberrant phenotype was observed in 35/62 (%56) out of AML (Ly + AML) and 8/18 (%44) out of ALL (My + ALL) patients. There were no difference between ALL patients with (My + ALL) and without aberrant phenotype (My – ALL) regarding to response to the remission induction therapy and overall survival. The most common myeloid antigen in ALL was CD33 (%29). There were no differences between CD33+ ALL patients and CD33 – ALL in terms of response to the remission induction therapy and overall survival. There were also no differences between AML patients with (Ly + AML) and without aberrant phenotype (Ly – AML) in response to the remission induction therapy and overall survival. Ly + AML and Ly – AML groups were comparable in terms of risk status based on cytogenetics (better-intermediate-poor). There was also no difference between My + ALL and My – ALL in terms of cytogenetic risk status (standart vs high). The most common lymphoid antigens in ALL were CD7 (%27), CD10 (%16), and Tdt (%15). CD7 + AML and CD7 – AML, Tdt+ AML and Tdt – AML, CD10 + AML and CD10 – AML groups had similar response rates to the remission induction therapy and overall survival.
Summary / Conclusion: In our study aberrant antigen expression did not have an impact on response to the remission induction therapy and overall survival of patients with acute leukemia. Although there is, recently, growing evidence about the prognostic importance of aberrant antigenic expression in acute leukemias during the diagnosis and monitorization of the disease, cytogenetic and molecular analyses remain to be main prognostic determinants. Studies combining cytogenetic and molecular features and aberrant antigenic expression profiles are needed.



B1283
PREDICTION OF CLONAL EVOLUTION UNRESPONSIVENESS TO THERAPY BY *ex vivo* CHEMOSENSITIVITY ASSAY IN A CASE OF ACUTE MYELOID LEUKEMIA
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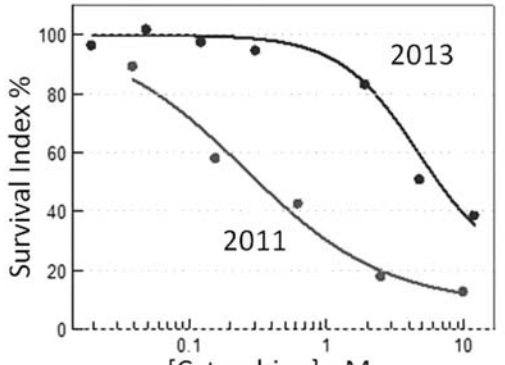
Background: Patient was part of a multicenter, prospective, non-interventional study of the PETHEMA group conducted by Vivia Biotech to determine the validity of an *ex vivo* personalized drug sensitivity test to predict clinical response based on the analysis of leukemic cell death *ex vivo*. Case study: A 23-year-old female patient was diagnosed with AML, M1 FAB type, in Aug-2011. She presented severe anemia, thrombocytopenia and leukocytoses with 80% blast cells in peripheral blood. BM aspirate showed 80% blast cells with myeloid phenotype and hyperploid kariotype. The molecular study was negative for PML-RARa, BCR-ABL, AML1-ETO, inv (cr16) translocations and FLT3-ITD, NPM1 and CEBPA mutations. The patient was classified at the intermediate risk group and received treatment according to PETHEMA-2010 protocol for patients <65 year-old with AML. After the induction course (idarubicin (Ida) plus cytarabine (Cyt), 3+7), she achieved complete remission with negative minimal residual disease (MRD) by immunophenotype, receiving then 2 consolidation courses followed by an autologous peripheral blood stem cell transplantation. First relapse was detected 6 months later; a rescue course of chemotherapy was started with FLAG-Ida schedule (fludarabine (Flu), Cyt, Ida). Still with MRD after recovery, treatment with clofarabine (Clo) and Cyt followed; however 10% of blast cells remained in BM. The patient did not have HLA compatible donor, so a reduced-intensity haploidentical transplantation was performed, with positive MRD (0.5%). After 2 months, patient progressed again. Chemotherapy with an IDICE course (Ida, Cyt and etoposide (Eto)) was then administered, with no further response.
Aims: For the patient presented here, a bone marrow (BM) sample was obtained at diagnosis in August 2011, and again in February 2013, allowing for the chance to examine the *ex vivo* patient response at two time points during the disease process.
Methods: BM samples were processed in Vivia'a lab according to protocol. The samples were diluted in its entirety and plated with the pharmacological agents, each at 8 concentrations. The plates were incubated for 48-hours, then ana-

lyzed by our automated flow cytometry-based ExviTech© platform. Malignant cell death was determined via labeling with appropriate monoclonal antibodies and AnnexinV-FITC. Dose-response curves for 9 drugs (including Cyt, Ida, Flu, Clo and Eto) were generated for both extractions. Key parameters are the efficacy of the drug to deplete cells, and potency measured as the concentration at which 50% of the cells are eliminated (EC50). The survival index calculates the percentage of malignant cells remaining. The Effective Maximum (Emax) is the maximum possible effect for the drug, thus an Emax of 0% indicates no surviving malignant cells.
Results: The patient's *ex vivo* response to 8 of the drugs was similar at both time points. However there was a marked change in the response to Cyt. Seen in figure1, the green line represents the data collected in 2011, where the sample was sensitive to Cyt with a high level of effectiveness. However the sample collected in 2013 displayed a marked change in response, with the drug losing both potency and effectiveness, with over 20% of the cells being resistant to the drug even at concentration that are not reached in the body.
Summary / Conclusion: Vivia's *ex vivo* results for Cyt mirror the clinical response in this patient. Upon diagnosis both the *ex vivo* sample and patient were sensitive to Cyt treatment, and then later displaying resistance.



Cyt	EMAX	EC50
2011	8.46%	0.26µM
2013	19.24%	4.74µM

B1284
THE COMPARISON OF TOXICITY AND OUTCOMES OF THE INDUCTION CHEMOTHERAPY DAC (DNR, ARAC, 2CDA) AND IAC (DNR REPLACED BY IDARUBICINE) IN THE ACUTE MYELOID LEUKAEMIA (AML) PATIENTS. RETROSPECTIVE STUDY
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Background: This study is a continuation of the previous studies concerning optimization of induction chemotherapy using purine analogues. (Hołowiecki J., Grosicki S., Robak T. et al. Leukemia 2004, Holowiecki J, Grosicki S, Giebel S. et al. J Clin Oncol. 2012).
Aims: The aim of this study was assessment of the toxicity and outcomes of the induction chemotherapy DAC (DNR, cytarabine, cladribine) and IAC (DNR was replaced by idarubicine) in the acute myeloid leukaemia (AML) patients. The primary aims were complete remisson rate (CR) and toxicity of the treatment, secondary was leukaemia free survival (LFS) and overall survival (OS).
Methods: DAC induction chemotherapy program is a standard in younger patients with AML. IAC program was used in those cases, when daunorubicin was not attainable in our center. Between 03.2009 and 03.2012 52 untreated AML patients in age 57 (20-72), women 54% and assessed as fit to intensive chemotherapy and alloHSCT, treated in Hematology Unit in Chorzow/Poland were included to this study. AML PML/RAR alfa positive patients were excluded.
Results: Materials and results

	DAC (n = 30)	IAC (n = 22)	p
Age (median)	20-72 (55)	38-70 (59)	NS
Sex (%)	W-53, M-47	W-55, M-45	NS
WBC at dgn. x10^3/L median (range)	27,7 (2,3 -257)	11,0 (0,9 – 210)	NS
Cytogenetic risk (%) low	0	0	NS
intermediate	50	64	NS
high	7	5	NS
CR	70	59	NS
2 years OS	31	12	NS
2 years LFS	43	22	NS

Study groups were well balanced according to age, sex, WBC at diagnosis and cytogenetics. CR rates in both groups were no differ and was similar to previous published after DAC induction. Whole patients reached deep thrombocytopenia and neutropenia WHO grade IV. The frequency and intensification of infection, mucositis, vomiting, diarrhea, alopecia, polineuropaty, as well as cardiologic, hepatic, or kidney failure were comparable in both arms, Statistically confirmed differences in LFS amd OS after 2 years were not reached. Number of alloHSCT in both groups was similar.
Summary / Conclusion: In conclusion, we must state, that results of this study authorize to alternative use of idarubicine and daunorubicine In induction chemotherapy based on antracycline, cytarabine and cladribine in fit AML patients without higher risk of additional toxicity.

B1285
SALVAGE WITH VERY HIGH DOSE CYTARABINE FOR RELAPSED/REFRACTORY AML IN THE SETTING OF INTENSIFIED ANTHRACYCLINE INDUCTION AND POST STEM-CELL TRANSPLANTATION – CLINICAL EFFICACY WITH ACCEPTABLE TOXICITY
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Background: Primary refractoriness to induction therapy as well as relapse of AML are associated with poor outcome. Cytarabine is considered one of the most potent anti-leukemic drugs and a cornerstone in most induction, consolidation and salvage regimens. The dose-response relationship of cytarabine in AML is complex and the question which is the most efficient and yet safe regimen is still open. Herzig et al. (Blood, 1983) demonstrated that a total dose of 36 g/m^2 delivered over six days achieves a high efficacy/toxicity ratio in patients with relapsed/refractory AML. Very high dose cytarabine (VHDAC) salvage may be of special relevance in the era of higher induction anthracycline dose. Safety and efficacy of VHDAC for AML patients refractory to intensified anthracycline induction regimen (with 90mg/m^2 daunorubicin [DNR]) and for those relapsing after hematopoietic stem cell transplantation (HSCT) has not yet been described.
Aims: We describe our experience on the efficacy and toxicity profile of VHDAC as salvage therapy for relapsed and refractory AML.

Outcome and major toxicities (n=8)	
Patient outcome n (%)	CR\CRp – 7 (87.5) Resistant disease – 1 (12.5)
Hematological toxicity	
Median duration of grade IV neutropenia (days, range)	20 (15-24)
Median duration of grade III-IV thrombocytopenia (days, range)	26 (12-48)
Non hematological toxicity (grade III-IV) n (%)	
Pulmonary	2 (25)
Severe infections	3 (37.5)
Ophthalmic	2 (25)
GI tract	1 (12.5) attributed to GVHD

Methods: Medical charts of patients treated in our institution with VHDAC as salvage for relapsed/refractory AML between January 2012 and January 2013 were reviewed. Treatment was administered according to Herzig et al. and was comprised of 12 doses of cytarabine 3g/m^2 administered over 1 hour every 12 hours for 6 days for a total of 36 g/m^2.
Results: Eight patients were eligible for the analysis. Median patient age at diagnosis was 54.5 years (40-56); 7 patients were males; 6 patients had unfavorable AML (by the ELN classification) and 2 patients were diagnosed with acute leukemia of ambiguous lineage. Five patients had primary refractory disease. Three patients relapsed after HSCT. Five patients received induction treatment with intensified anthracycline regimen with 90mg/m^2 DNR and 3 patients received induction with 60mg/m^2 DNR. Seven (87.5%) of the patients responded (CR-6 and CRp-1). This included 4 patients with primary refractory disease and all 3 patients following HSCT. One patient developed high fever and grade IV pulmonary toxicity and therapy was withheld after 8 out of the 12 scheduled doses. His day 24 BM demonstrated resistant disease, and he died on day 26 of treatment of multi-organ failure. In the 4 refractory patients that responded the toxicities were manageable. Serious infections (possible invasive fungal pulmonary infection and severe sepsis) developed in 2 patients

with refractory disease, both treated with intensified anthracycline induction before salvage. These 2 patients also developed grade III eye dryness (decreased visual acuity limiting self care) one of which also demonstrated superficial punctuate keratitis and subconjunctival hemorrhage. Of note, all patients were treated with prophylactic eye drops. A characteristic rash of grade II severity developed in 2 patients: palmar erythema in 1 and desquamation of the palms and soles in the other. One patient treated post HSCT developed grade IV diarrhea and hyperbilirubinemia on day 31 of therapy, attributed to GVHD and died on day 107 of treatment with no evidence for leukemia in BM. No CNS manifestations were noted in the cohort. Median duration of grade IV neutropenia and grade III-IV thrombocytopenia in patients who achieved CR/CRp were 20 days (15-24) (7 patients) and 26 days (12-48) (6 patients), respectively.

Summary / Conclusion: Salvage therapy with 36g/m^2 of cytarabine appears to be effective with acceptable toxicity in patients with AML refractory to intensified anthracycline induction treatment and in those relapsing after HSCT.

B1286

EFFECT OF HIGH DOSE CYTOSINA ARABINOSIDE I.V. ON EEG BACKGROUND ACTIVITY AND ON NEUROPSYCHOLOGICAL FUNCTIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Citosina arabinoside (Ara-C) is a nucleoside analog used in the treatment of hematologic malignancies. However, high doses of Ara-C i.v. (>2 g/m2) have been noted to produce a number of Central Nervous System (CNS) toxicities. A number of studies have evaluated CNS toxicity of others pharmacological treatments in patients suffering from leukemia, using either standard EEG or neuropsychological tests. To date, no reports have been published on the effect of Ara-C on the CNS, using both computerized EEG and neuropsychological tests in this patient population.
Aims: An open study to evaluate if high dose of Ara-C i.v can induce CNS side effects in adult patients affected by acute myeloid leukemia (AML), using computerized EEG analysis and neuropsychological tests.
Methods: 9 patients affected by AML (4 male, and 5 female, mean age 49.4 range 38-64 year); 5 treated with high dose of Ara-C i.v (≥ 2 g/m²die), and 4 treated with standard dose Ara-C i.v. (100g/m²die). We recorded EEG at rest with eyes closed (REST) and during hyperventilation (HP), mental arithmetic task (MA) and blocking reaction (BR). We used the following tests: Karnofsky Performance Status, Mini Mental State Examination, Rey Auditory Verbal Learning Test, Trail Making Test , Verbal Fluency Test, EORTC QLQ C30. We compared the EEG background activity and neuropsychological tests, at baseline and after six months of therapy, between the two groups.
Results: At baseline and at final follow-up, the comparison between the two groups revealed no significant differences, for the mean relative power for all frequency bands (delta, theta, alpha, beta), at REST and during BR, HP, MA, and for the Neuropsychological tests.
Summary / Conclusion: This is the first report indicating that high doses Ara-C do not induce CNS side effects, as revealed by EEG background activity and neuropsychological tests.

B1287

CLINICAL COURSE OF ACUTE PROMYELOCYTIC LEUKEMIA: SINGLE-CENTER REPORT FROM A DEVELOPING COUNTRY

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Background: Since the introduction of all-trans retinoic acid (ATRA) in 1980's acute promyelocytic leukemia (APL) has been the most curable variant of acute myeloid leukemias. However, recently International Consortium on APL and some other reports pointed out that the treatment outcomes of APL patients in developing countries are inferior compared to those of Western countries.
Aims: In this report we have retrospectively evaluated the clinical course and treatment outcomes of APL patients referred to our center between 2004 and 2012.
Methods: Molecular remission and bone marrow response was evaluated after completion of first consolidation chemotherapy. Overall survival and disease free survival were calculated with Kaplan-Meier analysis using SPSS 13.0 version.
Results: The prevalance of APL among all our acute myeloid leukemia patients was 2.8%. There were 22 patients, all of whom were treated according to AIDA protocol consisting of remission induction regimen with oral ATRA (45 mg/m^2 per day), divided into 2 daily doses and maintained until complete hematologic remission, and idarubicin (12 mg/m^2 per day) given on days2,4,6, and 8. After achievement of complete remission(CR), three cycles of consolidation chemotherapy with ATRA, idarubicin and cytarabine and then maintenance therapy with intermittent ATRA, 6-mercaptopurine and methotrexate for two years followed. Median age was 41.5 (range, 20-64) and 10 of the patients were male. Approximately 13.6 % (3) of patients had more than 10 x 10^9/L leukocytes at diagnosis and thus were classified as high risk for relapse; whereas only 6 (27.3%) patients presented with less than or equal to 10 x 10^9/L leukocytes and more than 40 x 10^9 /L platelets and were considered low risk. Thus, the majority (45.5%) of patients were classified as intermediate risk. The median time from the onset of symptoms until hospitalization was 7 days (range, 2-30). Coagulopathy was present in 32% of the patients at diagnosis. The median plasma level of fibrinogen was 226.9 mg/dL (range: 114-1083). Three of the patients relapsed at 24, 36 and 48 months after achievement of CR. There were 4 (18.2%) early deaths which occurred during induction therapy. At a median of 22 months overall survival and disease free survival rates were 68.9% and 59%, respectively. Among all patients differentiation syndrome, thromboembolic events and Sweet syndrome was seen in3, 4 and one patient, respectively during induction period.
Summary / Conclusion: In summary, when compared to reports from developed countries long-term disease-free survival was inferior (85% vs 59%) in our center. This analysis suggested that the delays in referral of these medically emergent patients to our tertiary medical center might explain the early deaths since hemorrhagic complications were the most common reason for the losses. It is clear that it is a must to improve early diagnosis and prompt medical intervention. Additionally, shortage of better anthracyclines such as daunorubicin and reimbursement issues for frontline usage of highly effective arsenic trioxide might have been likely contributed to somewhat worser outcomes.

B1288

THE EXPRESSION AND SIGNIFICANCE OF PD-L1 AND BCL-2 IN CHILDREN WITH ACUTE LEUKEMIA

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Background: Programmed cell deathligand-1(PD-L1) is a newly discovered apoptosis gene, and it plays an important role in children with leukemia development along with Bcl-2 gene.
Aims: To examine the expressions of PD-L1 and Bcl-2 in bone marrow cells in children with acute leukemia (AL) and to detect a relationship with the classification, clinical features, therapeutic effects and prognosis of AL.
Methods: Using the SABC method of immunohistochemical staining, expressions of PD-L1 and Bcl-2 in the bone marrow cells of 67 cases with AL were detected.
Results: The expressions of PD-L1 and Bcl-2 in the initial treatment, refractory relapse and relief groups were obviously higher than that in the control group (P<0.05). There was no significant difference in the expression of PD-L1 and Bcl-2 between the acute lymphoblastic leukemia ALL and acute nonlymphoblastic leukemia ANLL groups (t=1.015,t=1.032;P>0.05) . The expressions of PD-L1 and Bcl-2 in the complete remission(CR) group were lower than that in the initial treatment group(t=3.452,t=3.864;P<0.05). The expression of PD-L1 in the refractory relapse group was higher than that in the initial treatment group(t=2.885,P<0.05). However,high expression of Bcl-2 occurred both in the initial treatment group and the refractory relapse group, and there was no significant difference (t=0.932,P>0.05). Pearson rank correlation analysis indicated that there was a positive correlation between the expression of PD-L1 and Bcl-2 (r=0.617,P<0.05). Statistical analysis showed that the CR rates in patients with negative expression of PD-L1 and Bcl-2 were remarkably higher than that with positive PD-L1 and Bcl-2 expression(P<0.05).
Summary / Conclusion: Expressions of both PD-L1 and Bcl-2 play a role in onset, progression and prognosis of AL, and the two may act synergistically in the onset and development of AL.

B1289

PROGNOSTIC VALUE OF MICRO RNA92A IN ACUTE MYEOID LEUKAEMIA

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Background: The search for non invasive tools for diagnosis and management of cancer is extremely important for reducing the world wide health burden of cancer. MiRNAs show potential as biomarkers. MiR-92 is present in healthy individuals in the serum. qRT-PCR is superior to other methods of detection due to its high sensitivity, specificity and reproducibility. It requires less RNA input, as little as a single cell can be used for profiling. Since the expression levels of circulating miRNAs are very low, qRT-PCR is well adapted for analyzing circulating miRNAs profiles because of its sensitivity. Evidence implicating miRNA deregulation in the initiation and progression of cancer exposes opportunities for exploiting the miRNA system for therapeutic manipulations and development of novel therapies. Consequently oncogenic miRNAs can be targeted for down-regulation using anti-sense miRNA oligonucleotides to their precursor or mature

forms, and tumor suppressive miRNAs may be directly upregulated for an anticancer effect. the impact of some drugs such as DNA demethylating agents on miRNAs expression is also another therapeutic approach to modulate miRNAs expression.
Aims: To assess plasma level of micro-RNA 92a in acute myeloid leukemia patients and to correlate it with prognostic factors and therapeutic response
Methods: This study was carried out on fifty AML patients admitted to Hematology unit in Alexandria Main University Hospital as patients group as well as thirty five healthy subjects of matched age and sex as a control group. FLT3/ITD gene mutation using PCR were done to patients group. Measurement of plasma level of miR-92a using TaqMan quantitative RT-PCR and miR-638 as standardization was done to both groups. Cytogenetic study was done. Therapeutic response was assessed in patients group by doing bone marrow aspirate at day 28 of standard induction chemotherapy (3+7 protocol).
Results: The ratio (RQ) of plasma miR-92a to miR-638 in AML patients had a mean of 0.31±0.41 while in control group it had a mean value of 0.99±0.57 that confirmed statistical significance (p <0.001). Also there was negative correlation between RQ of miR-92a and bone marrow blast percentage on admission in patient group (r_s = -0.086). Patients who achieved complete response after induction chemotherapy had a median RQ higher than non-responder (0.36 versus 0.06 respectively) with statistical significance (P=0.001). There was no significant correlation between RQ of miR-92a and FLT3/ITD.

Correlation between plasma level micro RNA 92 and different parameters

	Number Of subjects	Plasma level micro RNA	r (p)
Age (patient) 42.90 ± 17.47	50		r_s = 0.165 0.251
Age (control) 35.09 ± 13.60	35		r_s = -0.063 0.762
FLT3			
-ve	32	0.12 (0.0 – 1.83)	0.693
+ve	18	0.15 (0.0 – 1.91)	
Response to therapy			
Non responder	16	0.06 (0.0 – 0.18)	
Partial remission	5	0.14 (0.01 – 0.50)	0.001*
Complete remission	29	0.36 (0.0 – 1.90)	
FAB			
M0	4	0.06 (0.0 – 1.91)	
M1	7	0.09 (0.2 – 0.24)	
M2	18	0.14 (0.01 – 1.83)	
M3	4	0.39 (0.0 – 1.08)	0.943
M4	8	0.23 (0.0 – 0.50)	
M5	5	0.08 (0.07 – 0.48)	
M5a	?	0.10 (0.13 – 0.10)	
M6	?	0.49 (0.18 – 0.79)	
Blast %			r_s = -0.086 0.554

r_s: Spearman coefficient

Summary / Conclusion: plasma level of miR-92a is decreased in newly diagnosed AML patients than normal subjects, and it was related to bone marrow blast percentage. This may be attributed to the package of microRNAs inside exosomes that are secreted from cells to be delivered to other cells and function in a new location. Thus, it might be possible that blast cells specifically take the exosomes that contain miR-92a and, as a result, miR-92a decreases from the plasma. Also resistance to induction chemotherapy is significantly related to lower RQ of plasma miR-92a. This may justify the possibility of its usage as a predictive to response to chemotherapy in newly diagnosed AML patients. Our data suggest the potential importance of microRNAs as noninvasive cancer biomarkers helping in diagnosis, clinical prediction of therapeutic response.

B1290
CONSOLIDATION CHEMOTERAPY WITH HIGH DOSE CYTARABINE FOLLOWED BY ALLOGENEIC HSCT IN *DE NOVO* ACUTE MYELOID LEUKEMIA PATIENTS: RESULTS FROM A SINGLE CENTRE EXPERIENCE ACCORDING TO DIFFERENCE RISK CLASSES
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Background: High-dose cytarabine applied during remission induction or as consolidation after attainment of a complete remission (CR) has become an established element in the treatment of adults with acute myeloid leukemia (AML). Recent evidences have challenged the need for these exceptionally high-dose levels of cytarabine. There is no direct evidence either to suggest that any particular genetically defined subset of AML would benefit from high-dose levels of the drug.
Aims: We report the outcome of 41 adult patients (pts) diagnosed as having AML between January 2009 and January 2013 and treated with high dose cytarabine (18 g/m2) for 2 or 3 courses as consolidation therapy followed by allogeneic hematopoietic stem cell transplantation (HSCT) and investigate the impact of this approach among different risk classes.
Methods: We consider 41 adults patients (mean age 54 years) with AML between January 2009 and January 2013. They were divided at diagnosis into low (LR, 15 pts), intermediate (IR, 17 pts) and high risk classes (HR, 9 pts) according to European Leukemia Net classification. Among LR pts, 4 were diagnosed as CBF leukemias (2 inv(16) positive, 2 t(8,21) positive, all of them c-KIT wild type) and 11 were NPM1 mutated/FLT3-ITD-. Patients allocated in HR class had complex karyotype (4 pts) or carried del(7q) (1 pt); we included in HR class also FLT3-ITD+/NPM1 wild type pts (4 pts). All other pts were defined as IR patients. All pts underwent induction therapy with cytarabine 100 mg/sqm twice daily (7 days) and idarubicin 12 m/sqm (3 days). 36 patients obtained complete remission (CR) after induction therapy and they underwent 2 or 3 cycles with high dose cytarabine (18 g/sqm per cycle, cumulative dose 36 g/sqm or 54 g/sqm). 12 pts underwent allogeneic HSCT in first or second CR (5 HR pts, 6 IR pts). Log-rank (Mantel-Cox) test applied to Kaplan-Meyer method was employed to estimate progression free survival (PFS) and overall survival (OS). OS, PFS and relapse rate were defined by the standard criteria.
Results: At 2 years 11 pts relapsed, 1 after HSCT and 10 without receiving HSCT: of them, 3 relapsed pts were not candidate to HSCT because they had a LR disease; they were all NPM1 mutated/FLT3-ITD-. Cumulative incidence of relapse at 2 years was 35.8%, 43.2% and 58.3% in LR, IR and HR pts respectively, with no statistical difference among risk classes. Overall, 12 pts underwent allogeneic HSCT in first (CR1) or second CR (CR2) No difference was found among different risk classes for PFS and OS: 2 year PFS was 64%, 42%, 57% for LR, HR and IR pts respectively (p 0.77) and 2 year OS was 65%, 50% and 53% for LR, HR and IR pts (p 0.83).
Summary / Conclusion: In our experience high dose cytarabine based consolidation regimen (36 g/mq or 54 g/mq) followed by HSCT in selected pts (IR pts in CR2 or in CR1 with a sibling donor and HR pts) seems to give an equal OS e PFS among different risk classes. Thus, in our experience this kind of approach seems to reduce the poor impact of adverse cytogenetic abnormalities and molecular markers such as FLT3 mutation in AML, without a significant difference in outcome among risk classes.

B1291
INFLUENCE OF FLT3/ITD MUTATION ON OUTCOME OF PATIENTS WITH DE NOVO ACUTE MYELOID LEUKEMIA: A SINGLE CENTRE EXPERIENCE
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Background: Survival of *de novo* acute myeloid leukemia (AML), particularly in younger patients (pts), has improved in recent years. Relapse continues to remain an important obstacle to successful outcome. This is particularly true in AML patients with FMS-like tyrosine kinase 3/internal tandem duplication (FLT3/ITD) molecular mutations. The FLT3/ITD mutations occur in about 30% of AML pts; when compared with their FLT3 wild type counterparts, FLT3/ITD+ pts are at particularly high risk of relapse when treated with chemotherapy alone. These pts are often referred for allo-SCT in first complete remission (CR1), which is becoming the preferred therapeutic option. However, there is no clear evidence that hematopoietic stem cell transplantation (HSCT) in CR1 decreases the relapse rate or improves overall survival (OS).
Aims: We retrospectively analyzed the impact of FLT3/ITD on outcome of citogenetically normal AML under 65 year-old pts treated at our institution between 2009 and 2013 in terms of progression free survival (PFS) and overall survival (OS).
Methods: We retrospectively considered 41 adults pts (median age 54 years) with *de novo* AML between January 2009 and January 2013. 39 pts who had FLT3/ITD mutation status available were included in the final analysis. The FLT3/ITD and NPM1 mutation assay was performed using fluorescent PCR with primers and amplification conditions. All pts received standard AML induction chemotherapy with cytarabine 100 mg/sqm twice daily (7 days) and idarubicin 12 mg/sqm (3 days) followed by consolidation chemotherapy with high dose cytarabine for 1–3 courses (18 g/sqm each course); among these pts, 11 underwent allogeneic HSCT. 8 pts were FLT3/ITD + (5 pts NPM1 mutated, 3 pts NPM1 wild type), 31 were FLT3/ITD- (12 pts NPM1 mutated, 19 pts NPM1 wild type). Log-rank (Mantel-Cox) test applied to Kaplan-Meyer method was employed to estimate PFS and OS. OS, PFS and relapse rate were defined by the standard criteria.
Results: At 2 years we observed a relapse after consolidation chemotherapy in 4 of 8 pts FLT3/ITD+ pts and in 5 of 31 FLT3/ITD- pts. Among FLT3/ITD+ and FLT/ITD- pts, 5 pts and 6 pts were transplanted in first or second remission, respectively. 2 years PFS was 16.7% for FLT3/ITD+ and 68.7% FLT3/ITD- pts (p 0.041); 2 years OS was 18.7% for FLT3/ITD+ pts and 72.2% for FLT3/ITD- pts (p 0.048).
Summary / Conclusion: We observed a significant difference in 2 years OS and PFS among FLT3/ITD+ and FLT3/ITD- pts, indipendently by NPM1 mutational status. In our experience FLT3/ITD mutation has a poor impact on AML patients, although most of FLT3/ITD+ pts underwent HSCT. It is debated from literature the role of HSCT in overcoming the poor impact of FLT3/ITD mutation; in different studies FLT3/ITD mutation seems to have an adverse effect on outcome of HSCT in the same direction it does after chemotherapy. Our data seem to confirm this hypothesis.

B1292

THE EFFECTIVENESS OF INTENSIVE CHEMOTHERAPY TO THE ELDERLY ACUTE MYELOGENOUS LEUKEMIA PATIENTS

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Background: Despite of advances in the treatment for acute myelogenous leukemia (AML), the response of chemotherapy for the elderly is still poor and there is no definite guideline for the elderly AML patients.
Aims: This study was conducted to verify the effectiveness of intensive chemotherapy in elderly AML patients, and to determine which sub-group of patients is more effective to the therapy.
Methods: The retrospective analysis with medical chart review was taken to the 82 patients over 65 years old and treated with AML from January 2003 to April 2012 at the Korea University Medical Center. The efficacy of chemotherapy for the elderly patients was evaluated and sub-group analysis was taken to determine which conditions are more effective to the chemotherapy.
Results: Median overall survival period of 82 patients were 3.9 months. 52 patients were treated with chemotherapy (33 patients were intensive chemotherapy with idarubicin plus cytarabine, 19 patients were low dose cytarabine) and 30 patients received best supportive care only. Among the intensive chemotherapy group, there were 16 cases of remission (48.5%) after induction chemotherapy. The treatment related mortality was 24.2% and the most common cause was pneumonia sepsis. Median overall survival period of intensive chemotherapy group was 7.9 months. In a multivariate analysis, significant factors for longer overall survival were performance status, no underlying heart disease, no fever at diagnosis, high platelet count, low blast count and type of induction chemotherapy. In the sub-group analysis, the efficacy of chemotherapy was observed in the relatively younger patients group (HR 0.437, *P*=.022), De novo AML group(HR 0.421, *P*=.025), no underlying heart disease group (HR 0.421, *P*=.031), high WBC count group (HR 0.306, *P*=.026), Low platelet count group (HR 0.12, *P*<.001), high blast counts group (HR 0.233, *P*=.005), high LDH group (HR 0.21, *P*=.001).
Summary / Conclusion: The result of chemotherapy in the elderly AML patients is poor. However the results of the chemotherapy will be better if the patients are selected appropriately through sub-group analysis.

B1293

IMMUNOPHENOTYPE DISTINCTION BETWEEN ACUTE PROMYELOCITIC LEUKEMIA AND HLA-DR-CD34 NEGATIVE ACUTE MYELOD LEUKEMIA: SINGLE CENTER EXPERIENCE

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Background: The availability of genotype-specific therapy for PML/RAR alpha positive acute promyelocytic leukemia (APL) requires exact diagnosis of this acute myeloid leukemia (AML) entity. The lineage assignment of the AML blasts cells guides to implementation of specific molecular analyses in those subtypes of acute leukemia and facilitate their further definition. APL blast cells usually do not expressed HLA-DR and CD34 antigens, but also a proportion of non-APL-AML sustain the same immunophenotypic characteristics.
Aims: In order to define more precise immunophenotypic criteria that delineate APL from non –APL-AML that are HLA-DR and CD34 negative we conduct retrospective study aimed to establish a surrogate marker profile for APL. We further evaluate antigenic and clinical features of confirmed APL by using Real time quantitative polymerase chain reaction (RQ-PCR) in comparison with the non-APL–AML cases.
Methods: We analyzed a series of 30 APL and 32 of non-APL-AML patients who were diagnosed in the last three years at the University Clinic for Hematology in Skopje, Macedonia. The diagnosis of acute leukemia was made by standard morphological examination and cyto-chemical analyses of bone marrow smears according to the criteria established by FAB Cooperative Study Group and confirmed by immunophenotyping of bone marrow aspirates and/or peripheral blood samples following the criteria of the European group for the immunological Classification of acute leukemia (EGIL). Flowcytometry analyses were performed by using the FAXS Canto II BD flow cytometer analyzer. Acquired data were analyzed with the software FACSDiva version 6.1.2 by using CD45 gating strategy. Slightly modified panel of monoclonal antibodies (McAb) against myeloid- and lymphoid-associated antigens as suggested by the EGIL was utilized.
Results: Our results showed significant differences between APL and non-APL patients in CD2, CD13, CD33 reactivity. Aberrant CD2 expression was absent in all non- APL AML. Moreover, all cases from non-APL-AML group did not expressed CD15. Mean florescence intensity of CD33, CD13 showed differences between the two groups. Regarding the clinical characteristic, non-APL-AML patients had higher leukocyte and platelets counts in comparison with APL cases.
Summary / Conclusion: Our results showed that expression of CD2, CD13, CD33 may be useful for initial delineation of APL from non-APL-AML patients that are HLA-DR and CD34 negative. However, cytogenetic and molecular characterizations are ultimate for to establishing the diagnosis of APL. Immunophenotypic characteristic could lead us prompt implementation of the specific molecular analysis in APL patients but implementation of multimodal diagnostic approach is essential for APL diagnosis and allows individual tailoring of the treatment of those patients.

B1294

HYDROXYUREA (HU) ASSOCIATED TO SUPPORTIVE THERAPY AS CONSERVATIVE APPROACH IS STILL A VALID OPTION FOR TREATING ELDERLY PATIENT WITH ACUTE MYELOID LEUKEMIA (AML)

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Background: In elderly, Acute Myeloid Leukemia (AML) is characterized by a poor prognosis, due to the presence of several negative prognostic factors as performance status (PS), comorbidities and high incidence of adverse cytogenetic abnormalities. Therefore the better treatment in this category of patients remains still controversial. During last years several studies have been performed in order to compare different therapeutic regimens, including intensive chemotherapy, low-dose of Citarabine, Hydroxyurea (HU) and other novel innovative drugs.
Aims: The aims of the present study were to evaluate clinical outcome of elderly AML patients considered "unfit" for intensive treatment and treated with conservative approach and to establish the clinical prognostic factors affecting survival in these patients.
Methods: From 10/2008 to 4/2012, 38 elderly patients with a median age of 78 years (range 63-87 years) were analyzed by a retrospective *"real life"* study. All patient were considered "unfit" to receive intensive chemotherapy and were treated with HU 30-50 mg/Kg daily and transfusions (n=21) or only whit transfusions (n=17). At diagnosis, 33 patients (86.8%) presented at least one severe comorbidity: cardiovascular disease (n=19), metabolic alteration (n=9), neurological diseases (n=6), respiratory disease (n=7) and active infection (n=8). Nineteen patients (50%) presented at diagnosis a poor PS (≥2), whereas the remaining patients had a PS of 0-1. Median value of White Blood Cells (WBC) at diagnosis was 10.150/µl (range 510-213.330). Out of 38 patients, 5 presented at diagnosis a WBC count higher than 50.000/µl, 13 between 10.000-50.000/µl and the remaining 20 lower than 10.000/µl. Median value of Platelets (PLTs) count was 55.000/µl (range: 9.000-511.000), whereas the median percentage of bone marrow blasts was 59% (range 20-100%).
Results: After a median of follow-up of 600 days (range 487-699), 4 of 38 patients are still alive. The global Overall Survival (OS) are 10.5% with a median of survival of 161 days (range 5-699) from the diagnosis. The 1-years survival rate is 21%. Poor PS (P<0.001), high WBC count (P=0.021), high percentage of bone marrow blasts (P=0.017) and older age (P=0.05) were negative prognostic factors for OS in univariate analysis. Multivariate analysis was not performed because of the low patients number. The median of hospitalization was 14 days (range 0-86) and treatment with HU was well tolerated without specific adverse effects and treatment discontinuation.
Summary / Conclusion: A conservative approach with HU and supportive therapy is still a valid option for treatment of elderly patients with AML. Our data suggest that this approach is well tolerated and it is able to obtain a fairly clinical outcome with a reduced hospitalization time in a non-selected cohort of elderly patients. Survival rate and median survival time were similar to that reported in the most important studies in which low-dose of Cytarabine and other novel innovative drugs have been investigated.

B1295

EVALUATION OF FEBRILE NEUTROPENIC EPISODES IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background: The most important cause of mortality in febrile neutropenic episodes (FNEs) which mature after chemotherapy is infections. Therefore fewer in neutropenic patients must be accepted as an infection until the contrary is proved and broad-spectrum empric antibiotherapy must be started immediately as a standard approach.
Aims: To evaluate febrile neutropenia episodes of AML patients to develop empiric antibiotic treatment policies.
Methods: Eighty-seven patients who have been treated because of acute myeloid leukemia in İnönü University Turgut Özal Medicine Center Adult Hematology Clinic between 2002 and 2010 was evaluated. The infection categories, isolated pathogen microorganisms, mortality ratios and antibiotherapy regimens in 236 febril neutropenic episodes which mature after chemotherapy were examined retrospectively in this study.
Results: Fifty-three (61.0%) of the patients were males and 34 (39.0%) were

females. The median age of the patients was 52.4. The median follow-up period was 9.5 months. In FNEs, fewer was evaluated as microbiologic defined infection (MDI) in 73 (30.9%) episodes, as clinical defined infection (CDI) in 95 (40.3%) episodes and as fewer of unknown origin (FUO) in 68 (28.8%) episodes. In forty-seven (19.9%) episodes efficient pathogen microorganism was isolated from blood cultures. 91.5% of the pathogens which isolated from blood cultures were bacteries and 8.5% was fungal agents. 55.8% of the pathogen bacteries were gram-positive and 44.2% were gram-negative. The predominant isolated gram-positive bacteria was *KNS*, gram-negative bacteria was *E. coli.* Pneumonia was the most clinical infection seen in CDI. The mean neutropenia duration was 13.3 days in all episodes, 16.7 days in MDI, 13.1 days in CDI and 10.0 days in FUO. The mortality rate was 8.5% in all episodes, 9.6% in MDI, 11.6% in CDI and 2.9% in FUO. The mean neutropenia duration in exitus patients were 21.6 days and 12.6 days in living patients. This difference was statistically significant.

Summary / Conclusion: Prolonged fever and neutropenia was more often accompanied with MDI and the lenght of the duration of neutropenia was found to be a risk factor for mortality in this study. For a better febrile neutropenia management process, medical centers must follow their infection agents closely and modify their empiric antibiotic treatment policies.

B1296

PROGNOSTIC IMPLICATIONS OF BIOLOGICAL PARAMETERS IN THE DIAGNOSIS OF ACUTE MYELOGENOUS LEUKEMIA. SINGLE INSTITUTION EXPERIENCE.

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Background: Acute Myelogenous Leukemia (AML) is a heterogeneous group of diseases, who is characterized by acquired genetic alterations in hematopoietic progenitor cell. Throughout the history it has been evaluated how different biomarkers, biochemical, cytogenetic and molecular correlate to the prognosis of the disease, survival and risk of relapse. The complete response after induction is an important goal in the prognostic of these patients.

Characteristics to diagnostic. (Table 1)

Age (years) Median (range)	Bimodal (32 y 70 years) 60 (0.23-88)
Sex (Men / Women)	57.5% / 42.5%
Subtypes FAB (%)	
- MO	4 (10)
- M1	3 (7.5)
- M2	10 (25)
- M4	13 (32.5)
- M5	8 (20)
- M6	2 (5)
- M7	0 (0)
Leukocytes	20760/mm³ (1070-296000)
- > 20.000/mm3	52.5%
- < 50.000/mm3	27.5%
Hemoglobin (g/dl) Median (range)	9.7 (5.4-14-9)
Platelets (/mm3) Median (range)	59.500 (13000-254000)
LDH (U/L) Median (range)	825 (276-13239)
Uric Acid (mg/dl) Median (range)	5.7 (1.7-12.5)
Infiltration MO % median (range)	25-98

Aims: Evaluating different diagnostic variables as potential predictors of response to induction.

Methods: We evaluated the diagnostic of AML non promyelocytic retrospectively; these were diagnosed from November 2006 to November 2011. We took the diagnosis data and specifically did a hematologic, biochemical and medular rating. We also wrote data in diagnosing bone marrow infiltration and the FAB classification; cytogenetic, immunophenotype, response rates after induction, complications and deaths. The patients chosen for chemotherapy received scheme 3+7 (idarrubicin plus cytarabine)treatment of the protocols PETHEMA (99 y 2007). In patients over 65 years it was opted for schemes 2+5 and in patients no eligible, supportive treatment and cytoreductive therapy with low dose thioguanine and/ or cytarabine.

Results: Data, related to the characteristics of the study population studied, is shown in the Table 1. We performed a comparison of variables about diagnosis to assess their impact on the response to induction therapy in candidates. We have seen significant association between the age on diagnosis and the survival at the end of the induction, as described in the literature. Superior hyperleukocitosis to 20000/mm^{3}leukocytes indicated a trend to significance for the estimation of survival to induction therapy (P=0.07). When the range was extended to 50000/mm^3, we observed statistically significant differences between groups (P<0.05). In evaluating others parameters showed no differences in the groups when considering the hemoglobin, plateletscountor uric acid to diagnosis. In the statistical analysis of LDH, there were values near to significance, as all patients, who died during induction, showed higher values of LDH. There were no statistically significant differences in the response rate in patients with different cytogenetic and molecular alterations. When phenotypes associated with leukemia measured by flow cytometry were described, the positivity or negativity of markers CD34 or CD117 did not have impact in the rates of response after induction treatment.

Summary / Conclusion: 1. Knowledge of cytogenetic and molecular alterations that influence in the development of acute leukemia allows the study of a higher number of factors in the survival. 2. Leucocytes levels to diagnostic LDH and uric acid are related, indicating an estimation of tumoral mass, replicative data y cellular turn-over. 3. In our study we have tried to identify predictors of response to induction. It does seem that citogenetic and molecular alterations to the diagnosis have not involved significant differences in obtaining response or not after induction. In conclusion, its effect on survival appears to be determinated in the medium term for possible selections of resistant clones. 4. The only factor clear induction response and survival is leukocytes level and age. 5. We are trying correlating phenotypes associated to leukemia with the success to treatment and survival. Relationship has not been established, although their range is limited in number. However, if it is clear that the depth in the characterization of the phenotype associated with leukemia is crucial for monitoring and accurate assessment of treatment response.

B1297

TUMOR FORMING PLASMACYTOID DENDRITIC CELLS ASSOCIATED WITH MYELOID NEOPLASM OR BLASTIC PLASMOCYTOID DENDRITIC NEOPLASM? A DIAGNOSTIC CHALLENGE: LITERATURE REVIEW AND CASE REPORT

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Background: Tumor forming plasmacytoid dendritic cells (TFPDC) associated with myeloid neoplasms is a clonal proliferation of mature plasmacytoid dendritic cells (PDC) associated with myeloid malignancies, normally chronic myelomonocytic leukemia and acute myeloid leukemia (AML) that can occur in lymph nodes, skin, spleen, or bone. Blastic plasmacytoid dendritic cells neoplasm (BPDCN) is derived from precursors of a specialized subset of dendritic cells, plasmacytoid dendritic cells, and hence is a myeloid-related neoplasm. It is a clinically aggressive neoplasm that is usually characterized at its onset by solitary or multiple skin lesions, often with associated regional lymphadenopathy. Case series of BPCN lacking cutaneous involvement was reported by Michael J Rauh et al in Leukemia Research 36 (2012) 81– 86. Although these two entities can mimic both show defining immunophenotypic characteristics described by Jegalian AG et al. in Adv. Anat. Pathol. Vol.16 Nº6 2009.Proliferation index Ki-67 <10% and CD56- are key data to differentiate TFPDC from a BPDCN.

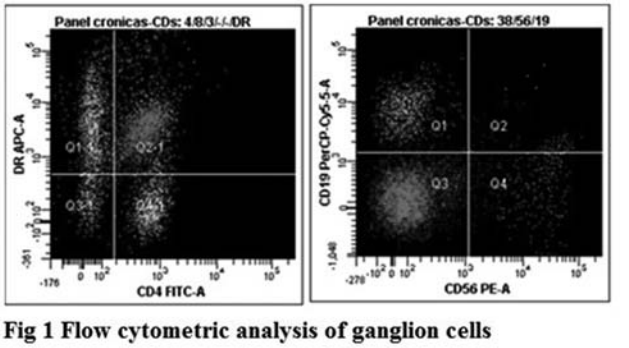

Fig 1 Flow cytometric analysis of ganglion cells

Aims: Present a case of tumor forming plasmacytoid dendritic cells associated with acute myeloid leukemia diagnosed in our institution this year.

Methods: A 68 years old woman with a history of cervical tumor and CT images compatible with multiple lymphadenopathy and splenomegaly of 14 cm was referred to hematology department for suspected lymphoma.

Results: Laboratory basal findings: normocytic anemia. Flow cytometric analy-

sis of peripheral blood was normal. Cervical node biopsy: plasmacytoid neoplasm with parafollicular and sinus distribution, immunohistochemical techniques reveal: CD68 +,CD123 +,CD4 +,CD74 +, CD43 +, CD31 + and CD56 - and granzyme B-. Flow cytometric analysis of ganglion cells: CD4 +, CD123 +,CD56-,CD11b- (Fig. 1). A TFPDC was diagnosed. Bone marrow aspiration: hypercellular bone marrow with 86% of blast cells, esterase and peroxidase were negatives. Flow cytometric analysis of bone marrow: 74% of myeloid blast cells, CD13 +,CD33 +,CD117 +,CD34 +,DR +. Bone marrow trephine : blast cells accumulations with immunohistochemical technical positivity for CD 34, CD 117, myeloperoxidase focally, CD31,CD7,negativity for CD38, CD4, CD123, CD TLE 1 and 56, compatible with AML with minimal differentiation . According to these findings, a diagnosis of TFPDC associated with acute myeloid leukemia was made. At that time there was no periphery expression of leukemia. The patient was admitted for start treatment and a new blood count and peripheral blood immunophenotype were made. The results were the following: hemoglobin: 8.9 g/dL; WBC: 24.0 10^9/L (3% neutrophils, 40% lymphocytes),LUC 3.63 10^9/L. Flow cytometric analysis of peripheral blood: 17% myeloblasts with two subtypes: one CD34+, DR+, CD13+, CD33+, CD117+, CD15-,MPO- and other CD34-, CD117+, CD13-, CD33, DR+, CD15- and 4.6% of cells with phenotype similar to ganglion cells CD4+, CD123+, CD56-, CD11b- . Standard chemotherapy induction cycle according to the PETHEMA LAM99 65 and older protocol: cytarabine and idarubicin based was started. The patient is still in hospital.

Summary / Conclusion: Although these two entities can mimic both show defining immunophenotypic characteristics. A panel of stains including CD56, TdT, and Ki-67, are recommended in cases of diffuse PDC proliferations that are clinically indistinguishable of BPDCN. Treatment and prognosis of these two entities is different so it is very important to reach the correct diagnosis.

B1298

ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS NOT CONSIDERED FIT FOR INTENSIVE TREATMENT; AZACITIDINE, LOW DOSE CYTARABINE AND ETOPOSID COMBINATION EXPERIENCE FROM A SINGLE CENTER

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Background: Acute myeloid leukemia (AML) is associated with poor prognosis in elderly patients with median survival estimated to be 2 months and a 2-year survival rate of 6%. Most of the patients cannot be treated with intensive chemotherapy (IC) because of comorbidities or poor performance status. For patients who are deemed unfit for standard induction or intermediate intensity therapy, low intensity treatments subcutaneous low dose ara-c (LDAC,), azacitidine, decitabine or best supportive care (BSC) can be chosen.

Aims: Despite marked activity in myeloid malignancy, monotherapy with azacitidine is limited by low response rates and median response durations. As with classical cytotoxic therapy, outcomes may be improved using a combination of agents azacitidine with LDAC and etoposid , which have been used to improve response rates and duration in our center.

Methods: We reviewed through medical records of 24 elderly AML patient diagnosed between 2010-13 who could not receive IC and treated with low intensity regimes including this combination or BSC. We reviewed medical records for response rates and overall survival.

Means and Medians for Survival Time

	Mean[a]				Median			
			95% Confidence Interval				95% Confidence Interval	
TREATMENT	Estimate	Std. Error	Lower Bound	Upper Bound	Estimate	Std. Error	Lower Bound	Upper Bound
AZACITIDINE	4,000	1,780	,512	7,488	2,000	1,500	,000	4,940
AZACITIDINE + ETOPOSIDE+ ARA-C	12,495	4,202	4,260	20,730	9,000	3,247	2,636	15,364
HYDROXYUREA	1,167	,441	,302	2,031	1,000	,408	,200	1,800
BEST SUPPORTİVE CARE	,867	,410	,064	1,669	1,000	,735	,000	2,440
ETOPOSIDE + ARA-C	,100	,000	,100	,100	,100			
LOW DOSE ARA-C	1,867	1,073	,000	3,970	1,000	,327	,360	1,640
Overall	6,051	1,900	2,328	9,774	2,000	1,220	,000	4,392

Results: Of the 24 elderly patients (age 61-84, mean age71,3)8 were female and 16 were male. All of them had poor performance status (ECOG ≥2), and mean ECOG was2,71. Patients' bone marrow blast ratios (BMBR) were between 20 and 93 (median 62). Four of them (16,6%) had azacitidine 75 mg/m^2/day for 7 days of every 28-day cycle, for 4 cycles. Ten of patients (41,7%) were treated azacitidine 75 mg/m^2/day for 7 days combined with cytarabine 40 mg/m^2/day for 7 days and etoposide 50 mg/m^2/day for 3 days and 1 patient (4.2%) treated etoposid and cytarabine in the same schedule. After a BMBR evaluation either continued same therapy or switched another one. Hydroxyurea alone, LDAC (20 mg twice day for 10 days) and BSC alone were each used at 3 patients (each 12,5%) BMBR was evaluated only at 8 patients that peripheral blast clearence observed, after 4 cycles in groups containing azacitidine. Complete and partial remission were only seen at combination group (44.5% and 11,1%), one patient from combinated and 2 from azacitidine alone group had progresive disease after fourth cycle and all other patients died before evaluation. Mean overall survival was 12,5 months (95% CI:4,26-20,7)at azacitidine combined with ARA-C and etoposide group and 4 months (95% CI: 0,51-7,49) at azacitide alone group. Men survivals were significantly lower in other groups (LDAC: 1.87, hydroxyurea: 1.17, BSC: 0,87, Etoposide + ARA-C:0.1 months).

Summary / Conclusion: Retrospective evaluation of elderly and medically unfit AML patients showed interesting complete remission rates of %44.4 and mean OS of 12.5 months of combination therapy of azacitidine, etoposide and LDAC but results were not from a randomized study. Azacitidine has better CR and median OS results (18% and24,5 months respectively) reported in elderly but with low (20-30%) BMBR AML before and new studies on combination of azacitidine and cytarabine reports its safety. We think additional trials about combinations ofazacitidine should be designed based on our experiences.

B1299

POSSIBILITY OF MOLECULAR REMISSION OBTAINING WITH NK AND T-CELLS RESTORATION USING AUTOLOGOUS LYMPHOCYTES INFUSIONS AND IL2 IN RELAPSED ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Chemotherapy together with the antitumor action leads to depletion of the lymphoid population which possesses a probable antileukemic potential. Recovery of lymphoid population takes a long period of time. More rapid lymphoid recovery after chemotherapy associated with good long term prognosis.

Aims: To assess antileukemic potential and recovery of the autologous lymphoid population in adult acute myeloid leukemia (AML) we initiated the pilot study performing multiple harvesting, storing and reinfusing of autologous lymphocytes in relapsed AML patients during second morphological remission in period of consolidating chemotherapy (CT) followed by interleukin-2 (IL-2) continuous infusions.

Subpopulations	Before lymphocytapheresis		After all autologous limphocytes infusions	
	M2 Pt	M4 Pt	M2Pt	M4Pt
T-cells	x10^9/L			
CD3+	0,36	1,082	0,739	0,147
CD4+CD8-	0,0827	0,26	0,236	0,054
CD4-CD8+	0,229	0,822	0,389	0,09
CD4+ T-cells subpopulations				
CD4+CD28+CD45RA+	0,0212	0,0439	0,055	0,00221
CD4+CD57+	0,00678	0,0078	0,0104	0,00448
CD4+CD25high	0,00132	0,0148	0,0046	0,0081
CD8+ T-cells subpopulations				
CD8+CD28+CD45RA+	0,0137	0,0518	0,0202	0,0099
CD8+CD57+	0,103	0,192	0,158	0,0519
NK subpopulations				
CD56+CD3-	0,0391	0,162	0,17	0,245
CD56highCD16-	0,0046	0,0225	0,0272	0,0108
CD56lowCD16+	0,0289	0,0951	0,107	0,211
CD56lowCD16-	0,00375	0,0433	0,025	0,0173

Methods: We treated two relapsed AML patients (pts): M2 t(8;21) and M4 inv(16). The second morphological remission in both pts was obtained after CT consisted of 7+3 Ida (conventional dose AraC + idarubicin) and HAI (high dose AraC + idarubicin) in M2 pt and two 7+3 Ida blocks in M4 pt. The consolidation CT consisted of 7+3 Ida two blocks in both pts and 5+2 Ida one block in M4 pt. The 1-3 lymphocytaphereses (LA) were performed straight before every consolidation CT block. The cells were stored frozen in liquid nitrogen. Unfrozen lymphocytes were transfused on 10th day after every consolidation CT block in profound cytopenia period. IL-2 started on lymphocytes reinfusion day and continued for 5 consecutive days 2-6 mln U per day as continuous i.v. infusion.

Results: In M2 pt 9,2x10^9 lymphocytes before 1st CT block and7,4x10^9 lymphocytes before 2nd CT block were harvested. In M4 pt 15,9x10^9 lymphocytes before 1st CT block, 17,1x10^9 lymphocytes before 2nd CT block and7,7x10^9 lymphocytes before 3rd CT block were harvested. After completion of consolidation CT with autologous lymphocytes infusions and hematological recovery

in both pts we observed increase of NK count in peripheral blood (see table), especially CD56lowCD16+ subpopulation (mature cytotoxic NK). The other NK subpopulations were changing in different ways. CD56highCD16- cells (immature NK) and CD56lowCD16- cells (mature NK) increased in M2 pt but decreased in M4 pt. In M2 pt CD3+ T-cells increased including CD4+CD8- (presumably) and CD4-CD8+ (to a small degree) together with their subpopulations: CD4+CD28+CD45RA+ (naive CD4+), CD4+CD57+ (terminally differentiated CD4+), CD4+CD25high (regulatory T-cells), CD8+CD28+CD45RA+ (presumably naive CD8+), CD8+CD57+ (terminally differentiated CD8+). In M4 pt all those CD3+ cell subpopulations decreased. In M2 pt relative amount of both T-cells and NK increased from 25,8% to 91%, NK compartment increased from2,5% to 17%. In M4 pt relative amount of both T-cells and NK decreased from 92% to 80%, but NK compartment increased from 12% to 50%. As a result of consolidation CT with autologous lymphocytes infusions in M2 pt, but not in M4 pt, molecular remission (mCR) was obtained.
Summary / Conclusion: The data of the study point out that in AML pts repetitive autologous lymphocytes harvesting before and reinfusion after consolidating CT blocks together with IL-2 stimulation could lead to significant increase of circulating NK cells, particularly mature cytotoxic NK subpopulations. T-cell subpopulations restoring correlated with mCR achievement. The lack of mCR was associated with all T-cell subpopulations decrease (especially naive CD4+, naive CD8+ and regulatory T-cells).

B1300
THE CLINICAL AND THERAPEUTIC FEATURES OF ACTUE PROMYELOCYTIC LEUKEMIA IN CHILDREN.
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Background: Acute myeloblastic leukemia (AML) is a heterogeneous group of malign diseases characterized by uncontrolled proliferation of myeloid progenitor cells in the bone marrow. It accounts for 15-25% of all childhood acute leukemia. Acute promyelocytic leukemia (APL) is an uncommon form of acute myeloid leukemia (AML). APL in children has constituted only 6-10% of AML. Molecularly, the disease is characterized by a fusion protein, PML/RARa that results from a balanced reciprocal translocation between the PML gene on chromosome 15 and the retinoic acid receptor alpha gene on chromosome 17. A major advance in the treatment of APL has been the use of all-trans-retinoic acid (ATRA). ATRA differentiates leukemic promyelocytes into mature granulocytes. Concurrent chemotherapy and ATRA have resulted in superior remission and survival rates so does the Arsenic in relapsed ones's.The aim of our study is to report the epidemiological, clinical and therapeutic features of APL in children.
Aims: The aim of our study is to report the epidemiological, clinical and therapeutic features of APL in children.
Methods: We report 9 cases of newly diagnosed APL in children patients. These patients were followed in the clinical hematology department at Farhat Hached university hospital (Sousse -Tunisia) over a period of 10 years.
Results: The median age was 13 years (3- 18 years). 44% of the patients were male with female/male ratio of 1.25. The motif of consultation was hemorrhagic syndrome of varying intensity in 8 cases and splenomegaly in one patient. One patient presented a left exophthalmia with homolateral facial paralysis. The blood cell count showed no leukocytosis in all patients and low levels of platelets so a high tendency for severe bleeding. The diagnosis is made after cytology, cytogenetic and molecular analysis (made in 5 patients). The t (15; 17) was detected in all patients. The research of PML/RARA mutation done in 5 patients was positive in 4 cases. All patients were treated with chemotherapy (induction + 3 consolidations courses) and ATRA according to AIDA protocol. Only one patient did not receive the ATRA due to its unavailability. 7 patients have completed the treatment and the other 2 patients refused to continue treatment (lost sight after the third cure of chemotherapy). The cytology, cytogenetic and molecular remission was achieved in 8 patients (89% of cases). The relapse was observed only in one patient (14 years old) 2 years after end of treatment. The patient received the same initial treatment due to the unavailability of arsenic with obtaining a complete remission. The bone marrow transplant was performed in this patient after the second course of consolidation with good results. Medium global survival rate was 43 months (24-84).
Summary / Conclusion: Since the introduction of ATRA, there have been remarkable advances at the laboratory and clinical level in treatment of APL. APL is the most curable disease and is a paradigm for successful targeted treatment. However, the treatment of APL in children is challenging because of the risks of early death and potential long-term cardiac toxicity resulting from the need to use high doses of anthracyclines. Prospective, randomized large clinical trials are needed to address several issues in APL and to possibly minimize and eliminate chemotherapy by combining ATRA and Arsenic.

B1301
ACUTE MYELOID LEUKEMIA WITH FLT3-ITD AND COMPLETE ANDROGEN INSENSITIVITY SYNDROME (MORRIS SYNDROME) –A CASE REPORT
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Background: In the classification of disorders of sex development Morris syndrome is included in the group of 46,XY disorders. It is a rare X-linked recessive condition, due to a complete or partial insensitivity to androgens, resulting in a failure of normal masculinization of the external genitalia in chromosomally male individuals.
Aims: We report a rare case of a patient with Morris syndrome, proven during late childhood, associated with acute myeloid leukemia. The described combination has not been previously reported in the literature.
Methods: A 41 year old phenotypically female patient was diagnosed in our department with acute myeloid leukemia with maturation, according to the WHO classification 2008. The diagnosis was established on the base of 56% blasts detected in peripheral blood and 74% blast population in the bone marrow samples. Cytochemical reaction for myeloperoxidase was positive. Multi color flow cytometric analysis of the bone marrow and peripheral blood blasts showed positivity for CD45, CD71, CD117; cyCD13 and cyMPO, while CD34 and CD15 were negative. Conventional cytogenetic analysis showed a male karyotype (46 XY), while molecular genetic tests revealed FLT3-ITD.
Results: A complete remission was achieved after a standard induction regimen, followed by consolidation and intensification therapy. Six months after the diagnosis the patient underwent a successful peripheral blood stem cell transplantation from a male sibling donor.
Summary / Conclusion: The described case is interesting on account of the combination between a rare genetic disorder and acute leukemia with molecular aberration.

B1302
CLINICAL RELEVANCE OF THROMBOSPONDIN RECEPTOR (CD36) EXPRESSION IN EGYPTIAN DE NOVO ADULT ACUTE MYELOID LEUKEMIA
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Background: Acute myeloid leukemias (AMLs) are a heterogeneous group of disorders that often present with different morphological, immunophenotypic, and cytogenetic patterns. Identification of these characteristics may be useful for a better prognostic evaluation and for a more appropriate therapeutic approach. CD36 is a transmembrane, highly glycosylated, glycoprotein commonly expressed on blasts in acute monocytic leukemia, megakaryoblastic leukemia, and erythroleukemia.
Aims: We evaluated CD36 surface expression in 97 newly diagnosed AML patients, and the results were correlated with the morphology, immunophenotype, cytogenetic pattern, and clinical outcome.
Results: CD36 antigen was recorded in 48 of 97 patients (49.5%) and particularly in those with M5 and M6 FAB subtypes. Moreover, CD36 expression was significantly associated with the expression of CD11b (P=0.001) and CD14 (P=0.0001), unfavorable cytogenetic abnormalities (P=0.001), shorter overall survival (P> 0.0001), and leukemia-free survival (P=0.03).
Summary / Conclusion: On the basis of the results of the study, it can be concluded that CD36 expression in AML patients may identify a subgroup with a poor prognosis, and may thus be a valuable adjunct to be added to the current prognostic factors.

B1303
LEUKEMIC TRANSFORMATION OF MULTIPOTENT HEMATOPOIETIC PROGENITOR CELL WITH T(4;11)(Q21:Q23) , MLL-AF9 FUSION PRODUCT AND LINEAGE SWITCH FROM B ACUTE LYMPHOBLASTIC TO ACUTE MONOBLASTIC LEUKEMIA (AML
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Background: Aim of the study. A chromosomal translocation t(4;11)(q21:q23) is associated with an aggressive mixed-lineage leukemia. We present a patient with B acute lymphoblastic leukemia and t(4;11)(q21:q23) az the diagnosis who during evolution of the disease changed immunophenotype to acute monoblastic leukemia, AML M5.
Aims: We have analysed the clinical course and responses to treatment of this so called mixed lineage leukemias which are mostly associated with a poor prognosis and obviously urges the search for better treatment modalities.
Methods: The diagnosis of the disease was based on cytology, cytochemistry

and immunophenotypic analyses with flow cytometry and . Cytogenetic analysis.
Results: A 37-year-old male presented in April 2012. with thrombophlebitis of deep veins on both legs, malaise and loss in weight. Laboratory data: Hb 118 g/l, WBC 254x109/l (with 91% blasts in differential leukocyte formula), platelets 60x109/l. In bone marrow aspirate hypercellularity was found with 90% blasts , which were myeloperoxidase negative. Flowcytometry of the blast cells detected the following immunophenotype: (HLA-DR, CD38,Tdt,CD19, CD22,cCD79a,cIgM, CD15)+ and (CD10,CD24-). Cytogenetic analysis: 46,XY, t(4;11)(q21:q23)[2]/62-82,XY,t(4;11)[18]. Molecular analysis showed MLL-AF4 rearrangment. The patient was on LMWH and chemotherapy according to protocol hyperCVAD. On day 11 a myocardial infarction was diagnosed with AV block II range. After recovering and achiving myocardial ejection fraction above 60% bone marrow aspirate showed presence of 63% blasts but with cytomorphologic monocytoid appearance which on repeated flowcytometry expressed the following immunophenotype (CD33, Lysozime, CD11b, CD64, CD24, CD56)+. Minor population of B lymphoblasts(CD19, CD79a, CD64, cLizozim, CD56)+ was detected. The cytogenetic evolution was also found: 46XYt(4;11)(q21;q23)[19],/47XY,t(4;11)(q21;q23),+C[1]. Chemotherapy with HiDAC+Daunoblastin (Ara-C 2 x 6 g days1,3,5,7 and DA 100 mg day2,4,6)-was given. The bone marrow aplasia lasted 20 days during which developed febrility, hepatosplenomegaly with huge abssces in the upper pole of the spleen, bronchopneumonia, anuria, hypotension. Control bone marrow aspirate showed hypocelullarity without blasts but again 2 weeks later bone marrow aspirate contained 17% of blasts of monocytopid morphology. He was treated with purinethol, mitoxantrone and vepesid. At this time cutaneous leukemic nodular infiltrates appeared. Again after period of aplasia 24% of blasts remained in bone marrow. Very soon the number of WBC rose to 45x109/l and in spite of the therapy with purinetol he died 8.10.2012 , 4 months after clinical presentation.
Summary / Conclusion: Conclusion.Analysis of cytoplasmatic and surface cell markers of leukemic cells permitt classification most of the leukemias depending on lineage belonging and maturation stage. Lineage switch is a phenomenon when acute leukemia express markers for one lineage at the diagnosis and meets the opposite phenotypic criteria upon relapse. The fusion MLL-AF9 is associated more commonly with acute myeloid leukemia. Lineage switch is nowadays considered as mixed lineage leukemia which occurs in 6-9% of cases at relapse. This phenomenon may occur due to clonal selection because chemotherapy eradicates the dominant clone present at diagnosis and enabling the expansion of lineage different clone at relapse.

B1304
PROGNOSTIC SIGNIFICANCE RBC TRANSFUSIONS' VOLUME INFUSED DURING THE FIRST INDUCTION CHEMOTHERAPY TO PATIENTS WITH DE NOVO ACUTE MYELOID LEUKEMIA (AML)
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Background: Long-term survival of patients with AML is dependent from different factors. R. Comrokji and co-authors [ASH 2011, Abstr. 3600] demonstrated the prognostic significance of 10 RBC transfusions during induction period on overall survival (OS).
Aims: The aim was to verify whether RBC transfusions are a potential predictor of OS.
Methods: A retrospective analysis of 38 patients' data treated with 7+3 scheme during the first induction chemotherapy was done.
Results: Median age of patients was 40.5 y. (17 – 61). Complete remission was verified in 24 patients (63.2%). The number of RBC transfusions was 3 – 20, median 7.5. There was no correlation between number of RBC transfusions and age of patients, and between number of RBC transfusions and variant of response to chemotherapy. The difference of OS was not statistical significance between the groups of patients received ≤7 RBC doses (n=21) and >7 RBC doses (n=17): 25.8 mo versus 9 mo; P=.334. But the difference of OS was significant when the borderline of RBC transfusions have been chosen like 10: 26.6 mo (n=27) versus 7.3 mo (n=11). The composition of 11 patients with the worst OS was very different. There were 4 patients with complete remission and 7 patients with no any kind of response after the first induction course of chemotherapy. According to ELN criteria there were 3 patients with good karyotype, 7 with intermediate karyotype, and 1 patient with poor karyotype.
Summary / Conclusion: The reason of poor outcome of patients with de novo AML received high number of RBC transfusions during the first induction course is not clear. We suppose 2 reasons at least. The first one is the biological phenotype of leukemic cells and its negative influence on recovery of hematopoesis in postinduction period. The second one is the injury of microenvironment that takes the active role in the progression of AML. Nevertheless we conclude that the patients transfused with more than 10 RBC doses during the first induction chemotherapy have to consider like potential candidates aggressive consolidation including to stem cell transplantation.

B1305
CONCOMITANT OCCURRENCE OF BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM AND ACUTE MYELOID LEUKAEMIA AFTER LENALIDOMIDE TREATMENT FOR DEL (5Q) MYELODYSPLASTIC SYNDROME: A COINCIDENCE OR MORE?
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Background: Lenalidomide, a thalidomide analogue with multiple mechanisms of action implicated in cancer, including inhibition of angiogenesis, modulation of cytokines, induction of apoptosis, inhibition of growth and cell adhesion, increase of T cells and NK cells *via* stimulation of dendritic cells, has been approved in 2005 by FDA specifically for the treatment of 5q- syndrome, a subtype of myelodysplastic syndrome (MDS) characterized by a specific hematologic phenotype and low rate of progression into acute myeloid leukemia in comparison to other subtypes of MDS.
Aims: Describe the concomitant occurrence of blastic plasmacytoid dendritic cell neoplasm (BPDCN), a rare and highly aggressive lymphoma deriving from the clonal proliferation of precursors of plasmacytoid dendritic cells (pDC), and of acute myeloid leukaemia observed in a MDS 5q- patient who had been previously treated with lenalidomide.
Methods: Literature search for: mechanisms of action of lenalidomide and of resistance to it; mechanism of MDS transformation; AML and BPDCN pathogenesis.
Results: The main findings are the following: 1) Up to 20% of BPDCN are associated with or subsequently develop myeloid disorders: this fact could be explained by the hypothesis that malignant pDC cells and myeloid blast might have a common origin at an early stage of differentiation. 2) Lenalidomide increases the expression of the ARPC1B gene, that is both an activator and a substrate of aurora A: its depletion inhibits aurora A, which impairs the ability of mammalian cells to enter mitosis. Moreover, ARPC1B regulates the centrosome integrity and has been described as a regulator of cell cycle. Because overexpression of aurora A leads to polyploidy and chromosomal instability, the augmented expression of ARPC1B induced by lenalidomide may have a negative impact on the stability of 5q- patients remission. 3) The mechanism of MDS transformation is thought to be due to clonal expansion of an abnormal pluripotent stem cell. In fact, most of the patients, including ours, acquired new chromosomal aberrations in addition to the 5q-. In the specific case of 5q- MDS it has been speculated that lenalidomide, while suppressing an indolent clone, prolonging the disease free survival period, can potentially favour the selection of a more aggressive clone, subsequently evolving to acute leukaemia. 4) It has been shown that there is persistence of rare but distinct stem cells harbouring the 5q- in patients even in complete remission after lenalidomide treatment. These cells provide a reasonable explanation both for relapses and clinical and cytogenetic progression. Actually, both the propensity toward clonal evolution and transformation of these persistent MDS stem cells and the role of lenalidomide in this process (inhibiting or accelerating it), are unknown.
Summary / Conclusion: It is possible that our patient presented cells resistant to lenalidomide that continued to expand slowly during treatment with lenalidomide, even when the patient appeared to be in complete cytogenetic remission, and that constituted the basis for AML progression. Lenalidomide, through induction of ARPC1B gene, may have contributed to this process; moreover, for its known stimulatory effects on dendritic cells, it may have triggered the expansion of malignant pDC from the common clone they share with myeloid blasts.

B1306
THE USE OF AZACITIDINE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA UNFIT FOR INTENSIVE TREATMENT OR RESISTANT TO CHEMOTHERAPY
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Background: The effectiveness of hypomethylating agents for treatment of high risk myelodysplastic syndromes has raised the question of its potential use in acute myeloid leukemia (AML) patients unfit to receive intensive chemotherapy or with relapsed/refractory disease.
Aims: To analyse the use of azacitidine in patients with acute myeloid leukemia unfit for intensive treatment or resistant to chemotherapy
Methods: Herein, we retrospectively analyzed the outcomes of a series of 15 AML patients treated with 5-Azacitidine in a single centre, between 2009 and 2012.
Results: The median age was 64 (range, 45-76). Five patients (33.3%) received 5-Azacitidine as first-line treatment. Seven (46.7%) medically unfit for intensive chemotherapy or 8 (53.3%) relapsed/refractory patients were given a 7-day regimen of 5-Azacitidine every 28 days. A median of 4 (range 1-15) Azacitidine cycles were given to each patient. Cytogenetic risk was intermediate in 7 (46.7%) and poor in 8 (53.3%) patients. Of 10 patients with evaluable

FLT3 and NPM1 mutations, 1 (6.7%) patient had isolated FLT3-ITD, 1 (6.7%) patient had isolated NPM1 mutation, and 8 (53.3%) patients had no FLT3 and/or NPM1 mutations. Six (40%) patients presented with more than 30% of blasts before initiating treatment. The median overall survival (OS) of this cohort was 12 months. The overall response rate was 40% (6 of 15 patients), and included 2 (13.3%) complete remissions, 2 (13.3%) partial responses (less than 20% blasts) and 2 (13.3%) patients had any kind of hematological improvement. The best cumulative response was seen at a median of 4 cycles (range, 2-8). The median number of blasts at diagnosis and post-treatment was 50% (range: 30-90) and 17.5% (range: 2-60), respectively. The median OS for patients who achieved a response was 18 months versus 4 months for non-responders (P=.029). Of note, it was observed a response in two (13.3%) patients with pre-treatment relapsed/refractory AML: one patient achieved complete remission (< 5% blasts) and other patient showed a reduction in the number of blasts to 12% (evaluated at the 4th cycle of 5-Azacitidine). No significant differences were found for OS with regard to age, cytogenetics, number of pre-treatment blasts and "de novo" versus relapsed/refractory AML. During treatment, five patients (33.3%) presented adverse events: the most common was febrile neutropenia, observed in 3 (20%) patients. Two (13.3%) of these patients required hospitalization due to febrile neutropenia. None of the remaining 13 (86.7%) patients needed to be admitted in hospital throughout 5-Azacitidine treatment.

Summary / Conclusion: In conclusion, our analysis demonstrates that 5-Azacitidine is generally well tolerated by patients unfit for intensive chemotherapy. Although our series is small, it was possible to observe an anti-leukemic activity of 5-Azacitidine in a significant number of poor prognosis patients.

B1307

MULTIPLE INVOLVED SITES OF EXTRAMEDULLARY MEGAKARYOBLASTIC LEUKEMIA IN A CHILD

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Background: Extramedullary acute megakaryoblastic leukemia is a rare entity. To our knowledge there are 10 pediatric case report in the literature.These patients tend to be younger than 2 years of age and accompanying lytic bone lesions can be seen. Bone marrow involvement was noted at the time of diagnosis in all these case reports except one. Literature review revealed multiple sites of bone infiltration and bone + skin involvement however here we report a patient with multiple sites of infiltration (iris,orbita,gingiva, maxilla, skin, testis) without a prominent bone lesion and bone marrow involvement.

Aims: Diagnosis of the extramedullary presentation of acute megakaryoblastic leukemia is somehow challenging. Atypic morphology of megakaryoblasts outside the bone marrow and sometimes similar morphological features with small blue cell tumors may cause confusion. However, immunohistochemical analysis(CD41, CD61) detection of t(1:22) which is characteristic among infants with AML-M7 may help in diagnosis.We aimed to present our unique case.

Results: Case: A 8- month- old boy was admitted to our hospital for a swollen, red left eye. His symptoms began 2 days prior and his mother recognised painless nodularity on his forehead and eyebrow beginning with an erythematous papul 6 days ago. He was treated with an antibiotic treatment with the initial diagnosis of preseptal cellulitis. An orbital MRI was not compatible with preseptal cellulitis however a mass with a 1x2x1 cm in diameter exhibit homogeneous contrast enhancement and another mass with a 3x2x2 cm in diameter which was invaded the right maxillary sinus and obliterated right pterygopalatine fossa were detected. His past medical history revealed intermittently appearing skin lesions localised on the lower extremities for 4 months and a skin biopsy detect atypical T lymphocyte infiltration at the local hospital. Physical examination showed mild gingival hypertrophy, multiple painless nodules on his extremities, skulp, left eyebrow and forehead. Laboratory examination revealed Hb:12.9 g/dl, Htc:39.3 %, MCV:71.6 fl, WBC: $9x10^{-9}$/l Plt: $594x10^{-9}$/l peripheral smear showed 65% lymphocyte, 25% PMNL, 10% inmature lymphocyte with an hypochromic microcytic erythrocyte morphology. Orbital examination under general anestesia showed a stromal infiltration of the iris which may suggest a leukemic infiltration.Bone marow aspiration, biopsy and flowcytometry did not confirm leukemic infiltration. Incisional biopsy of the maxillary mass was suggestive of neoplastic infiltration of megakaryoblasts with different maturation stages and stromal fibrosis(immunohistochemically CD61, CD68, CD31, CD43 and Factor 8 were positive , MPO, Tdt, CD20, CD3 and CD117 were negative).Cytogenetic studies and FISH were normal. During his follow up scrotal enlargement and nodularity was detected and excision of these nodules again showed a megakaryoblastic infiltration. AML-BFM 2004 protocol was given and after receiving chemotherapy skin and testicular lesions disappear.

Summary / Conclusion: Our previous experince with the extramedullary infiltration showed that 40% of pediatric AML had EMI at the time of diagnosis and 27% demonstrated multiple involved sites wheras 73% had single site involvement and EMI was detected in children with all subtypes of AML except in patients with AML-M7. Therefore, this patient is unique for us who had the unusual presentation of multiple involved sites with no peripheral and bone marrow abnormalities.

Chronic lymphocytic leukemia and related disorders – Biology

B1308

STUDY OF ANGIOGENESIS ON LYMPH NODES OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background: An increasing body of evidence support the hypothesis that angiogenesis plays an important role in the leukemogenic process in CLL. Among angiogenic factors VEGF has a key-role in promoting tumor angiogenesis.There are only a few reports with small series of patients on the evaluation of angiogenesis on lymph nodes of CLL patients.

Aims: To evaluate the angiogenic status including the measurement of microvessel characteristics and VEGF expression in lymph nodes and other tissues apart from bone marrow specimen of Chronic Lymphocytic Leukemia (CLL) patients and correlate it with clinical and laboratory parameters as well as with patients' survival.

Methods: Sixty-two patients fulfilling the diagnostic criteria of CLL-SLL were enrolled in this study. The specimen analysed was lymph node in 57 cases and spleen in 5 . This analysis was performed in formalin-fixed, paraffin-embedded tissue. Microvessel characteristics were evaluated using CD31 staining and parameters regarding microvessel caliber (area, perimeter, Feret diameter and major and minor axis length), microvessel shape (shape factor and compactness) and the extent of microvascular network [total vascular area (TVA) and microvascular density (MVD)] were assessed in the region of most intense vascularization using image analysis software. VEGF assessment was obtained separately in the proliferation centers (PCs) and in the non proliferation areas (nPC) using proper antibody. A Histo-score (H-score) based on the percentage of stained neoplastic cells multiplied by staining intensity was calculated. Moreover, the extent of vascularization within and outside the PCs was assessed manually.

Results: Median patients' age was 58 years (36-78) with male predominance. Thirty-seven (60%) had disease stage A while 17(27%) and 8(13%) had B and C respectively. Seventeen patients presented with splenomegaly, 7(11%) with bulky lymphadenopathy and 6 (10%) with B-symptoms. Eighteen patients had elevated LDH. 29 and 25 out of 50 patients presented with CD38 and ZAP-70 positivity respectively. VEGF expression in the PCs ranged from 0,5 to 70% (H-score 0,75-285)with a median value of 25% (H-score 30) and was significantly higher from that observed in the non-PC areas (range 0-90%, median 20%, H-score 20-200, p value <0.0001). Non-PC areas showed higher extent of vascularisation compared to PCs in 55,6% of the examined cases, which also displayed higher major and minor axis length (P=0.0128 and P=0.0253), as well as higher vascular area(P=0.0119) and perimeter (P=0.0383).Those cases that displayed higher VEGF in the PCs showed also lower MVD (P=0.0345), TVA (P=0.0257) and vascular shape factor (P=0.0431). Moreover, VEGF expression in the PCs was negatively correlated with TVA and MVD (r=-0.03536, P=0.0344 and r=-0.02992 and P=0.0762 respectively). VEGF expression within and outside the PCs was not correlated with any of the patients' clinical parameters. Finally, higher VEGF H-score in the PCs (>90) was correlated with increased time to relapse (median time 100.3 months) when compared to decreased VEGF H-score (median time 39.3 months).

Summary / Conclusion: This is the largest study concerning the assessment of angiogenesis in lymph node of CLL patients. VEGF expression is higher in the PCs compared to the non-PC areas CLL lymph nodes are not uniformly vascularized Lower VEGF expression in the PCs associated with shorter time to relapse.

B1309

UBLITUXIMAB (TG-1101), A NOVEL ANTI-CD20 MONOCLONAL ANTIBODY, DISPLAYS MARKED IN VITRO ACTIVITY IN LOW-CD20 EXPRESSING TUMORS

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Background: In contrast to normal B-lymphocytes, malignant B cells in CLL patients (pts) generally express low CD20 levels on their cell surface. Rituximab (RTX) has shown limited activity in CLL pts as a single agent, often attributed to low CD20 expression. Importantly, RTX exposed pts have also exhibited B cells with decreased CD20 levels. Ublituximab (UTX) is a novel, chimeric mAb targeting a unique epitope on the CD20 antigen. UTX has been glycoengineered to enhance affinity for all variants of FcγRIIIa receptors, demonstrating

greater ADCC activity against NHL/CLL cell lines compared to RTX and ofatumumab (OFA), an anti-CD20 mAb optimized for high CDC activity. UTX has displayed single agent activity in a Phase I trial in Rel/Ref CLL pts (ORR=45%).
Aims: Herein, we analyzed the CDC and ADCC activity of UTX vs. OFA and RTX in cell lines exhibiting varying levels of CD20.
Methods: CD20 expression/binding were quantified in MEC-1, SUDHL-8, Raji, JY E5.1 and patient CLL cells by flow cytometry. C1q-dependent-CDC triggered by OFA, RTX and UTX was determined. ADCC in various cell lines was observed after incubation with mAbs at various concentrations in the presence of PBMCs.
Results: Higher CDC activity was observed for OFA vs. UTX and RTX on several cell lines expressing high CD20 levels (MEC-1 cells: 90% cell-lysis by OFA vs 60% by UTX). In contrast, CDC activity against low CD20 cells was significantly lower and almost identical with all three mAbs (≤5%). UTX was shown to mediate higher ADCC than OFA and RTX against all cell lines (MEC-1 cells: 55% UTX lysis vs <30% RTX or OFA lysis; Raji cells: UTX EC_{50}= 0.93 ng/ml vs RTX EC_{50}=14.20 ng/ml). Importantly, UTX consistently mediated potent ADCC against low CD20 cells, whereas OFA and RTX ADCC activity was diminished (SUDHL-8 cells: 20% UTX lysis vs <10% RTX or OFA lysis; B-CLL cells: UTX EC_{50}= 5.0 ng/ml vs RTX EC_{50}=125.0 ng/ml).
Summary / Conclusion: While CDC activity of OFA was diminished in low-CD20 cells, ADCC activity of UTX was robust in cells with low and high CD20 expression. Results support the potential therapeutic use of UTX in hematologic malignancies with low CD20 expression such as CLL, Marginal Zone lymphoma, and other lymphoma subtypes in addition to RTX or OFA Rel/Ref pts.

B1310

PHOSPHORYLATION OF ROR1 RECEPTOR TYROSINE KINASE AT TYROSINE 641, 646 AND SERINE 652 RESIDUES: THE IMPACT ON THE SURVIVAL OF LEUKEMIC CELLS OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Receptor tyrosine kinases (RTK) are important for different cellular processes as cell proliferation and survival, as well as for the malignant transformation of many types of cancer. ROR1, as a member of RTK families, has shown to be overexpressed in chronic lymphocytic leukemia (CLL), mantle cell lymphoma and other hematological malignancies. ROR1 inhibition in CLL leukemic cells induced apoptosis of the cells. In this study, we studied the effects of an anti-ROR1 mAb for specific dephosphorylation at the tyrosine kinase domain of ROR1 in CLL cells.
Aims: To investigate phosphorylation of tyrosine and serine residues, within the kinase domain of ROR1, of importance for survival of CLL cells.
Methods: Bioinformatic analysis of the ROR1 structure revealed that three amino acid residues in the tyrosine kinase domain might be critically phosphorylated. Based on this prediction, a 19 amino acid long peptide, phosphorylated at two tyrosine (tyrosine $_{641, 646}$) and one serine (serine $_{652}$) residues was designed and used for immunization of rabbits. An anti-phospho-ROR1 (pROR1) polyclonal antibody (pAb) was purified, using phospho-peptide affinity chromatography. The specificity of anti-pROR1 pAb was determined by ELISA, immunoprecipitation (IP) and western blot experiments. An anti-ROR1 mAb (IgG) (CRD 1D8 clone) was used to investigate the effects on ROR1 phosphorylation in CLL cells at tyrosine $_{641, 646}$ and serine $_{652}$ residues preceding apoptosis. ROR1 phosphorylation was investigated by western blot and IP of ROR1 probed with anti-pROR1 pAb, from untreated and CLL cells treated with the anti-CRD 1D8 mAb. Quantitative intracellular staining of ROR1 by flowcytometry in time kinetics experiment after treatment with anti-CRD 1D8 mAb was also used to check phosphorylation of ROR1. Annexin V/PI staining (flowcytometry), MTT assay, PARP and caspase 8 cleavage as well as MCL-1 protein (western blot) were used for detection of apoptosis. To investigate phosphorylation and localization of 64-130 kDa ROR1 isoforms in various compartments of CLL cells, lysates were prepared from the nucleus and cytoplasmic proteins of CLL cells.
Results: Two tyrosine (641, 646) residues and one serine (652) residue of the tyrosine kinase domain were phosphorylated in CLL cells. As previously described (Mellstedt et al, Abstract No: 1771, 53th ASH annals meeting, 2011), the 64, 105 and 130 kDa ROR1 isoforms were shown to be constitutively phosphorylated at tyrosine and serine residues in CLL leukemic cells. Treatment of CLL cells with an anti-ROR1 mAb against the CRD domain induced rapid dephosphorylation of ROR1 at tyrosine $_{641, 646}$ and serine $_{652}$ residues within 20 min and gradually increased up to 4 hours. The phosphorylated 64 kDa ROR1 isoform was localized to the nucleus of CLL cells and probably represents an intracellular part of ROR1, while the ROR1 130 kDa isoform was presented both in cytoplasm and nucleus of CLL cells.
Summary / Conclusion: Our data show that the ROR1 molecule is phosphorylated at tyrosine $_{641, 646}$ and serine $_{652}$ residues. The presence of 64 and 130 kDa ROR1 isoforms in the nucleus of CLL cells may suggest a role of these isoforms as transcription factors. Collectively, the data might suggest that phosphorylated ROR1 may be an important protein for the growth of CLL cells as well as an interesting structure to target in a therapeutic intervention.

B1311

THE REPERTOIRE OF HEAVY CHAIN IMMUNOGLOBULIN GENES IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA IN UKRAINE AND RUSSIA

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Background: Mutation status of the heavy chain variable region genes (IgVH) is known as an important factor in long-term prognosis in B-cell chronic lymphocytic leukemia (B-CLL). A more detailed study of IgVH genes led to the discovery of stereotyped antigen receptors (SARs) which carry highly similar or almost identical amino acid sequences. Narrowing of the repertoire of IgVH genes in CLL and SARs indicate that influence of antigen (at least in some cases) occurs during the development of the disease. SARs have been reported previously to represent 20 to 30% of all B-CLL cases in different regions. Here we present the data concerning SARs repertoires in CLL patients from eastern slavic countries.
Aims: To measure and compare repertoire of IgVH genes in representative sample of B-CLL cases from Ukraine and Russia.
Methods: DNAs were PCR amplified in 6 separated reactions using primers specific for VH-families, and a consensus JH primer [Campbell et al. 1992] or primer sets recommended by the BIOMED-2 [van Dongen et al. 2003]. PCR products were sequenced using family-specific primers and Big Dye Terminator v3.1 kit (Applied Biosystems). Sequences were analyzed with IMGT/V-QUEST (http://www.imgt.org/IMGT_vquest/vquest). 98% homology cut-off was used to discriminate between mutated and unmutated cases.
Results: Study included 564 Russian and 390 Ukrainian patients with B-CLL. Among Russian patients 350 (62%) were without mutations and 214 (38%) with mutations. In the Ukrainian cohort 260 (66.7%) patients were with unmutated VH-genes and 130 (33.3%) with mutated. There is almost identical VH-, D-, JH-gene usage in B-CLL patients from Russia and Ukraine. Stereotyped receptors found in 20% and 27% of Russian and Ukrainian B-CLL cases respectively. Most prevalent in both countries were$_{7,6}$, 3 and 1 subsets according to published classification [Tobin et al. 2004, Stamatopoulos et al.2007]. VH1-69 gene is found in 19-21% of all cases of B-CLL, and almost always (95%) in unmutated cases. This may explain the predominance of$_{7}$, 6 and 3 SARs subsets compared with European countries. VH3-21 typical for Nordic countries occurs in Russia and Ukraine less frequently (about 5%). Accordingly, SARs subset 2 is also infrequent.
Summary / Conclusion: The main features of the repertoire of the immunoglobulin heavy chain genes in the Russian and Ukrainian patients are almost the same. Compared to western Europe higher occurrence of VH1-69 and SARs subsets$_{7}$, 6 and 3 has been detected.

B1312

THE HDAC INHIBITOR TRICHOSTATIN A INDUCES EFFECTIVE CELL KILL IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL).

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Background: Chronic lymphocytic leukemia is characterized by an accumulation of mature, functionally incompetent B cells. In CLL B cells the WNT pathway is constitutionally active with upregulated levels of LEF 1. Proteins bind to the frizzled receptor, the pathway is activated, β-catenin can translocate to the nucleus and start the transcription of effector proteins by activation of the transcription factor LEF 1. For the inactive state of the WNT pathway, a multiprotein complex is formed to phosphorylate β-catenin, which is further ubiquitinated and degraded. This multiprotein complex is formed by APC, AXIN 1 (acting as a scaffold protein), GSK3β (a kinase) and the pre-kinase CK Iα.
Aims: The aim of this study is to find and discribe WNT modifying drugs for experimental CLL therapy.
Methods: In order to examine the functionality of the WNT cascade we tested a compound library containing 75 substances. Divided in activators and inhibitors the substances have different targets along the pathway. Testing of the library was performed via ATP assay on CLL cells, PBMCs and healthy B cells extracted from human peripheral blood. Survival analysis on effective substances was performed via FACS analysis with Annexin/PI staining.
Results: The ATP assay revealed several substances that selectively affect CLL cells. A positive survival effect was seen for QS 11, an inhibitor targeting ARF-GAP1, which showed a survival advantage of up to 150% at 10 µM. The most selective effects on CLL cell viability were achieved by the NO-donators NO-ASA, JS-K and the HDAC inhibitor Trichostatin A. The NO-donator NO-ASA resulted in an IC50 of$_{6}$,7 µM (n=17) on CLL cells and 47,25 µM (n=9) on healthy PBMCs after 24h. Via FACS survival analysis the NO-donator JS-K leaded to CLL cell death with an IC50 of$_{3}$,58 µM (n=5) after 24h and$_{2}$,87 µM (n=5) after 48h compared to healthy PBMCs with an IC50 of 18,03 µM (n=3) after 24h and$_{8}$,1 µM (n=3) after 48h. The HDAC inhibitor Trichostatin A showed a stronger effect compared to JS-K. Whereas JS-K showed a high potency after 24h, Trichostatin had its maximum after 48h. Here we saw via FACS analysis an IC50 of 166,7 µM (n=4) after 24h and 157,4 nM (n=5) after 48h on CLL cells compared to an IC50 of$_{4}$,21

mM (n=3) on PBMCs after 24h and 145 μM (n=3) after 48h.
Summary / Conclusion: Besides the know targets of the WNT pathway three substances with a slight link to the WNT pathway showed interesting results. As we saw NO donators show a potent effect on CLL. But comparing results of CLL cells and PBMCs Trichostatin A showed the most selective effect on CLL cells while not harming the healthy PBMCs or B cells.

B1313

REDUCED EXPRESSION OF TOLL-LIKE RECEPTOR 4 IN B-CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH AUTOIMMUNE COMPLICATIONS

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Background: B-chronic lymphocytic leukemia (B-CLL) is characterized by the accumulation of mature-like monoclonal B lymphocytes, defective apoptosis, progressive hypogammaglobulinemia with immunodeficiency, and high prevalence of autoimmune phenomena.Toll-like receptors (TLRs) are a pattern of type I integral membrane glycoproteins expressed on cells of the immune system and represent major agents of innate immunity and initiators of adaptive immunity.
Aims: To evaluate TLR4 and TLR9 genes and protein expression in B-lymphocytes from B-CLL patients, and its relationship with stage, therapy, and known prognostic markers (IgVH region mutational status, cytogenetic alterations, CD38 and ZAP70). Further aim was to correlate TLR4 and 9 expression with infections, autoimmunity and disease progression.

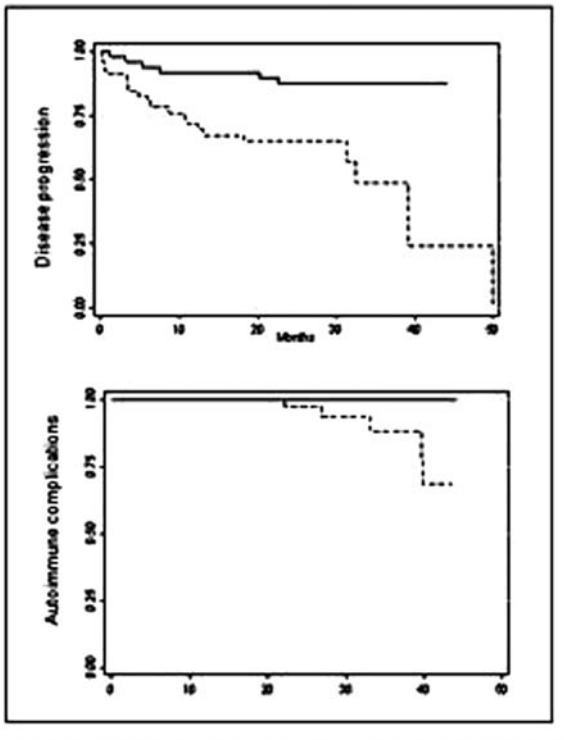

Figure 1 - Disease progression (upper panel) and occurrence of autoimmune complications (lower panel) in patients with TLR4 gene expression higher/equal (continuous line) and lower (dotted line) than the median value, estimated by Kaplan-Meier method.

Methods: TLR4 and TLR9 gene expression was evaluated in total RNA of B cell from 95 B-CLL patients and controls (pool of 10 healthy donors) by TaqMan® Gene Expression Assays on the model 7300 real-time PCR system (Applied BioSystems-Life Technology, Foster City, CA, USA). The results of relative quantitation (RQ) assays were analyzed using SDS Software (Applied BioSystems-Life Technology). Surface TLR4 and TLR9 expression on B-lyphocytes was determined by standard flow cytometry. TLR4 expression was evaluated in cultured B cells, obtained with RosetteSep (purity >97%) either unstimulated or stimulated with 1mg/mL lypopolysaccharide (LPS). Culture supernatants were assayed for IL-10 and TGF-β production using commercially available ELISA kits.
Results: Patients (41 females and 56 males, median age 74 years, range 41-89) were 78.9% in Binet stage A, 11.6% in B, and 9.5% in C and were followed for a median of 27.6 months, range 19-44. Quantitative real-time PCR revealed that TLR4 gene expression was decreased (RQ=16.1±1.56) and TLR9 increased (RQ=2725±165) in all B-CLL patients versus controls (RQ=100). Consistently, the percentage of CD19+ cells expressing TLR4 by cytofluorimetric analysis was lower (1.70±0.2% versus 3.93±0.68%, P=0.004) and TLR9 greater (5.5±0.6% versus 1.39±0.31%, P=0.04) in patients compared to controls. TLR4 reduction was more pronounced in advanced and multi-treated disease, and in patients with unmutated IgVH status and unfavorable cytogenetic abnormalities. Univariate Cox regression model showed that patients with reduced TLR4 gene expression had an increased risk of disease progression (HR 4.6, 95% CI 1.8-11.6; P=0.001) (Figure1, upper panel), suggesting that an impaired innate immunity identifies a subset of B-CLL patients with poor prognosis. Interestingly, patients with reduced TLR4 gene expression had an increased risk to develop autoimmune complications (P=0.02) (Figure1, lower panel). Finally, TGF-β production by B cells was increased in patients who thereafter developed autoimmune complications, compared with those that did not (1092±158 versus 793±18 pg/mL, mean±SE, P=0.07).
Summary / Conclusion: Reduced expression of TLR4 in B-CLL patients correlates with an advanced and multi-treated disease and is a risk factor for disease progression and development of autoimmune complications. Moreover, B-lymphocytes from patients with autoimmunity produced high levels of TGF-β. These findings suggest that interactions between the B cell receptor and TLRs signaling, and an abnormal cytokine pattern play a critical role in the maintenance of self-tolerance.

B1314

IMMUNE CO-STIMULATION (BY CD40L) IS NOT SUFFICIENT TO INDUCE DIFFERENTIATION OF CLL B-CELLS INTO IMMUNOGLOBULIN SECRETING CELLS

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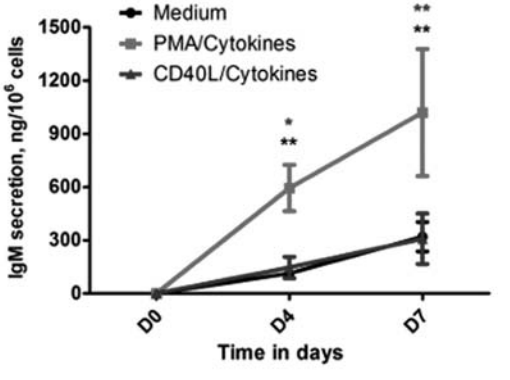
Background: B cell chronic lymphocytic leukemia (B-CLL) is a monoclonal proliferation of CD5+ B lymphocytes blocked at a stage of differentiation. However, the normal counterpart from which these cells evolve is still not identified. Gene expression profiling and IGVH genes mutation status relate B-CLL cells to memory B cells or to pre-germinal center B cells. In vitro, memory B cells differentiate into plasma cell in CD40L differentiation system. Efficiency of this system has not been studied on CLL B-cells. Previous study has shown that phorbol myristate acetate (PMA) induces activation and differentiation of CLL B-cells into Immunoglobulin secreting cells (ISC), however this differentiation is not well characterized.
Aims: Thereby, in this study we have tried to answer three questions, the first, how CLL B-cells behave following CD40L differentiation model? The second, is antigenic stimulation necessary for the differentiation of CLL B-cells into ISC? And the third, what can behaviour of differentiated CLL B-cells teach us about their normal counterpart?
Methods: Therefore, CD19+ CD5+ CD38- B lymphocytes of 13 patients with A-stage CLL were purified with a purity >98%, and then activated in vitro with PMA or CD40L in the presence of a mixture of cytokines for four days. Cells were washed and incubate in the presence of a second combination of cytokines for three days. Morphological, phenotypic and molecular changes were examined after 7 days of culture.



Results: Both stimulations resulted in dramatic phenotypic and molecular changes in CLL B-cells. Surface expression of CD19, CD20, CD5, CD27 and CD45 decreased whereas the expression of IgM, HLA-DR and CD25 increased. Unexpectedly, CD38 surface expression and mRNA was induced in only 40% of cases. We also observed that the expression of CD138 mRNA was induced in some cases. Transcriptions factors studied by quantitative RT-PCR showed that immunophenotypical changes involved a decrease in the expression of B cell transcription factor PAX5, LEF1, BACH2, IRF8 and an increase

in the expression of plasma cell markers BLIMP1, IRF4, XBP1s and ERN1. Changes in transcription factor profile were confirmed by western blot. Importantly, Gas6, BATF and AID transcriptional expression were induced. Furthermore, we also observed that stimulated cells displayed proliferation and prolonged survival in culture. However, the difference between PMA and CD40L stimuli was more evident at two levels, cell morphology and IgM secretion. Our data show that only CLL B-cells stimulated by PMA can differentiate into ISC. IgM secretion levels were highly significant in PMA differentiated cells while IgM levels in CD40L treated cells were comparable to control condition. Morphologically, PMA differentiated cells developed plasmacytoid/plasma cell morphology with an eccentric nucleus and a developed cytoplasm whereas CD40L treated cells resembled to that of activated lymphocytes.

Summary / Conclusion: All these results strongly suggest that CLL B-cells can be driven to a more mature stage and that antigenic signals are critical for ISC differentiation of these cells. Describing events and behaviour of malignant B cells differentiation may be relevant for understanding the biology of B-cell malignancies. Based on our data we speculate that memory B-cells cannot be a normal counterpart of B-CLL.

B1315

IMMUNOGLOBULIN GENE REARRANGEMENTS IN ASIAN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world, with 4 new cases x 100,000 individuals per year. In Asia this figure is 5-10 times lower and does not change in Asian residents in the US and their descendants. The underlying environmental and genetic bases remain obscure. Limited data on Asian CLL seem to confirm the prognostic impact of conventional biologic markers, while suggesting a different distribution of immunoglobulin heavy chain variable (IGHV) gene families in comparison with Western CLL.

Aims: To characterize IGHV-D-J rearrangements of a series of Asian CLL and compare them with those in Caucasian CLL, focusing on gene family usage and stereotype of the HCDR3 region.

Methods: A cohort of 77 untreated CLL patients fromHong Kong has been analyzed. There were 57 men and 20 women, with a median age of63 years (range: 43–94). Forty-five cases were in Binet stage A (58.4%), 17 in stage B (22.1%) and 15 in stage C (19.5%). IGHV-D-J gene rearrangements were sequenced and analyzed by the IMGT database (http://imgt.cines.fr). We evaluated: IGHV mutational status; IGHV-D-J gene usage; HCDR3 length and composition analyzed by the Clustal X software (http://www.clustal.org). The published series by Agathangelidis A. et al (Blood 2012) on Caucasian CLL was used as control.

Results: Of 77 Asian CLL patients, 32.5% and 67.5% harbored unmutated and mutated IGHV genes, respectively. The most frequent IGHV families were: IGHV3 (53.2%), IGHV4 (22.1%), IGHV1 (14.3%), IGHV5 (6.5%), IGHV2, IGHV6, and IGHV7 (1.3% each). The most frequent IGHV genes were V3-7 (15.6%), V1-69 (7.8%), V5-51 (6.5%), V4-34 (6.5%), V3-74 (6.5%), V4-39 (5.2%), whilst V3-21 was very low (2.6%). Compared with Caucasian CLL the frequency of V3-7 (P=0.0015), V3-74 (P=0.013), V5-51 (P=0.019) appeared to be significantly higher. This different distribution does not seem related to the higher proportion of mutated cases among Asians, as shown by a dedicated analysis (V3-7, P=0.022; V5-51, P=0.001). Overall, the most frequent IGHV genes employed by Caucasian CLL (V1-69, V4-34, V3-23, V3-33, V3-7, V4-39, V3-30, V1-2, V3-48, V3-21), accounting for 62% of the total, are significantly less represented (37/77, 48%, P=0.01) in Asian CLL. IGHD genes were identified in 76/77 sequences. Among them, the IGHD3 (42.1%) was the most frequent followed by IGHD6 (19.7%), IGHD2 (11.8%), IGHD1 (11.8%), IGHD4 (9.2%), IGHD5 and IGHD7 (2.6% each), at variance from the frequency shown by Caucasian CLL i.e. IGHD3>IGHD2>IGHD6>IGHD5>IGHD1>IGHD4>IGHD7. Among IGHD genes, D3-10 (13.0%) and D3-22 (13.0%) were the most recurrent. D5-18, displayed by 3/77 (4%) Asian CLL, was never found among Caucasian cases (P=0.0001). No difference was found in IGHJ families. Only 2.6% of Asian CLL carried known stereotyped receptors (subset #59, subset #77) and no new subset has been identified so far.

Summary / Conclusion: Our data suggest a different frequency in IGHV and IGHD gene usage in Asian CLL: IGHV4 genes were more represented than IGHV1, although the frequency of V1-69 was similar.In addition, the frequenciesof V3-7, V3-74, V5-51 genes werehigher than in Caucasian CLL. By extending this study to other collaborators in China, it will be possible to corroborate this distinctive pattern, evaluate the frequency of known subsets and identify potential new subsets among Asian CLL. Overall, these results may ultimately translate into pathogenetic hypotheses on Asian CLL.

B1316

ABERRANT EXPRESSION OF TOLL-LIKE RECEPTORS HIGHLIGHTS IMMUNE DEREGULATION WITH SERIOUS CLINICAL CONSEQUENCES IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background: In CLL patients who suffer from recurrent infections the nonspecific immune response could be continuously stimulated. The response of the innate immune system could eliminate infectious agents and is crucial for developing pathogen-specific adaptive immunity mediated by B and T cells. Toll-like receptors (TLRs) are responsible for host defense against pathogens by recognizing a wide variety of pathogen-associated molecular patterns. These receptors regulate innate immunity and determine the polarization and function of adaptive immunity. Triggering TLRs results in increased expression of inflammatory genes, which then play a protective role against various infections. Functional TLRs are expressed in a most of tumors and suggest that activation of tumoral TLRs induces the releasing of proinflammatory as well as immunosuppressive factors. This process increases the resistance of tumor cells to cytotoxic lymphocyte assault and further results their escape from immune surveillance. However, it has been suggested that the stimulation of TLRs expressed on CLL cells could increase immunogenicity of tumor cells and thus potentially contribute to the induction of tumor-specific immune response.

Aims: In the current study we performed comprehensive characterization of expression pattern of *TLR2, TLR7, TLR9* as well as splicing variants expression of *TLR4 (TLR4(1), TLR4(3), TLR4(4)*) in peripheral blood and bone marrow compartments/samples. Additionally, we assessed the protein expression level of TLR 9 in independent group of 41 patients and evaluated its impact on the treatment-free survival (TFS).

Methods: For 94 CLL patients samples qRT-PCR was performed. Expression levels of *TLR2, TLR7, TLR9, TLR4(1), TLR4(3)* and *TLR4(4)* were assessed and normalized against housekeeping gene *GAPDH*. The protein expression of TLR9 was assessed using flow cytometry.

Results: We found that the expression of *TLR7* and *TLR9* were significantly higher in CLL patients compared to healthy volunteers (HVs) and correlated with prognostic factors such as CD38 and ZAP-70 expressions or *IGVH* mutational status. In contrast, expression levels of *TLR2, TLR4(1), TLR4(3), TLR4(4)* were decreased compared to HVs. *TLR2*, *TLR7* and *TLR9* possessed higher expression in III and IV stages according to Rai classification. Moreover, protein expression of TLR9 strongly categorized patients for good and bad prognosis. Patients with higher expression of intracellular expression of TLR9 have significantly longer TFS than patients with lower expression of TLR9 (P=0.004). While TFS in group with lower expression of TLR9 (below median expression of 84.5%) was 8 months, in group with higher expression of TLR9 (above median expression of 84.5%) was 57 months.

Summary / Conclusion: The mRNA expressions of *TLR7* and *TLR9* were significantly higher in CLL patients compared to HVs, while the expressions of *TLR2, TLR4(1), TLR4(3)* and *TLR4(4)* were decreased. The protein expression level of TLR-9 was found to be associated with clinical outcome. Obtained data suggest that TLR-9 might provide a prognostic value in CLL. Furthermore, higher expression of certain TLRs may manifest deregulation of immune system in advanced stages of CLL.

B1317

***IN VITRO* EFFICACY OF PARA-NITRIC OXIDE DONATING ACETYLIC SALICYLIC ACID TOWARDS LYMPHOMA CELL LINES AND PRIMARY CHRONIC LYMPHOCYTIC LEUKEMIA CELLS HARBORING TP53 MUTATIONS OR DELETION 17P.**

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Background: Chronic lymphocytic leukemia (CLL) is characterized by a remarkable heterogeneity in the clinical course among patient subgroups with distinct genetic characteristics. Some patients have a rapidly fatal disease despite therapy, which is accompanied by the incidence of deletion of chromosome 17p and mutation of TP53. Non-steroidal anti-inflammatory drugs (NSAIDs) possess anti-neoplastic activity in CLL. Moreover, nitric oxide donating derivatives of NSAIDs, most notably para-nitric oxide acetylic salicylic acid (NO-ASA), show superior anti-neoplastic efficacy compared to their parent compounds. The mechanism of the anti-neoplastic action is still not clarified.

Aims: Our main objectives were to determine whether NO-ASA is a specific therapeutic substance in high risk CLL and to illuminate the mechanism of its cytotoxic effect.

Methods: p-NO-ASA was synthesized by standard organic chemistry procedures. Primary cells were isolated from peripheral blood of CLL patients or healthy individuals. Further, the human B-cell lymphoma cell lines HT (B-cell non-Hodgkin lymphoma, TP53 mutated), U2932 (treatment resistant B-cell lymphoma), GRANTA-519 (relapsed high-grade B-non Hodgkin lymphoma, Cyclin D1 activation),

JVM3 (B-prolymphocytic leukemia, expresses proto-oncogene Bcl2) and MEC-1 (chronic B-cell leukemia, del17p) were used. Primary CLL cells with or without TP53 mutation, selected cell-lines as well as PBMCs and B-cells of healthy volunteers were tested in a luminometric assay (ATP content) after 24 h incubation with p-NO-ASA. Caspase-dependency on the cytotoxic effect on CLL cells was determined in a luminometric assay after 6 h drug-incubation as well as in immunoblot analysis after 24 h drug-incubation. Impact on NFκB-pathway was investigated via immunoblot analysis after 3 h drug-incubation.
Results: p-NO-ASA effectively reduced ATP content in the cell lines HT (LD50 9.20 μM), U2932 (LD50 4.81 μM) and JVM3 (LD50 3.33 μM). LD50 values were comparable with those achieved for CLL cells from a mixed patient population (LD50 4.34 μM). Primary CLL cells from TP53 mutated patients (n=5) showed a slightly increased LD50 of 25.56 μM, which was still significantly lower than that for healthy PBMCs (n=5) (LD50 63.72 μM). GRANTA-519 and MEC-1 were less sensitive (LD50 53.44 μM and 22.21 μM, respectively). In addition a concentration-dependent activation of the caspase cascade could be shown via luminometric assay as well as an apoptosis induction via western blot analysis. Phosphorylation of the NFκB p65 subunit at Ser 536 was reduced by NO-ASA around 58% (n=3), which implies an inhibition of NFκB pathway and a reactivation of apoptosis protein p53.
Summary / Conclusion: p-NO-ASA shows potent reduction of ATP content in cell-lines derived from treatment resistant cell lines featuring several bad prognosis markers such as TP53 mutation or Bcl2 overexpression. Further, TP53 mutated patients are about three fold more sensitive towards p-NO-ASA treatment as PBMCs derived from healthy individuals. The cytotoxic effect depends on caspase-activation and might be influenced by inhibition of NFkB-pathway. Hence, p-NO-ASA is worth further evaluation as a treatment for poor prognosis patients unresponsive to conventional CLL treatment regimens.

B1318
INCIDENCE AND PROGNOSTIC SIGNIFICANCE OF TCL-1 ONCOGENE IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: TCL1 oncogene has been shown to be highly expressed in most B-cell tumors including CLL and contributes to the pathogenesis of CLL as a regulator of signaling of B-cell receptor, directly involved in activation of kinase cascade. Deregulation of this oncogene has implicated in the pathogenesis of the aggressive form of B CLL.
Aims: To evaluate TCL1 expression in a series of CLL patients (pts) and correlate its activity with patients' clinical and laboratory findings as well as other prognostic factors and survival.
Methods: 97 pts diagnosed with CLL based on standard criteria were included in this study. Baseline clinical and laboratory features were recorded. All pts underwent bone marrow aspiration and biopsy at diagnosis. Analysis of the IgVH mutational status as well as FISH for the detection of 11qdel and 17pdel were performed also at diagnosis. TCL1 as well as ZAP70 expression were evaluated by immunohistochemistry (IHC) using standard techniques in bone marrow paraffin embedded sections in all pts.
Results: Patients' median age was 60 years (30-85) with male predominance(56). According to Binet staging system, 79 pts (81%) had disease stage A, 12 (12%) B, and 6(6%) C 12 pts (12%) presented with splenomegaly and 4 had B-symptoms. Increased LDH values had 15/93 pts, abnormal $\beta_2$microglobuline 18/45, while 15 presented with rapid lymphocyte duplication time. Thirty-two (33%) had nodulal pattern of bone marrow infiltration while 32 (33%), 10(10%) and 24(25%) interstitial, diffuse and mixed pattern respectively. IgVH mutational analysis was performed in 55(57%) pts and 23/55 were unmutated. FISH analysis for 11q del and 17p del were evaluated in 44 and 48 pts respectively. In 3 pts 11q del was detected while 4 pts were positive for 17p del and 45 pts were ZAP70 (+). Fifty-nine pts (61%) were TCL1 positive. Male patients tended to present with higher TCL1 positivity compared to female ones [38 out of 56 (68%) male disclosed TCL-1 positivity against 21/41 females (51%) (p:0.097)]. TCL-1 expression was higher in ZAP-70(+) pts compared to ZAP-70(-) and this difference was statistically significant [39/45 (87%) ZAP70 (+) pts were TCL1(+) against 20/52 (39%) ZAP(-) (P<0.001)]. Further on, unmutated CLL patients presented with higher TCL-1 expression. Analytically, 19/23 (83%) unmutated pts expressed TCL1 against 14/32 (44%) mutated pts (P=0.004). TCL1 expression was more frequent in pts with non nodular pattern of bone marrow infiltration. Median Treatment Free Interval (TFI) was132 months, 7-year TFI was 51% and 10-year overall survival was 71% (median not reached). TCL-1(+) patients had marginally lower 7-year TFI(45% vs. 65%, P=0.09). No differences in overall survival were observed between TCL1 (+) and TCL1(-) patients (P=0.38).
Summary / Conclusion: TCL1 expression correlates with adverse prognostic factors in CLL such as ZAP70 and IgVH unmutation status and could be an additional easily applicable, reliable and reproducible marker for the disease. TCL-1(+) patients tend to have shorter TFI compared to TCL-1(-) patients

B1319
COMBINED PATTERNS OF IGHV REPERTOIRE AND CYTOGENETIC/MOLECULAR ALTERATIONS IN MONOCLONAL B LYMPHOCYTOSIS VERSUS CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Chronic lymphocytic leukemia (CLL)-like monoclonal B lymphocytosis (MBL) with (MBL^{hi}) or without (MBL^{lo}) absolute B-lymphocytosis precedes most CLL cases, the specific determinants for malignant progression remaining unknown.
Aims: To investigate the potential existence of unique cytogenetic/molecular profiles associated with specific IGHV repertoires which could confer an increased risk of progression from MBL to CLL.
Methods: Simultaneous analysis of well-known abnormalities of chromosomes 11, 12, 13, 14 and 17 in CLL-like cells by iFISH and molecular pattern of rearrangements of IGHV genes of both MBL and CLL cases.
Results: Our results based on 78 CLL-like MBL and 117 CLL clones from 166 subjects living in the same geographical area and whose written informed consents were obtained; show the existence of three major groups of clones with distinct but partially overlapping patterns of IGHV gene usage, IGHV mutational status and cytogenetic alterations. These included a group enriched in MBL^{lo} clones expressing specific IGHV subgroups (e.g. VH3-23) with no or isolated good-prognosis cytogenetic alterations, a second group which mainly consisted of clinical MBL^{hi} and advanced stage CLL with a skewed but different CLL-associated IGHV gene repertoire (e.g. VH1-69), frequently associated with complex karyotypes and poor-prognosis cytogenetic alterations, and a third group of clones with intermediate features.
Summary / Conclusion: These findings suggest that the specific IGHV repertoire and IGHV mutational status of CLL-like B-cell clones may modulate the type of cytogenetic alterations acquired, their rate of acquisition and/or potentially also their clinical consequences.
Further studies investigating the IGHV gene repertoire of MBL^{lo} clones in distinct geographic areas and microenvironments, may shed light on the role of some antigen-binding BCR specificities contributing to clonal evolution.

B1320
VEGF AND BFGF SINGLE-NUCLEOTIDE POLYMORPHISMS IN CHRONIC LYMPHOCYTIC LEUKEMIA: IMPACT ON SUSCEPTIBILITY
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Background: B-cell chronic lymphocytic leukemia (B-CLL) is a heterogeneous disease with a highly variable clinical outcome. Recent studies have identified a number of different molecular prognostic markers (including mutational status of the *IgVH* gene, ZAP70 and CD38 expression) that allow to discriminate patients in prognostic subgroups. However, different expression patterns of angiogenic factors as *VEGF*, *VEGFR1* and *bFGF* have been related with B-CLL susceptibility and treatment requirements.
Aims: We have analyzed the polymorphisms: -710 C/T in *VEGFR1,* rs1109324, rs1547651, rs3025039 (936C/T) and rs833052 in *VEGF* and rs1449683 (223 C/T) in *bFGF* in order to determine the possible association with susceptibility in B-CLL.
Methods: Peripheral blood samples from 224 B-CLL patients and 476 healthy controls were genotyped using probes TaqMan SNP Genotyping Assays. Samples were providing from the Hospital Clinic of Valencia. Four SNPs in the *VEGF* gene, one SNP in the *bFGF* gene and one SNP in the *VEGFR1* gene were evaluated. Statistical analysis was performed using SNPStats program (Catalan Institute of Oncology) and Fisher's exact test was applied to evaluate the significance.
Results: We have observed an increased frequency of the T allele in the rs1449683 SNP [OR 1.62 (95% CI: 0.98-2.66) p-value =0.063] and in the rs1547651 SNP [OR 0.72 (95% CI: 0.51-1.03), p-value=0.072] in our B-LLC patients when compared to control subjects. Moreover we observed that T allele carriers of rs3025039 (*VEGF)* have a significant protective effect concerning this disease [OR 0.59 (95% CI: 0.39-0.89) p-value=0.009].
Summary / Conclusion: Our data indicate an increased frequency of the T allele in polymorphisms rs1449683 (*bFGF)* and rs1547651 (*VEGF)* in the group of patients, which possibly account for the individual susceptibility to develop B-CLL. On the other hand the data provided suggest that the T allele of *VEGF* rs3025039 is likely important genetic marker of susceptibility to B-CLL.
Further studies regarding the role of pro-angiogenic markers in B-CLL would be beneficial to help elucidate pathogenic pathways in this disease.

B1321

ASSOCIATION BETWEEN THE MDM2 SNP309 POLYMORPHISM AND CLINICAL OUTCOME IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH UNMUTATED IGHV GENES

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Background: Data concerning prognostic significance in chronic lymphocytic leukemia (CLL) of the single nucleotide polymorphism 309T>G polymorphism in the promoter region of the MDM2 gene (SNP309) are still controversial (Willander et al., 2010; Asslaber et al., 2010; Kaderi et al., 2010; Zenz et al., 2010).
Aims: To evalute impact of the MDM2 SNP309 polymorphism in CLL prognosis.
Methods: The study of the MDM2 SNP309 polymorphism was performed in 231 CLL patients (178 males and 53 females). Median duration of follow-up period was 62.4 months. The following patient characteristics were analyzed: clinical data at diagnosis, time-to-treatment period (TTT), progression-free survival (PFS), overall survival (OS), immunoglobulin heavy chain variable (IGHV) gene mutation status. Polymerase chain reaction with restriction of product reaction by MspAI was used. Statistical analyses were performed using SPSS 13.0 for Windows.
Results: The distribution of the SNP309 genotype in observed patients was as follows: T/T carriers, n = 110 (47.6%); T/G carriers, n = 96 (41.6%); and G/G carriers, n = 25 (10.8%), that was comparable with other CLL cohorts (Gryshchenko et al., 2008). CLL patients with G/G genotype vs T/T+T/G genes were more likely to have an advanced stages (B or C according to the Binet classification) at the moment of diagnosis 72% vs 49%; P=0.03), shorter TTT period (medians 5 and 14 months correspondingly; P=0.035) and PFS (medians 30 and 50 months correspondingly; P=0.024), and a tendency to shorter OS (medians 61 and 98 months correspondingly; P=0.099) regardless the IGHV gene mutation status. Any significant differences in these parameters were found in CLL patients with mutated IGHV genes. Impact of the SNP309 genotype was found in CLL patients with unmutated IGHV genes only. Namely, 18.8% of CLL patients, carriers of G/G genotype, had A stage at the moment of diagnosis comparing with 43.4% patients among carriers of T/T+T/G genotypes (P=0.024). Medians of survival parameters in CLL patients with unmutated IGHV genes were as follows: TTT period 3 months in G/G carriers vs 10 months in T/T+T/G carriers (P=0.04); PFS 24 months in G/G carriers vs 45 months in T/T+T/G carriers (P=0.002); and OS 43 months in G/G carriers vs 86 months in T/T+T/G carriers (P=0.005).
Summary / Conclusion: The MDM2 SNP309 polymorphism may be an additional unfavorable prognostic marker for CLL patients with unmutated IGHV genes.

B1322

COMPARISON OF DIFFERENT RQ-PCR DESIGN STRATEGIES FOR MINIMAL RESIDUAL DISEASE EVALUATION IN LYMPHOPROLIFERATIVE DISORDERS: CORRELATION BETWEEN IMMUNOGLOBULIN GENE MUTATION LOAD AND RQ-PCR PERFORMANCE

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Background: Minimal residual disease (MRD) evaluation in B-cell acute lymphoblastic leukemias (ALL) and chronic lymphoproliferative disorders (B-CLD) requires the identification at the time of diagnosis of patient-specific molecular targets, i.e. immunoglobulin heavy chain (IGH) genes, on which primers and probes are constructed, and used to monitor MRD during the follow-up.
Aims: In the context of the MRD network of the Fondazione Italiana Linfomi (FIL), we compared two different approaches for the design of specific primers/probes for RQ-PCR, to assess their applicability, specificity and sensitivity for the MRD evaluation of B-CLD characterized by a variable mutational load of IGH genes.
Methods: A total of 25 samples of peripheral blood or bone marrow mononuclear cells from patients with chronic lymphocytic leukemia (CLL) (n=18) or mantle cell lymphoma (MCL) (n=7), were analyzed and sequenced to identify the functional germline V-D-J segment and the IGH mutation load, at the time of diagnosis. RQ-PCR quantitative analysis was performed using specific primers/probes generated with two design strategies, whose difference mainly consists in the positioning of the primers/probes on the V-D-J regions. In one method (method 1), the sense and antisense primers, both patient-specific, are positioned on the CDR2 and the CDR3 regions, respectively, while the probe is placed on the FR3 (Voena et al, Leukemia 1997); in the other (method 2), the sense primer, the only patient-specific, is positioned on the CDR3 region and the antisense primer together with the probe, both in germline configuration, on the FR4 region, where a portion of the JH is included. Method 2 derives from the European Study Group guidelines on MRD detection for ALL.
Results: Based on the IGHV mutation analysis, 22/25 (88%) samples carried >2% mutations and were classified as mutated; 20/25 (80%) samples showed a mutation load >5%. IGHJ genes showed a percentage of mutations between 0 and 29.41%, with a median value of 10.42%. 21/25 (84%) samples carried >2% mutations and 18/25 (72%) showed a mutation load >5%. 92% (n= 23) of samples resulted evaluable using the method$_1$, while 24% (n= 6) were evaluable with the method 2. Samples evaluable by both methods were 20% (n= 5), while samples evaluable with at least one method were 96% (n= 24). The poor performance of the method 2 was evident in the present series, enriched of mutated IGHV/J cases; on the contrary, selecting a CLL series with a germline IGHV/J configuration, the method 2 was successful in 21/23 cases (91%). Nevertheless, we could not find a specific mutation load of the IGHV/J region above which the RQ-PCR performance failed.
Summary / Conclusion: IGH genes mutations in B-CLD are a phenotypic marker which influences the analysis. In this context, the success or failure of RQ-PCR are strongly influenced by both the positioning of the primers/probes and their specificity i.e. the method 1 is based on 2 patient-specific primers, while the method 2 on one patient-specific and one germline. However, it was not possible to identify a specific mutation load determining the RQ-PCR failure. Therefore, it is necessary to reformulate the molecular strategies to evaluate MRD according to the B-cell receptor features of the disease investigated.

B1323

IMPACT OF THE BIOLOGICAL AND CLINICAL FEATURES OF THE DISEASE ON THE REAL LIFE OF THE CLL PATIENTS. A RETROSPECTIVE SINGLE CENTRE STUDY.

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Background: Chronic Lymphocytic Leukemia (CLL) is the most common lymphoproliferative disorder of the elderly population in Western countries. Clinicians collect many clinical and biological data useful for prognosis and management of the disease. No comprehensive data on epidemiological, clinical and biological characteristics and evolution of the B-CLL population are available in a single centre analysis.
Aims: This retrospective study has the purpose to analyse demographic, clinical and biological characteristics of our B-CLL population, diagnosed and followed at Hematology Institute of Catholic University of Rome.
Methods: From our database consisting of 507 diagnoses during the last 15 years, we excluded 105 patients previously treated in other centre or referred to our centre for consultancy. Thus we selected 402 patients affected by B-CLL diagnosed from January 1997 to December 2011 and followed until December 2012. The diagnosis of B-CLL was made according to criteria from the National Cancer Institute. For each patient in the database we reported clinical and biological features at diagnosis, comorbidities, any relevant clinical condition developed during the B-CLL history, time to progression (TTP), time to treatment (TTT), overall survival (OS) and cause of death.
Results: We recorded a mean of 27 B-CLL diagnosis per year.The median age at diagnosis was 66 years (range 35-89 years) with a ratio M:F of 1.68:1 (249 males, 153 females). The Binet stage was A in 286 patients (71.1%), B in 82 pts (20.4%) and C in 34 pts (8.5%). The median value of absolute lymphocyte count was 10.100 lymphocytes/mmc (range 1000 to 312.000). Fifty-four patients (13.4%) were diagnosed as Monoclonal B lymphocytosis because clonal lymphocytes were less of 5000/mmc. Positivity of biological parameters was detected for ZAP-70 in 133 pts (36.0%), for CD38 in 94 pts (24.9%), for CD49d in 71 pts (40.6%) and for CD69 in 35 pts (26.1%). Fish analysis was performed in 364 patients: del(13q14) resulted in 97 pts (26.6%), +12 in 52 pts (14.3%), del(11q22) in 30 pts (8.2%) and del(17p13) in 23 pts (6.3%). The analysis of the IgVH mutational status showed 200 pts (60.1%) mutated and 133 pts (39.9%) unmutated.
Analysing the time dependent outcomes, the median TTP was 4 years with 220 patients who showed progressions (63%), the projected rate of progressions at 5-years and 10-years was 57% and 76% respectively. The median TTT was 5 years:193 patients (55%) were treated in our series; 51% of all patients were untreated at 5 years and 32% after 10 years. All 34 patients lost at follow-up were considered censored and excluded by the analysis for OS (median observation time of 5 years). At last follow-up (December 2012) ninety-six patients were dead (28%); median overall survival was 14 years: 56% of the deaths due to B-CLL, 31% to extra-haematological diseases (especially neoplasia, cardiovascular and respiratory diseases), 8% to infections and 4% undetermined. The 5 and 10-year OS was 86% and 68% respectively. Most of the deaths seem to be related to CLL: among the extra-haematological deaths only 13% did not experience progression. ZAP-70, del(17), unmutated-IgVH, beta-2-microglobulin and CD49d identified a group of patients with significantly shorter TTP and OS, while CD38, del(11) and CD69 only for TTP.
Summary / Conclusion: By comparison with the data reported in the literature,

our cohort is representative of the B-CLL population. A comprehensive characterization of the clinical features and these standard biological prognostic parameters of the disease is essential to allow to the clinicians the best management of the patients.

B1324

COMPARISON OF THE PI3K-DELTA INHIBITORS TGR-1202 AND GS-1101 IN INDUCING CYTOTOXICITY AND INHIBITING PHOSPHORYLATION OF AKT IN CLL CELLS IN VITRO

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Background: The PI3K pathway is a central pro-survival mechanism in chronic lymphocytic leukemia (CLL). Expression of the delta isoform of PI3K is largely restricted to lymphocytes with *in vitro* inhibition inducing CLL cell apoptosis and death. Clinical evaluation of PI3K-δ inhibitors, such as GS-1101, has been promising, with responses seen in Rel/Ref CLL patients (pts). TGR-1202 is a novel PI3K-d specific inhibitor previously demonstrated to inhibit Akt phosphorylation and induce apoptosis in B-cell lymphoma cell lines.
Aims: Given the efficacy of other PI3K-δ inhibitors observed in CLL, we assessed the ability of TGR-1202 to induce cytotoxicity and apoptosis, and inhibit Akt phosphorylation in primary CLL lymphocytes.
Methods: We collected blood from consented CLL pts seen at the Duke Center for CLL and enrolled in IRB approved protocols at the Duke University and Durham VA Medical Centers. CLL lymphocytes were isolated using negative selection (> 95% purity). Primary CLL cells were incubated with serial dilutions of TGR-1202 or GS-1101 for 48 hours and tested for apoptosis by activated caspase-3 and 7AAD staining measured by flow cytometry. After 72 hours of incubation, CLL cells were evaluated for cytotoxicity using the colorimetric MTS reagent. Phosphorylated Akt (S473) was measured by flow cytometry after one hour of incubation with either compound and ten minutes of incubation with anti-IgM or anti-IgD. Akt phosphorylation was quantified by median fluorescent intensity (MFI).
Results: We evaluated TGR-1202 and GS-1101 in CLL lymphocytes collected from 7 pts. Five had mutated IGHV, 5 had 13q deletion or normal cytogenetics determined by FISH, 3 were ZAP-70 negative, and 7 were CD38 negative. IgM expression ranged between 13% and 90%, whereas IgD expression was uniformly elevated. Both TGR-1202 and GS-1101 significantly induced apoptosis (caspase-3+/7AAD+) and cytotoxicity in a dose-dependent manner in concentrations between 0.1 and 25.6 μM (P<0.05 in pairwise Wilcoxon signed rank tests). There was no significant difference observed between the compounds in terms of induction of apoptosis or cytotoxicity at any of the concentrations tested (0.1 – 25.6 μM) except 0.4 μM, where GS-1101 induced more apoptosis and cytotoxicity (median of 18.6 vs. 13.7% caspase-3+/7AAD+; median of 55.2 vs. 48.6% cytotoxicity, P=0.03 for both, Wilcoxon signed rank test). Incubation with anti-surface immunoglobulin significantly induced Akt phosphorylation compared to media alone (median MFI 1011.5 and 369, respectively, P=0.03, Wilcoxon rank sum test). The addition of either TGR-1202 or GS-1101 significantly abrogated this effect (P=0.03 for 0.1, 0.4, and 1.6 μM, Wilcoxon rank sum test) and returned Akt phosphorylation to baseline (P> 0.05 comparing drug treatment to media control, Wilcoxon rank sum test).
Summary / Conclusion: TGR-1202 is a potent PI3K-δ inhibitor that suppresses Akt phosphorylation and induces apoptosis-dependent cytotoxicity in primary CLL lymphocytes. These effects are comparable to those seen with GS-1101. Differences seen at the 0.4 μM concentration were not observed at higher or lower concentrations. Given the improved selectivity for the delta isoform of PI3K seen with TGR-1202 compared to GS-1101, these results suggest that TGR-1202 may have benefit in treating CLL while inducing fewer off-target effects and toxicities. Additional *in vitro* studies in high risk cytogenetic CLL patient samples are underway. TGR-1202 is currently being evaluated in a Phase I clinical trial in patients with hematologic malignancies.

B1325

POLYMORPHISM OF APOPTOSIS REGULATORY AND GROWTH FACTOR GENES IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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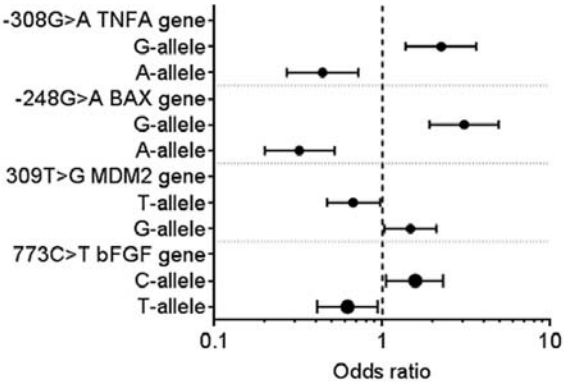
Background: Introduction
Chronic lymphocytic leukemia (CLL) is an oncohematological disease of the lymphatic tissue which is characterized by accumulation of lymphocytes in the peripheral blood, bone marrow and lymph nodes. CLL has a B□cell origin and is the most common type of all leukemias in adults.
Characteristic clinical symptoms of CLL include anemia, thrombocytopenia, enlargement of lymph nodes and hepatosplenomegaly. It has been established that at the time of diagnosis such symptoms appear to be absent in approximately 40% of patients. In most cases CLL is diagnosed in the later stage safter the development of complications. Thus, the important task of oncohematology is to determine the prognostic criteria and to assess the risk for the development of the disease at the earliest stages possible.
Like any other oncological process, CLL is most commonly caused by the disorder of apoptosis (tumor necrosis factor□α (TNFA), main p53 protein regulator (MDM2), proapoptotic protein (BAX) and by interferences with cell proliferation control (vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF)).
Aims: The purpose of this study was to make a comparative analysis of polymorphic loci of TNFA gene (□308G>A), BAX gene (□248G>A), MDM2 gene (309T>G), VEGF gene (936C>T) and bFGF gene (773C>T) in patients with CLL and healthy individuals from Bashkortostan Republic. The study also aimed to search for possible associations of polymorphic variants of these genes related to the development of CLL.
Methods: Molecular and genetic analysis of DNA samples was performed in 329 persons, all residents of the Bashkortostan Republic. The group of patients with CLL included 133 patients hospitalized to the hematology unit of the Republican Clinical Hospital of the Ufa city (2008□2010). All patients were selected at random. Clinical examination of the patients was carried out by hospital doctors and combined essential and optional methods of examination. DNA samples were isolated from lymphocytes of the peripheral venous blood by the method of phenol□chloroform extraction. The polymorphic loci were studied by the method of polymerase chain reaction (PCR) of DNA synthesis. Mathematical processing of the results was performed with the use statistical programs BIOSTAT.



Results: In the group of patients with CLL the GG□genotype was significantly more common than that compared with the control group (82 and 65% respectively, χ2= 10.67, P=0.002). The frequency of G□allele of polymorphic locus□308G>A of TNFA gene in patients with CLL was higher than the corresponding result in the control group (91 and 81% respectively, χ2= 10.41, P=0.002). The comparative analysis of the frequencies of polymorphic variants of locus□248G> A of BAX gene revealed significant differences in GG□genotype, which accounted for 74.30% in CLL patients and 46,30% in the control group (χ2= 16.25, P=0.001). Analysis of the polymorphic locus 309T>G of MDM2 gene revealed that the frequency of G□allele in patients with CLL was 46% compared to 37% in healthy individuals (χ2= 4.14, P=0.042; OR = 1.47; 95% CI: 1.03□2.11).
Comparison of genotype and allele frequencies of polymorphic locus 936 C>T of VEGF gene in patients with CLL and in the control group did not reveal any significant differences. TT genotype frequency of polymorphic locus 773C>T of bFGF gene was higher in the control group than that in the group of patients (40.65% vs. 56.44%, χ2= 4.92; P=0.027). In contrast, the allele T was more frequent in the group of patients (35.00% vs. 25.25%, χ2= 4.48, P=0.03).
Summary / Conclusion: Thus, as a result of the study the following risk markers for CLL have been identified: GG□genotype of the polymorphic locus □308G>A of TNFA gene (OR = 2.46), GG□ genotype and G□allele of the polymorphic locus□248G>A of BAX gene (OR =3,35; OR =3,07, respectively), G□allele of the polymorphic locus 309T>G of MDM2 gene (OR = 1.47), allele T of the polymorphic locus 773C>T of bFGF gene (OR = 1.57).

B1326

FACTORS THAT MAY INFLUENCE THE KINETICS OF B LYMPHOCYTE DEPLETION IN RELAPSED CLL PATIENTS TREATED WITH OFATUMUMAB – A SINGLE CENTRE EXPERIENCE

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Background: Chronic lymphocytic leukemia (CLL) is the leading cause of leukemia in Western countries and preferentially affects men after age of 65s. The advent of monoclonal antibodies has led to a more effective management

of this disease. Ofatumumab is an anti-CD20, second-generation humanized monoclonal antibody with anti-tumor activity mainly dependent on complement. The activation of the classical complement pathway is assumed as a key mechanism of B cell depletion.
Aims: We conducted a retrospective, monocentric, cohort study, to characterize the kinetics of C4 and C3 fractions of complement during treatment with Ofatumumab for relapsed, refractory CLL. We also analysed the phenotype of FcgammaR3 and the impact on leukaemia cell depletion.
Methods: Between 2009 and 2011 we identified12 patients treated with Ofatumumab for a relapsed CLL. We analyzed complement data from blood assays: C4 fragments for the classical pathwayand the C3fragments for alternative pathway activation, data available for 7 patients.A patient had 2 courses of Ofatumumab, a year apart, thanks to an excellent initial, therapeutic response.We also collected the phenotype of FcgammaR3 (CD16) and we then analyzed, based on the phenotype, objective responses and the kinetics of blood leukemic cell depletion, by flow cytometry (double staining CD19, CD5).
Results: Of the7 patients analyzed for complement fragments, 5 patients obtained a biological response.We identified 3 complete remissions and 2 partial remissions. Patients who had a biological response had a strong activation of the classical complement pathway (C4). The alternative complement (C3) pathway is not enabled for any patient. The initial values and the variations during the treatment of C4 fragment does not influence complement activation or the response to ofatumumab.
We identified 4 patients with a VF and 8 with a FF phenotype of CD16. Of the 4 patients VF,2 patients presented an objective response: complete response and partial response. All the FF phenotype patients had a response: 3 patients had a complete remission and 5 patients had a partial remission. The kinetics of the response is quite variable, weidentified 3 early complete responses, from the second cycle of Ofatumumab and a later response, after the 6th Ofatumumab perfusion. Partial responses obtained in the VF group stabilized after the fourth infusion of Ofatumumab, those of the FF group had continued beyond , arriving to stabilization during the monthly (maintenance) perfusions.
Summary / Conclusion: This descriptive analysis of our cohort confirm the fact that Ofatumumab has cytolytic activity by complement activation. The Ofatumumab will activate the classical pathway of complement; the alternative pathway is not involved in any of the biological response.The values of C4 does not influence complement activation or responseto Ofatumumab. The descriptive analysis also highlights differences between patients treated with Ofatumumab for relapse CLLby looking to FcγRIII receptor phenotype (CD16): VF compared to FF.Interestingly, response rate is moreimportant and kinetics of the response more pronounced into the FF group Our data suggests that ADCC may be influenced by the phenotype of CD16 (VF / FF).Further, larger studiesare required to confirm these observations.

B1327
POSSIBLE MODIFYING ROLE OF PDE4D SNP83 POLYMORPHISM FOR IGHV REPERTOIRE IN CLL PATIENTS
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Background: T cells are important component of microenvironment for chronic lymphocytic leukemia (CLL) B-cells and may influence on their activation, proliferation and apoptosis (Stevenson, Caligaris-Cappio, 2004). Type 4 phosphodiesterases (PDE4), expecially PDE4D, are critical regulators of T-cell activation (Peter et al., 2007).Opposite, expression of PDE4D isoforms in B-CLL cells decreased (Peiro et al., 2011). Genetic variation in PDE4D family members are associated with some clinical disorders, while a functional relevance for any of these single nucleotide polymorphisms (SNPs) and distribution in CLL have not been reported so far.
Aims: To evaluate the distribution of the PDE4D SNP83 genotypes in CLL patients depend on mutational status of immunoglobulin heavy chain (IGHV) genes.
Methods: The study of the PDE4D SNP83 polymorphism and IGHV gene mutation status was performed in 171 CLL patients and 271 age- and sex-matched controls (only SNP83). Polymerase chain reaction with restriction of product reaction by Tail was used for the PDE4D SNP83 polymorphism. IGHV-D-J rearrangements were amplified according to the BIOMED-2 consortium rules (van Dongen et al., 2003).
Results: The distribution of the SNP83 genotypes in CLL patients was as follows: T/T carriers, n = 60 (35.1%); T/C carriers, n = 77 (45.0%); and C/C carriers, n = 34 (19.9%). The frequencies of the SNP83 genotypes were not significantly different in CLL patients and controls: T/T carriers, n = 93 (38.1%); T/C carriers, n = 115 (47.5%); and C/C carriers, n = 34 (14.0%); P=0.287. CLL patients with T/T genotype comparing with T/C and C/C carriers were more likely to have mutated IGHV genes (T/T genotype - 50.0%; T/C genotype -62.3%; C/C genotype - 71.7; P=0.076) and expressed mutated IGHV4 gene family (T/T genotype - 50.0%; T/C genotype - 42.9%; C/C genotype - 7.1; P=0.006). Expression of IGHV3-21 gene was lower in T/T+T/C carriers comparing with C/C carriers (3.6% and 11.7%, correspondingly; P=0.04).
Summary / Conclusion: Our preliminary data showed a possible modifying role of PDE4D SNP83 polymorphism for IGHV repertoire in CLL patients.

B1328
CELL SURFACE CD20 ANTIGEN EXPRESSION ANALYSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Chronic lymphocytic leukemia (CLL) is an heterogeneous disease with variable outcome. The identification of factors that could predict the clinical course of CLL is a crucial objective. Flow cytometry makes it possible to evaluate malignant B cell immunophenotypic characteristics and study important prognostic factors, such as either CD38 or ZAP 70 expression.
Aims: We investigated the relationship between immunophenotypic variables such as the CD20 intensity of expression and clinical outcome in CLL. Correlation between time to progression, measured as the time elapsed between diagnosis and first treatment, was addressed.
Methods: We retrospectively evaluated 102 patients (pts) with *de novo* CLL treated at the University Hospital of Bari (Italy) between April 2006 and February 2011. At diagnosis all pts were studied for clinical characteristics and peripheral blood multicolor flow cytometric analysis. The intensity of CD20 Antigen expression was analyzed by means of flow cytometry. A cut-off of 70% was considered to define weak (<70%) and strong (≥70%) expression of the antigen. Median time to progression was calculated for all pts and related to CD20. In addition among these pts two groups were choosed: the first group included 38 pts treated within 3 months from diagnosis; the second group included 11 pts treated after 5 years of observation. Univariate analyses of each group were performed to evaluate the correlation between CD20 antigen expression and time to treatment.
Results: Among all pts a statistically significant difference was found in median time to progression comparing pts with weak (<70%) and strong (≥70%) cell surface antigen (P< 0,05). Moreover, in the group of pts treated within 3 months from diagnosis 10 % pts presented CD20 expression <70% and 90 % CD20 expression ≥70%; in the group of pts treated after 5 years of observation 67% presented weak CD20 expression and 33% strong; the difference was statistically significant (P: 0.005).
Summary / Conclusion: In this study a lower CD20 expression was associated to a shorter time to first treatment. CD20 is a transmembrane phosphoprotein involved in the activation, proliferation, and differentiation of B lymphocytes. The immunophenotypic intensity of CD20 expression could help to recognize at diagnosis that subset of patients that may show rapid evolution to progressive disease. Multicentric studies and more consistent cohorts of patients are warranted to confirm these preliminary data.

Chronic lymphocytic leukemia and related disorders

B1329

DESCRIPTION OF A FAVORABLE GROUP OF PATIENTS WITH CD5(+) MONOCLONAL B-CELL LYMPHOCYTOSIS AND CHRONIC LYMPHOCYTIC LEUKEMIA RAI-0

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Background: CLL-like MBL has been recently recognized as a distinct entity and displays many similarities with B-CLL Rai-0. Prognosis is variable and the distinction between these two entities is not well established.
Aims: To describe the clinical, laboratory and biological features of patients with CLL-like MBL and CLL-Rai 0 with favorable clinical outcome.
Methods: Selection of patients was based on the following criteria: Patients with a diagnosis of CD5(+) MBL or CLL RAI-0 who had available data and were not in need for treatment after a follow up time of at least 5 years.
Results: 177 patients were included in this study: 56 with MBL and 121 with CLL Rai-0. The median age was 63 years (37-81). Females predominated (54%). All patients were asymptomatic in a good performance status, without lymphadenopathy or organomegaly as these were assessed by physical examination and CT scanning. No cytopenias were identified. LDH was elevated in only 1 pt. Hypoglobulinemia was observed in 40/126 pts (32%) and paraproteinemia in 7/62 (11%). The median lymphocyte count was 10.290/μL (1650-121.440) and the median absolute B-cell count was 7700/ μL (820-98.366). Bone marrow was evaluated in 124 pts. The median percentage of infiltration was 35% (5-95), while the pattern of infiltration was interstitial in 34 (27%), nodular in 24 (19%), diffuse in 3 (2%) and mixed in 63 (52%). FISH analysis for the identification of del11q and del17p was performed in 70 pts and all were negative for the presence of these cytogenetic abnormalities. 45 pts were evaluated with PCR analysis for the identification of the monoclonal IgVH rearrangement and sequence analysis for the identification of mutational status (table). All studied cases were mutated, while the most common used VH families were: VH3-23 (5/45), VH4-34 (4/45), VH3-7 (4/45), VH1-2 (3/45). After a median follow-up time of 101 months (60-361), 5 unrelated deaths were recorded, 50 pts remained stable, 119 presented with increase in ALCs, while 3 pts had a decrease in ALCs.

VH FAMILY	% HOMOLOGY	VH FAMILY	% HOMOLOGY	VH FAMILY	% HOMOLOGY
VH3-23*01	5.56	VH4-b*02	10.38	VH3-74*03	8.05
VH4-b*02	6.72	VH3-33*01	6.00	VH3-23*01	8.8
VH3-15*01	10.32	VH3-48*02	6.69	VH3-13*01	14.17
VH3-66*01	5.31	VH2-5*10	6.93	VH1-2*02	8.51
VH1-2*02	8.51	VH3-23*01	8.24	vH3-07*01	9.02
VH3-7*01	3.43	VH4-34*01	8.11	VH1-3*01	6.1
VH1-2*02	4.51	VH3-74*01	4.86	VH3-23*01	7.6
VH4-34*01	3.28	VH3-7*01	9.5	VH4-59*08	9.88
VH3-30*03	9.65	VH2-05*10	5.42	VH3-23*01	7.02
VH3-48*03	9.24	VH3-53*02	8.00	VH2-26*01	3.3.2
VH3-33*03	2.79	VH4-34*01	5.71	VH4-39*07	9.6
VH3-15*01	11.15	VH1-46*01	4.45	VH3-53*01	6.67
VH3-7*01	10.53	VH1-08*01	4.9	VH4-34*01	6.61
VH4-61*02	3.6	VH6-1*01	6.27	VH3-72*01	4.25
VH2-70*12	7.66	VH5-51*01	6.45	VH4-59*02	5.33

Summary / Conclusion: This study presents the clinical and biological characteristics of pts with MBL CD5(+) and Rai-0 CLL with a favorable outcome. In all cases the IgVH genes were mutated while no adverse risk cytogenetic findings were identified. These results are preliminary and the study is ongoing in order to compare this favorable group with a similar group of patients [CD5 (+) MBL, CLL Rai-0] who were in need of therapy. Furthermore to identify differences between CD5 MBL versus CLL Rai-0 patients.

B1330

CHEMOIMMUNOTHERAPY WITH RITUXIMAB AND LOW-DOSE ORAL FLUDARABINE AND CYCLOPHOSPHAMIDE (R-FC-LOW DOSE OS) AS INITIAL TREATMENT FOR ELDERLY PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Current standard therapy for fit young patients with chronic lymphocytic leukemia (CLL) is rituximab, fludarabine ,cyclophosphamide (R-FC). This therapy is highly effective in standard risk patients with CLL, however in the elderly it can be troublesome since it can give unacceptable myelotoxicity and increased risk of infection. Because median age at diagnosis for CLL patients is 72, the majority of patients cannot receive RFC, the best therapy available. Fludarabine and cyclophosphamide (FC) given intravenously at low doses in relapsed or refractory indolent lymphoid malignancies (CLD) can decrease the incidence of severe myelosuppression and infectious complications. Based on this background in the past we demonstrated that low-dose oral fludarabine and cyclophosphamide (FC-low dose-os) given to pretreated elderly patients with CLD enabled response rates that were comparable to those obtained with standard i.v. regimens with very few side effects and complications. These responses were confirmed in a population of untreated elderly low grade non Hodgkin lymphoma patients. Finally we showed that in 26 elderly CLL patients who cannot benefit of more aggressive schedules, FC low-dose-os was very effective especially in the untreated population. The regimen was easy to administer on an outpatient basis with mild haematological and extra-haematological toxicity.
Aims: To test efficacy and safety of the addition of rituximab to the oral regimen of FC (R-FC).
Methods: Biological prognosticators were assessed at diagnosis and at disease relapse. In particular IGHV gene use and mutational status, ZAP-70 protein and CD38 expression by flow cytometry, deletions of 11q22.2, 13q14.1, 17p13.1 loci and chromosome 12 trisomy were determined by fluorescent in situ hybridization (FISH) as previously reported. Rituximab 375 mg/m^2 was administered day 1 of each 28 days cycle. Fludarabine 40 mg total dose was given orally (os) days2,3,4,5; cyclophosphamide 200 mg total dose was given orally days2,3,4,5. Bacterial and antiviral prophylaxis were given during all period of treatment. For cycles were planned, when possible (unsatisfactory response) patients received a maximum of 6 cycles.
Results: Fifty one patients were treated. In particular 25 patients received R-FC, while 26 patients received FC. Median age was comparable in the two groups of patients (75, range 67-88). A median of 4 cycles was received by each patient (range1-6). R-FC led to an overall response in 21/25 (89%) patients; in particular a CR was achieved in 13/21 (61%) patients a PR in 8/21 (28%). Median PFS was 39 months. Poor prognosticators for PFS were IGHV unmutated, del11q/del17p, ZAP70 positivity (19 months, 15 months, 19 months respectively; P=0.002). Toxicity was acceptable and manageable and mostly hematological (grade III-IV leucopenia and thrombocytopenia in 15% of the patients). FC led to an overall response in 21/26 (80%) patients. 6/21 (29%) obtained a CR, while 15/21 (71%) a PR. Toxicity was comparable to the RFC regimen (grade III-IV hematological 10%). Median PFS was 19 months and poor prognosticators were IGHV unmutated, del11q/del17p, ZAP70 positivity (15,12,18 months respectively; P=0.001). Including patients with chronic lymphoproliferative disorders such as low-grade Non Hodgkin Lymphomas we treated with the oral formulation of fludarabine and cyclophosphamide at our Institution 107 elderly patients, with good efficacy and good safety profiles.
Summary / Conclusion: Considering the median age of the patients, the R-FC regimen was well tolerated and led to impressive durable responses.

B1331

EFFICACY AND SAFETY OF RITUXIMAB AND HIGH-DOSE DEXAMETHASONE (R-DEX) IN THE TREATMENT OF RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Refractory chronic lymphocytic leukemia (CLL) has an extremely unfavourable prognosis with overall survival frequently shorter than 12 months. High-dose methylprednisolone (HDMP) in combination with monoclonal anti-CD20 antibody rituximab is active in treatment of relapsed/refractory CLL but infectious toxicity is serious. Recently published pilot data suggest that high-dose dexamethasone might be equally effective but less toxic than HDMP.
Aims: To assess efficacy and safety of high-dose dexamethasone combined with rituximab (R-dex) in relapsed/refractory CLL.
Methods: We retrospectively evaluated medical records from patients (pts) with relapsed/refractory CLL treated with R-dex at 4th Department of Internal Medicine – Hematology, Charles University Hospital and Faculty of Medicine, Hradec Kralove, Czech Republic, between September 2008 and October 2012. A total of 52 pts were included (38 males [73 %], median age, 66 years [range,

37-86], Rai III/IV stage in 61%). The median number of therapies prior to R-dex was 2 (range, 1-6). The schedule of R-dex was as follows: rituximab, 500 mg/m^2 i.v. day 1 (375 mg/m^2 in 1st cycle), dexamethasone 40 mg orally on days 1-4 and 10-13, cycles repeated every 3 weeks for the maximum of 8 cycles. All pts received antimicrobial prophylaxis with sulfamethoxazole/trimethoprim and aciclovir. The median number of R-dex cycles was 5 (range, 1-8).
Results: The overall response (ORR)/complete remissions (CR) were achieved in 70/4%. With regard to side effects, serious infections (grade III/IV according to Common Terminology Criteria for Adverse Events) occurred in 29% of the patients; 19% pts developed steroid diabetes requiring temporary use of short-acting insulin. At the median follow-up of 12.7 months, median progression-free survival was 7.6 months and median overall survival 22.6 months.
Summary / Conclusion: Our data show that R-Dex is an active and feasible treatment for patients with relapsed/refractory CLL; however, major infections remain relatively frequent despite combined antimicrobial prophylaxis. In addition, significant and long-term disease control can be expected in a minority of patients only. Updated results will be presented.

B1332
REDUCTION IN IL-33 PLASMA LEVELS MIGHT BE INVOLVED IN T-CELL DYSREGULATION IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: It is now clear that chronic lymphocytic leukemia (CLL) is a proliferative disorder that requires the help of its microenvironment to be maintained and to progress. In CLL neoplastic B cells inhibit normal T lymphocytes, and the alteration of several cytokines may also contribute to this T-cell dysregulation. In this context particular relevance may have some recently discovered cytokines, such as interleukin (IL)-33 and IL-31. IL-33 can activate dendritic cells directly driving polarization of naïve T cells towards a Th2 phenotype. IL-31 coordinates the interaction of different immune cells, including T-cells, mast cells, and eosinophils, with epithelial cells.
Aims: We analyzed the plasma levels of IL- 33, and IL-31, in patients with B-CLL. In the same subjects we also evaluated the lymphocyte immunophenotypical pattern, and we performed IgVH gene analysis, CD38 positivity and ZAP-70 expression to evaluate a possible correlation between interleukin concentrations and biological risk.
Methods: The study population included 77 patients with B-CLL (38 F –39 M) with a median age of 72.38±11.1 years. In a small group of patients the dosage of the cytokines was performed before and after different treatment protocols. Plasma from 63 normal subjects were also included as controls. IL-31 and IL-33 protein levels were measured using the commercially available ELISA kits. Data were presented as interquartile range IQR and range except age presented as mean±Standard deviation.
Results: The IL-31 was detectable in 40/77 (40.05%) CLL patients and in 36/63 (57.14%) controls, and there was not statistical difference between patients and controls (c2 = 1.15, P=0.28). The IL-33 was detectable in 50/77 (64.94%) CLL patients and in 35/63 (55.56%) controls. There was no statistical difference in detectability between them (c2 = 1.28, P=0.26) therefore the two groups were statistically comparable. There was a significant difference (P<0.0001) between the levels of IL-33 in patients affected by CLL (411.5 and 617.2 pg/ml) and those measured in controls (1,375.3 and2,035.4 pg/ml). There was not a significant difference between the levels of IL-31 in patients affected by CLL (1,783.5 and 10,692.8 pg/ml) and those measured in controls (3,278.4 and 10,067.1 pg/ml). There was a significant difference, although not in a statistically way (P=0.072), between the IL-33 levels in CLL patients before and after therapy (83.67 and1,421 vs. 837.22 and1,790.38 pg/ml). There was a positive correlation in CLL patients between IL-31 plasma levels and IL-33 (rho = 0.962, P<0.0001), while we found a negative correlation in patients between IL-31 levels and IL-33 and CD20 expression (respectively rho = -0.6, P=0.014 and rho = - 0.43, P=0.031). There was a positive correlation in patients between levels of IL-33 and CD3 expression (rho = 0.81, P=0.027).
Summary / Conclusion: : In leukemia patients, T-cell function has been suppressed with the disease progress. Cytokines play a critical role in the control of the immune responses. To the best of our knowledge, ours is the first study that demonstrated decreased concentrations of IL-33 in patients with CLL, and this reduction might justify the reduction of the Th2 response observed in these patients. In conclusion, our study might contribute to better understand the cellular immune features in CLL patients, confirms the value of multicytokine analyses for the evaluation of CLL patients, and could open a new scenario in understanding the pathophysiology of CLL.

B1333
THE EFFICACY AND SAFETY OF FIRST-LINE TREATMENTS FOR CHRONIC LYMPHOCYTIC LEUKEMIA - A SYSTEMATIC REVIEW OF CLINICAL EVIDENCE
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Background: Fludarabine/cyclophosphamide/rituximab (FCR) is the standard of care as first line treatment for healthy, fit patients with chronic lymphocytic leukemia (CLL). However, a majority of patients with CLL are elderly and suffer from comorbidities which tend to make them inappropriate for fludarabine-based therapies. A number of therapeutic options are available for these medically less-fit or 'slow-go' patients.
Aims: The objective of this systematic review is to identify evidence for the clinical efficacy and safety of therapies for the first-line treatment of CLL patients for whom fludarabine-based therapies are not appropriate.
Methods: The following electronic databases were searched (without limitations on publication date or language): MEDLINE, MEDLINE In-Process, Embase, BIOSIS, and the Cochrane Library. Search terms included combinations of free-text and Medical Subject Heading terms, including terms for CLL, and the interventions (ofatumumab, chlorambucil (Chl), bendamustine (B), rituximab (R), lenalidomide, and GA-101) and study types of interest (RCTs; open-label, follow-up studies, nonrandomized, controlled trials; single-arm trials; and prospective cohort studies). Conference proceedings from January 2010 to January 2012 and clinical trials.gov also were searched. Articles were screened for relevance by two researchers using predefined inclusion and exclusion criteria.
Results: A total of1,650 records were identified for manual screening; 124 publications were progressed for full text screening and 14 were included: 8 primary and 6 secondary reports. The 8 primary articles included a randomized trial comparing B with Chl (Knauf et al., 2009); an ongoing randomized trial comparing R + B with R + Chl (MaBLe; Leblond et al., 2012); and five single-arm studies investigating R + B, lenalidomide, R monotherapy, R + Chl, and B. One study comparing fludarabine (F) + Chl with F + cyclophosphamide in a population eligible for F therapy was included in this review, as it was the only retrieved frontline study which reported quality of life data (Else et al., 2012). Knauf et al. (2009) reported a median progression-free survival of 21.6 months for B and 8.3 months for Chl (P<0.0001). Overall response (OR; 68% vs. 31%) and complete response (CR) rates (31% vs. 2%) were higher for B than for Chl (P<0.0001). In the MaBLe study, the CR rate was higher in the R + B arm (30%) than in the R + Chl arm (13%) (P=0.054). ORR also was higher in the R + B arm (88%) than in the R + Chl arm (80%). Seven of the included studies reported adverse events. Knauf et al. (2009) reported a greater incidence of adverse events with B than with Chl, with the most common adverse event being neutropenia or granulocytopenia. The MaBLe study (Leblond et al., 2012) reported a similar rate of serious adverse events for R + B (35%) when compared with R plus Chl (34%). Conversely, the rate of withdrawals due to adverse events was higher for R + Chl.
Summary / Conclusion: This review highlighted a paucity of RCT evidence for the treatments of interest in CLL patients who are unsuitable for fludarabine-based treatments. Only two published RCTs were identified. One of these studies was an abstract presenting interim results of an ongoing RCT and thus contained little data. The management of patients with CLL who are unsuitable for fludarabine-based therapies remains a primary unmet clinical need.

B1334
NEW ANALYSIS STRATEGIES MAKE FLOW CYTOMETRY MORE RELIABLE IN THE CLASSIFICATION OF B-CELL CHRONIC LYMPHATIC LEUKEMIA
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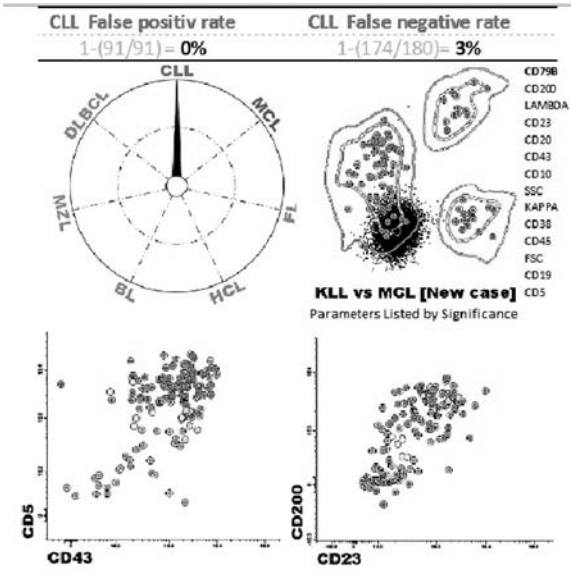
Background: The the old way of analyzing flow cytometry data requires years of experience and still consistent interpretation is challenging.
Improved software makes it now possible to simplify instrument settings and compensation which enable comparison of mean fluorescence intensity(MFI) between samples. With new analysis software you can build a reference database which simplifies comparison and automatic separate cases based on the expression of all analyzed markers. New cases can be matched and compared with the reference cases which should make the interpretation more consistent and flow cytometry more reliable in disease classification.
Aims: Develop a database with diagnosed B-cell lymphoma cases. Develop an analysis strategy that focus on simplicity and reproducibility. Use the database and the new analysis strategy to classify Chronic lymphatic leukemia cases and evaluate the diagnostic usefulness.
Methods: Bone marrow, peripheral blood and cell suspension from glands were prepared with a lyse-wash-incubate-wash procedure.
FACSCanto II (BDBiosciences) were used for flow cytometry analysis, instrument setup and compensation according to Euroflow and BD userguide.
Infinicyt 6.0 were used for analysis. Tube-1 and 2 were merged and calculed according to Infinicyt manual. The lymphoma were classified as CLL if the

immunophenotypic fingerprint matched the database, or if MFI matched CLL variants.



Results: The reference file are based on 50 CLL, 30 MCL, 10 FL, 4 HCL, 1 BL, 9 MZL, 10 DLBCL
Of the 180 CLL cases 162 cases matched the refference cases (RED), 5 cases expressed low levels of CD5 but were classified as CLL (BLUE), 7 cases expressed low levels of CD43 but were classified as CLL (PURPLE). 6 cases did not match the CLL criteria (YELLOW).
Summary / Conclusion: In this study we show that with new software and analysis strategy the majority of all CLL cases can be classified, reproducible and with high reliability using only flow cytometry. We conclude that using a software to analyze MFI and match with reference cases a high consistency in classifying CLL is obtained. Using MFI and reference cases as background in dot plots a high consistency in interpretation of staining intensity is obtained. We propose that the new analysis strategy make CLL classification more reliable but also simplifies identification of cases with poor prognosis. To further validate this procedure more cases have to be analyzed and all different lymphomas have to be included in the reference database.

B1335
THE DIAGNOSTIC VALUE OF CD123 IN HCL AND OTHER B-CELL DISORDER WITH HAIRY LYMPHOCYTES SINGLE CENTER EXPERIENCE
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Background: Hairy cell leukemia (HCL) and hairy cell leukemia-variant (HCL-v) are rare B-cell lymphoproliferative disorders (BC-LPD) with overlapping clinico-pathological features. However, certain morphological features of HCL, such as villous cytoplasmic projections or characteristic tissue specific infiltrative patterns, including red pulp expansion with pseudosinuses, may be seen in other B-cell lymphoproliferative disorders. A methodical and comprehensive approach including the evaluation of the immunophenotypic features further improves diagnostic work-up. CD123 is an antibody that identifies and binds to the Alfa chain of the human interleukin – 3 receptor. It is expressed at the normal hematopoietic cells at most of the hairy cells. The coexpression of CD123, in conjunction with bright CD11c, bright CD20, bright CD22, CD25, and CD103, is the immunophenotypic sine qua non in diagnosing HCL.
Aims: The aim of the study was to determinate the diagnostic value of CD123 expression in HCL and other B-cell lymphoproliferative disorder cases with hairy lymphocytes.
Methods: We investigated the diagnostic value of CD123 expression in neoplastic cells from 50 patients with B – cell disorder with circulating hairy lymphocytes We performed flow cytometry analysis (FCM) of 50 cases (30 HCL, 5 HCL-v, 15 splenic marginal zone lymphoma (SMZL)), correlating results with available corresponding clinical and morphological data. Immmunophenotype analyses were performed by using the FAXS Canto II BD flow cytometer analyzer on 50 samples, from peripheral blood (23) and bone marrow (27). Acquired data were analyzed with the software FACS Diva version 6.1.2 by using CD19 gating strategy according to revised guidelines for the diagnosis and management of hairy cell leukemia and hairy cell leukemia variant according to British Committee for standards in hematology (BCSH).We used the panel recommended by which incorporates the following markers : CD11c, CD25, CD103, CD123, that are specific for HCL combined with common B-cell markers (CD19, CD20, CD22) and T cell markers..
Results: Our findings show that cells from 100% of typical HCL expressed CD123 with strong intensity, while cells from other B – cell disorder with hairy lymphocytes did not expressed CD123. HCL expressed bright CD20, bright CD22, bright CD11c, bright CD25, CD103, and bright homogeneous CD123(100%). HCL-v expressed bright CD20, bright CD22, CD11c(20%) and uniformly lacked CD103(100%), CD123(100%),CD25(100%) antigens. SMZL cases were CD103(-) and CD123(-). Detection of BRAFV600E mutation by using PCR methods was examined in a subset of cases from our study group. All HCL-v cases were negative for BRAFV600E mutation, in contrast to HCL (50% positive for BRAFV600E mutation).Those results further validate our FCM diagnostic criteria.
Summary / Conclusion: We conclude that CD123is a useful marker for distinguishing B cell disorder with villous lymphocytes from HCL with high sensitivity and specificity.

B1336
THE PROGNOSTIC VALUE OF THYMIDINE KINASE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA IN THE VARIOUS TYPES OF MODERN THERAPY
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Background: Thymidine kinase (TK) is recognized as the predictor of disease progression in chronic lymphocytic leukemia (CLL). But the answer to the current chemotherapy and immunochemotherapy has not been still estimated according to the contents of TK.
Aims: Determine the prognostic value of TK in patients with CLL in different types of modern therapy.
Methods: The research included 230 patients with CLL. The median age was 59 years. Patients were divided into 3 groups according to the type of received treatment. The first group included 36 patients, who were prescribed leukeran, the second one – 96 patients who received treatment FC (fludarabine, cyclophosphamide), the third – 98 patients who received RFC (rituximab, fludarabine, cyclophosphamide). The enzyme content, exceeding the maximum rate of its normal level twice, was used as a cut-off value (20 U/L).
Results: The answer to the therapy manifested the achievement of partial remission (PR) among 16 (73%) patients and complete remission (CR) among 2 (9%) patients from 22 ones (61%) of the first group with TK levels < 20 U/L. There was no treatment effect among 13 (93%) patients from 14 ones (39%) with TK ≥ 20 U/L (P=0.001). Median overall survival (OS) in patients with TK levels < 20 U/L has not been reached after 62 months of follow-up, but it was 31 months in patients with TK levels ≥ 20 U/L (P=0.041). Median time to progression (TTP) was 7 months with the concentration of TK < 20 U/L, and 2.7 months with the rate of TK ≥ 20 U/L (P=0.023). CR and PR were achieved among 22 (40%) and 29 (53%) patients accordingly from 55 ones (57%) of the second group with the content of TK < 20 U/L. The lack of therapeutic effect was observed in 4 (7%) patients. At the same time, CR and PR were achieved in 7 (17%) and 16 (39%) patients accordingly from 41 ones (43%) with TK levels ≥ 20 U/L, there was no response to therapy in 18 (44%) patients (P=0.001). Median OS in patients with TK levels < 20 U/L has not been reached, whereas it was equal to 56 months among patients with TK levels ≥ 20 U/L (P=0.015). Median TTP in patients with TK levels ≥ 20 U/L was 28 months, but it was 18 months in ones with TK ≥ 20 U/L (P=0.028). The effect of the therapy was independent of RFC TK content in blood serum. CR was achieved in 41 (76%) and 28 (64%) cases among the patients of the third group with TK levels < 20 U/L and ≥ 20 U/L accordingly (P=0.692). Medians OS of the patients with different levels of TK and treated by the program RFC have not been achieved (P=0.367). Median TTP of patients with TK levels < 20 U/L and ≥ 20 U/L was 60 and 44 months accordingly (P=0.156).
Summary / Conclusion: TK maintains its prognostic value among patients with CLL treated with leukeran and FC: OS and TFT depend on the level of this enzyme. At the same time immunochemotherapy RFC eliminates the negative effect of TK as a tumor marker for the direct effect of treatment and duration of response. The contents of TK among patients with CLL can be used as additional prognostic factors of response, as well as for the selection of appropriate treatment programs.

B1337
AUTOIMMUNITY AND CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Chronic Lymphocytic Leukemia (CLL) is frequently related with autoimmune diseases. The relation between CLL and autoimmune cytopenias, like autoimmune hemolytic anemia (AIHA) or immune thrombocytopenia (IT), is well known. The presence of these phenomena was considered as an adverse prognostic factor. However, recently, it was demonstrated that patients with immune cytopenias at diagnosis have better prognosis than those with cytopenias secondary to diffuse bone marrow involvement.
Aims: Identify clinical, laboratory, immunophenotypic and cytogenetic parameters potentially related with autoimmune complications in a group of patients with CLL-B.
Methods: Review of demographic, clinical and laboratorial data, particularly the Rai/Binet staging, immunophenotypic and cytogenetic characterization (FISH) of patients diagnosed with CLL-B in our department, from January 1st, 1988 to July 30th, 2012.
Results: 382 patients were identified, M/F ratio 1.6:1, median age 71 years (38-95) and median lymphocytosis 21 G/L (5-1050). Accordingly to the Rai/Binet staging system, 245 were low risk patients, 60 were intermediate risk and 77 were high risk (64, 16 and 20%, respectively). The most frequent autoimmune disease observed was AIHA in 51 patients (13.4%), followed by IT in 22 patients (5.8%) and pure erythroid aplasia in 9 patients (2.3%). Other non-hematological autoimmune diseases were observed, as rheumatoid arthritis (n=9), autoimmune thyroiditis (n=7), and others (n=9). After statistical analysis, we observed a relationship between autoimmune cytopenias and advanced stage diagnosis (P=0.02), beta-2 microglobulin two times higher than the normal level (P=0.02) and immunophenotypic atypia (P=0.02). We dit not find any relationship between cytogenetic abnormalities and immune cytopenias. Non-hematologic autoimmune pathologies are not related with the factors described. Overall survival (OS) is 75%, 58% and 35% at5, 10 and 15 years, respectively. The 5 years OS is 62% in the group of patients with autoimmune cytopenias and 80% in the other patients (P=0.024).
Summary / Conclusion: The results suggest that patients in advanced stage at diagnosis, high beta-2 microglobulin and immunophenotypic atypia have a higher risk for autoimmune cytopenias. Patients with autoimmune disease seem to have lower survival.

B1338

VEMURAFENIB IS HIGHLY ACTIVE IN ADVANCED HAIRY-CELL LEUKEMIA: A REPORT OF TWO CASES

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Background: Hairy cell leukemia (HCL) is a rare indolent B-cell lymphoma characterized by splenomegaly, profound pancytopenia and frequent infectious complications. Standard therapy consists of purine analogs±rituximab and for most patients survival is not compromised. However, in rare cases HCL may become chemoresistant resulting in a more aggressive course. Recently, it was shown that almost all HCLs harbor a distinct mutation of the BRAF gene (BRAF V600E). Thus, targeted treatment with BRAF inhibitors may represent an attractive treatment option for refractory HCL.
Aims: To report preliminary clinical data on the use of vemurafenib in advanced HCL.
Methods: We herein report two cases of HCL treated with the BRAF inhibitor vemurafenib.
Results: Case one is a 79 years old woman refractory after 2-CdA + rituximab as 4th line treatment suffering from pronounced pancytopenia and repeated life-threatening infectious complications despite repeated G-CSF administration. Within one week after the initiation of vemurafenib (960mg/d) blood counts improved significantly and after 105 days a complete remission was achieved and vemurafenib was stopped. With a follow-up of currently seven months the patient is still in complete remission. The second case is a 68 years old man with subtotal HCL bone marrow infiltration resulting in G-CSF refractory °IV neutropenia, transfusion-dependant anemia and °III thrombocytopenia. In view of a concomitant life-threatening invasive pulmonary aspergillosis and a one week history of STEMI requiring dual antiplatelet therapy with ASS and clopidogrel the patient received firstline treatment with vemurafenib (960mg/d) to avoid prolonged pancytopenia and immunosuppression commonly observed after 2-CdA therapy. One week after the initiation of vemurafenib blood counts began to improve and after 80 days the patient had achieved an excellent remission with near-complete normalization of blood counts and splenomegaly, recovery from pulmonary aspergillosis and only residual HCL infiltration in the bone marrow. Treatment with vemurafenib is planned to continue until a complete remission is achieved.In both patients treatment with vemurafenib was safe and well tolerated
Summary / Conclusion: Vemurafenib is highly active in advanced HCL and represents an attractive novel treatment option in particular for refractory patients.

B1339

AN OPEN-LABEL, SINGLE-ARM, PHASE I STUDY TO ASSESS SAFETY AND EFFECT OF OFATUMUMAB ON B-CELL COUNTS, COMPLEMENT, AND CYTOKINES/CHEMOKINES IN PATIENTS WITH REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: Ofatumumab is a human monoclonal antibody against CD20 approved for treatment of fludarabine- and alemtuzumab-refractory CLL. Clinical and lab correlative data from a study conducted to collect QTc data in fludarabine-refractory CLL patients are presented here.
Aims: Assess the safety and effect of ofatumumab on complement, B-cell counts , and cytokines/chemokines in patients with refractory CLL.
Methods: Adult subjects with active CLL refractory to at least one fludarabine-containing regimen received 300 mg at Week1, followed by 11 infusions of 2000 mg (Weeks 2-8, 13, 17, 21 and 25). Infusion rate started at 12 mL/h (first infusion) or 25 mL/h (later infusions) and escalated by doubling the rate every 30 min up to 400 mL/h to administer 1000 mL. Biomarker samples were collected prior to dosing at Weeks1,2,5, 9, 13, 17, 21, 25, 29, 37 and 49. The study assessed safety and the effect of ofatumumab on circulating B-cell counts ($CD5^+CD19^+$, $CD5^-CD19^+$), complement (C2, C3, CH50), cytokines (IFNg, TNFa, IL-1b, IL-2, IL-6, IL-8, IL-10, IL-12 p70), and chemokines (MCP-1, GM-CSF, MIG, IP-10) at the maximum studied dose of 2000 mg.
Results: Fourteen patients were enrolled. Median number of prior treatments was 3. Five (36%) patients completed the study, 2 (14%) patients died of pneumonia, 5 (36%) patients withdrew due to disease progression, and 2 (14%) withdrew consent. Thirteen patients completed the first 8 weekly infusions, and 8 patients completed all 12 infusions. Median duration of the first infusion dose was 4.9 h (range 4.7-8.9 h) and for later doses was 4.1 to 4.4 h. Infusion reactions were seen in 79% of patients at first dose, 57% at second dose, and 0-30% of patients at later doses. No grade 4 or 5 infusion reactions were observed; two subjects (14%) had grade 3 infusion reactions, which resolved. No patient withdrew from treatment due to infusion reactions. Most common AEs and SAEs included diarrhoea, headache, decreased appetite, fatigue, pyrexia and neutropenia. Overall response rate was 43% (0 CR, 6 PR, 7 SD, 1 NE). Circulating B-cell counts were greatly reduced by Week 8 and remained low during treatment. By the 6-month follow-up visit, median normal B-cell counts had returned to near-baseline, while median tumor B-cell counts remained below baseline. Baseline C2, C3, and CH50 levels did not appear to differ between responders and nonresponders. Median C2 and CH50 levels decreased in both groups initially and remained below normal for responders; levels returned to normal range during monthly dosing in nonresponders. C3 had smaller changes. Median levels of TNFa, GM-CSF, and IL-2 appeared higher and median levels of MCP-1, IL-6, and IL-8 appeared lower in responders than in nonresponders throughout the study. Median IFNg and IL-10 levels were initially higher in responders than in nonresponders, then became lower after Week 9. No trends emerged with other cytokines/chemokines.
Summary / Conclusion: Maximum infusion rates of 400 mL/h at the first two doses were well tolerated; infusion reactions were similar in frequency and severity to previous results with ofatumumab. Overall response rate was similar to previous data (42-51%). B-cell depletion was observed along with complement consumption; median C2 and CH50 levels remained lower during monthly dosing in responders. Responders also appeared to have higher median levels of certain pro-inflammatory cytokines and lower median levels of certain immunotolerant cytokines during the course of the study than nonresponders. Further investigation of the role of complement and cytokines in patients treated with ofatumumab is warranted.

B1340

MULTIVARIABLE MODEL CONSISTED OF CLINICAL AND BIOLOGICAL MARKERS FOR TIME TO FIRST TREATMENT IN CLL PATIENTS: PRELIMINARY RESULTS FROM SINGLE CENTRE EXPERIENCE

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Background: The clinical course for patients with chronic lymphocytic leukemia is extremely heterogeneous, one of the most important challenges in the clinical management of those patients is the decision of initiating their treatment, but there is no available prognostic system that will resolve this issue. Usually, criteria for active disease are used to initiate therapy. Recently, some authors proposed prognostic models, scoring systems involving a set of clinical and biological risk factors and estimates individual patient survivals.
Aims: Here, we report our initial results from a study designed to evaluate the statistical association of the distinct clinical and biological parameters with the

prognosis and time to initiating treatment for patients with CLL.
Methods: Our study incorporated 100 consecutive, treatment naïve CLL patients. In each patient all traditional laboratory, clinical and biological prognostic factors were evaluated at their first visit to our Institution. Than we combined the following independent characteristics: age, β-2 microglobulin, absolute lymphocyte count, sex, Rai stage, and number of involved lymph node groups, which are included in some of the already published CLL prognostics index, in association with the CD38 expression and mutational status of the IGVH gene regions. Further, we correlate those factors by multivariable analysis with time to first treatment. This multivariable model was used to develop a nomogram-a weighted tool to calculate 5- and 10-year survival probability and estimate median time to first treatment.
Results: According to prognostic index a classification tree was built that identified three subsets of patients who scores were 1-3 (low risk- 32pts- 32%), 4-7 (intermediate risk-48pts- 48%) and >8 (high risk-20pts- 20%). Estimated median survival at low risk subset of patients is 14,1years, 10,7 and4,6 years respectively at intermediate and high risk subsets of patients. Projected survival in respectively low, intermediate and high-risk groups are 100%, 100%, 25%, and 34%, 43%, 25% at 5-year and10-year, respectively. Also, statistical analyses showed that three involved lymph node sites, increased size of cervical lymph nodes, increased serum lactate dehydrogenase ,CD38 expression and unmutated IGHV mutation status are associated with shorter time to first treatment.
Summary / Conclusion: Our prognostic model that combines and correlates the distinct clinical and biological markers of CLL patients enables identification of the patients that are at high risk for progression. This prognostic model may facilitate clinical decision for initiating treatment.

B1341
CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): EPIDEMIOLOGY, COMORBIDITIES AND TREATMENT PREFERENCE IN DAILY CLINICAL PRACTICE
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Background: CLL diagnosis and management data come mainly from clinical trials. Little is known about the spectrum of the disease as well as comorbidities in daily practice.
Aims: To assess the CLL epidemiology, comorbidities and treatment preference in daily clinical practice.
Methods: A questionnaire including demographics, disease characteristics, comorbidities, and treatment from the date of diagnosis, was distributed to all outpatient and inpatient hematologists who take care of CLL patients in a tertiary healtcare facility Vilnius University Hospital with the catchment area of about ⅓ of Lithuanian population of 3 million people. Data were collected during 6 month period of 2012, presuming that all CLL patients were about to visit a hematologist during this interval at least once.
Results: 455 CLL patients were registered. The CLL diagnosis was confirmed in 86% of cases by flow cytometry or histology. Estimated prevalence was 30 patients per 100 000 inhabitants. Male to female ratio was 1:1. Median age at the time of visit was 70 years (range 38-90), and 16% were > 75 years old or older. Sixty-four persent of the patients had comorbidities, mainly hypertension (66%), coronary heart disease (34%) and musculoskeletal disorders (27%). Six (1.3%) patients had concomitant cancer. Cumulative illness rating scale (CIRS) was evaluated and the median score was 2 (range 0-14). FISH analysis of 17p deletion/*TP53* mutations was carried out in 128 (67%) of treated patients and was positive in 13 cases (10%). Three persent of the patients had autoimmune hemolytic anemia and 2% had immune thrombocytopenia.Treatment was administered in 58% of the patients at any point of their disease and 20% received more than 3 lines of therapy, 11 (2.4%) patients were allografted. First line treatment was mainly chlorambucil (60%), FCR was given to only to 6%. Combinations containing rituximab were more often used in relapsed patients. Treatment preference (chemoimmunotherapy (CIT) vs alkylating agents) did not differ much according to CIRS (≤6 vs >6), but differences were noted according to age: 65 years old or younger patients were more often treated with rituximab containing regimens compared to older ones: first line treatment 15% vs 2%, second line 28% vs 11%, respectively. There was a trend of more use of CIT in 2012 compared to previous years: first line FCR 23% vs 6%, second line FCR 26% vs 10%, respectively.
Summary / Conclusion: CLL prevalence in Lithuania is comparable to Western countries (SEER). More than half of the patients are followed untreated. Though many CLL patients have comorbidities, intensity of treatment (alkylating agents vs CIT) is more related to age. There is a trend for more CIT use in 2012, which can be explained by the fact that rituximab was approved for CLL in Lithuania only in 2011. Data from this study will help to refine CLL management in different patient groups.

B1342
INTEGRATION OF AUTOMATED MORPHOLOGICAL FEATURES RESOLVES A DISTINCT GROUP OF ATYPICAL CHRONIC LYMPHOCYTIC LEUKEMIAS WITH CHROMOSOMAL ABERRATIONS
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Background: In recent years, automated morphological assessment of peripheral blood slides has become a powerful modality that allows better characterization and quantification of cells in a uniform, fast and robust manner, associated with a reliable management of illustrative data. However, the capacity of this automated laboratory approach in the diagnosis of lymphoproliferative disorders has not been established yet.
Aims: To evaluate the morphological diversity in peripheral blood films of patients with chronic lymphocytic leukemia (CLL)
Methods: Blood films of 80 patients with CLL were analyze using the DM1200 CellaVision automated microscopy system. Aberrant lymphocytes and smudge cells were enumerated and correlated with CLL immunophenotype, prognostic parameters and clinical outcome.
Results: An increased proportion of aberrant lymphocytes were associated with trisomy 12 and an atypical immunophenotype. CLL patients with $\geq$7.5% aberrant lymphocytes had a shorter time from diagnosis to first treatment compared to patients with <7.5% lymphocytes with atypical morphology. While the percentage of smudge cells was extremely variable among patients, a low percentage of smudge cells correlated with trisomy 12 and atypical immunophenotype. The ratio between percentages of smudge cells to aberrant lymphocytes appeared to be the most powerful index for morphological CLL scoring. Low ratio values highly correlated with an atypical immunophenotype and trisomy 12.
Summary / Conclusion: Automated morphological analysis of peripheral blood leukocytes is a powerful and robust tool for the quantitative morphological stratification of CLL. Integration of the automated morphological features discriminates between different CLL phenotypes and distinct chromosomal aberrations.

B1343
SMALL LYMPHOCYTIC LYMPHOMA/ CHRONIC LYMPHOCYTIC LEUKEMIA : A RETROSPECTIVE SURVEY OF PROGNOSIS FACTORS
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Background: Small lymphocytic lymphoma (SLL) is a mature (peripheral) B-cell neoplasm characterized by a progressive accumulation of monoclonal B lymphocytes. It is considered to be identical to chronic lymphocytic leukemia (CLL) with similar pathologic and immunophenotypic features. Distinction is usually made based upon clinical presentation and an arbitrary cut-off of $5x10^9$/L lymphocytosis.
Methods: We retrospectively reviewed 117 patients (pts) (20 SLL and 97 CLL patients) registered in our data, between 1995 and 2012, comparing outcomes with emphasis on cytogenetic data and biological prognostic factors. Beta-2 microglobulin, flow cytometry (CD19, CD20, CD5, CD23, CD38, CD11c,FMC7), IgV_H mutation status and caryotype or FISH were studied in both groups. The χ^2 test was used to identify significant differences between groups. Overall survival (OS) curves were estimated using the Kaplan-Meier.
Results: Median age of SLL pts (58.5 y.o.) was somewhat younger than the median age (65 y.o.) in CLL pts. The 97 CLL pts were classified according to the Binet staging (55 stage A, 21 stage B, 21 stage C). SLL were classified according to the AA staging (stage II, n=2 - stage III-IV, n=18). 5 and 10/yrs OS were respectively 71% and 19% in SLL pts. 5 and 10 yrs OS of CLL was 90% and 82% in stage A, 83% and 75% in stage B, 53% and 40% in stage C. Advanced stage SLL behaves such as CLL Stage C in terms of OS. Further analyses on biological factors showed that SLL and CLL share similar patterns in terms of phenotypes. We confirm the prognostic relevance of the disease staging system (Binet) for CLL. Biological prognostic factors such as CD38+, β2microglobulin >3mg/dl, $IgGV_H$ mutational status, cytogenetic abnormalities were also significantly discriminant in OS for pts with SLL/CLL. In our small series, cytogenetic abnormalities such as 11q or 17p del were more frequent in SLL (30%) than in CLL (14%) (P=0.01). The CD11C expression had no significant prognostic value in SLL pts (P=0.54) but a good prognosis factor in CLL pts (P=0.03).
Summary / Conclusion: SLLs express similar phenotypic features, similar biological prognostic markers and similar overall survival for advanced diseases. However, at the time of diagnosis, our small series suggests that median age is lower and poor cytogenetic abnormalities are more frequently described.

B1344

RITUXIMAB IN COMBINATION WITH CLADRIBINE IN HAIRY CELL LEUKEMIA (HCL)

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Background: HCL is characterized by exquisite responsiveness to purine analogues, cladribine and pentostatin. Therefore, despite the fact that it is one of lymphoid neoplasms with highest density of CD20, there are only limited data on the efficacy of rituximab in combination with purine analogues.
Aims: We performed this study to analyze the toxicity and efficacy of the combination of rituximab and cladribine in patients with HCL.
Methods: We retrospectively analyzed outcomes of patients who were treated with this combination at our center.
Results: We identified 10 patients, 7 men and 3 women, 44-82 years old (median 53). Three were treated up-front and 7 for refractory or relapsing disease. Patients in the latter group have previously received between 1 and 2 treatment lines (median 2), at least one including purine analogues. Cladribine was administered at the standard dose, continuously in 8 and intermittently in 2 patients, rituximab was administered concurrently in 8 and after cladribine in 2 patients for 2-8 (median 6) cycles. Cotrimoxazole and acyclovir were administered for infection prophylaxis. Duration of severe granulocytopenia was 0-17 days (median 7), 2 patients received RBC transfusions and 1 platelet transfusions. Five had serious infections, one died early due to an infection that was present at time of treatment start. All 9 evaluable patients responded. One frail elderly lady died 4 months after treatment start of cerebrovascular disease. The median follow-up of survivors is 37 months (range 8-84 months), none relapsed. Three-year OS and PFS is 79%. Duration of remission was longer than the previous in 4/4 patients with sufficient follow-up.
Summary / Conclusion: The combination of rituximab and cladribine is an effective treatment for HCL relapsing after or refractory to cladribine monotherapy. In comparison to historical controls treated with cladribine monotherapy, time to hematological recovery seems shorter (possibly due to increased tumor cell killing) but infectious complications seem more frequent.

B1345

CHOLESTEROL LEVELS IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The cholesterol levels may be low in solid tumors and hematological malignancies such as multiple myeloma. The cholesterol levels in the patients with chronic lymphocytic leukemia (CLL) decreased in some experimental studies. Some patients have indolent disease and never need treatment, but in others the clinical course is aggressive and soon after diagnosis requires intensive treatment.

	CLL (n:73)	Control (n:71)	P value
TC(mg/dl) 179±38	217 ± 36	0.000	0.000
HDL-C(mg/dl)	37 ± 12	53 ± 14	0.000
LDL-C(mg/dl)	113 ± 30	131 ± 29	0.000
Triglycerid(mg/dl)	142 ± 78	147 ± 68	> 0.05
VLDL-C(mg/dl)	28 ± 15	31 ± 17	> 0.05

Aims: In this study, according to the International CLL study group, 73 patients with newly diagnosed CLL (38 male and 35 female with mean age of 67±11 years) were retrospectively evaluated for lipid parameters.
Methods: 71 (43 females and 28 males with mean age of 55±9 years) healthy subjects were enrolled to the study as control group. Architect C800 instrument and enzymatic/ calorimetric method was used to measure lipid parameters including total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C), and triglyceride levels. Low-density lipoprotein-cholesterol (LDL-C) values were calculated according to the formula of Friedwald. Lipid parameters were compared between Binet A and C stages using with student's t test.
Results: While 59% of the patients with Binet A, while 36% were Binet C. TC, HDL-C, LDL-C values in the patients with CLL lower than control group (P<0.001), Triglyceride and VLDL-C levels were not significantly different (P>0.05) between two groups (Table 1).There was no difference for lipid parameters between Binet stages.
Summary / Conclusion: Low cholesterol levels in patients with CLL may occur due to increased use of cholesterol by lymphocytes.

B1346

THE ASSOCIATION OF FLUDARABINE AND CYCLOPHOSPHAMIDE AS FRONT LINE TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA: HAS IT STILL A ROLE IN THE IMMUNE THERAPY ERA?

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Background: The combination of Fludarabine and Cyclophosphamide (FC) was widely used in Chronic Lymphocytic Leukemia (CLL) for its tolerability and higher response rate in respect to the alkylator based regimens. Nowadays the association of monoclonal antibodies with chemotherapy is considered the standard treatment of CLL.
Aims: Here we report the long term results and late toxicities of FC combination administered orally or intravenously, taking into account biological profile of patients, in order to establish its role in the era of monoclonal antibodies.
Methods: We retrospectively enrolled 65 CLL patients with a median age of 65 years (range 44-78 years) treated with oral FC (30/250 mg/m^2) (38 patients) or intravenous FC (25/250 mg/m2), administered for 3 consecutive days every 4 weeks for 6 cycles. All patients were studied at baseline for IgVH, CD38, Zap 70, FISH abnormalities.
Results: No statistical differences were noticed in terms of efficacy between the two routes of administration. Seven patients did not complete the treatment: two developed immune thrombocytopenia (ITP), one pure red cell aplasia and one skin toxicity. Two patients in the intravenous arm died of pneumonia and one for sudden cardiac death. We observed a good compliance, especially in the oral FC group. Overall response rate (ORR) was 84% (53% complete response); median progression free survival (PFS) was 37 months (range 2-199); median time to re-treatment (TTR) was 42 months (range 2-199). At a median follow-up of 6 years (range 1-199 months) median overall survival (OS) was reached at 96 months. Thirty-two patients are still alive. Three patients developed lymphoproliferative diseases: two of them a Richter's syndrome respectively 11 and 51 months after FC (the second patient died) and one a Hodgkin lymphoma 74 months after FC. One patient showed a myelodysplastic syndrome 36 months after FC. One patient with previous surgery for gastric ulcera developed a gastric cancer 32 month after FC and died. Eighteen patients died of progressive disease at median of 47 months after FC and five due to infective late complications. One patient, who received only 2 cycles of FC and immune therapy for ITP, died of hepatic cancer. Other late fatal events unrelated to CLL were two heart diseases, one liver disease, and one respiratory failure. Among patients who required salvage therapy, our data suggest that the combination of Rituximab with chemoterapy (Chlorambucil, FC, Bendamustine) is the best strategy as second line therapy. A significantly lower OR (HR 0,093; CI 0,015-0,580; P=0,011) and reduced PFS (HR$_{3,857}$; CI$_{1,766}$-8,424; P=0,001) was noticed in the high risk (Del 17p- and/or Del 11q-) FISH group compared to standard risk. Unmutated IgVH (HR$_{2,857}$; CI$_{1,207}$-6,762; P=0,017) and high risk FISH (HR$_{4,641}$; CI$_{1,929}$-11,164; P=0,001) emerged as independent prognostic factors on TTR. Patients with high risk FISH showed reduced OS (68 months versus 96 months standard risk), without statistical significance.
Summary / Conclusion: These results confirmed the safety and tolerability of FC. Even if in the era of monoclonal antibodies in which BTK and PT3K inhibitors showed surprising results, this association could be still used especially as oral formulation in patients not eligible for more aggressive protocols, allowing a home therapy.

Chronic myeloid leukemia - Biology

B1348

BCL-XL EXPRESSION AS A POTENTIAL PROGNOSTIC PARAMETER IN CHRONIC MYELOID LEUKEMIA

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Background: Chronic myeloid leukemia (CML) is characterized by the presence of the Ph chromosome (*BCR/ABL* chimeric gene) in hematopoietic stem cells. Clinically, it is manifested in three distinct phases: chronic, accelerated, and blastic. *BCR-ABL* expression results in constitutive activation of STAT5 which contributes to increased expression of the anti-apoptotic Bcl-2 family member *Bcl-xL*.
Aims: The aim of this work was to investigate the role of *Bcl-xL* expression in CML progression into advanced phases and its possible significance as a prognostic parameter.
Methods: The study was conducted on 32 CML patients including 12 males and 20 females with an age range of 21-79 and a median of 41.5 years. They included 18 in chronic (Group I), 3 in accelerated and 11 in blastic crisis phase (Group II). Hasford score was available for 30 patients. They were divided into 3 risk groups: Low risk group: score 780 (8 patients), Intermediate risk group: score 781-1480 (13 patients) and High risk group: score > 1480 (9 patients). Patients received standard therapy. *Bcl-xL* expression was assessed by RT-PCR; it was studied in relation to various hematological and clinical parameters. The study was approved by the IRB of the NCI, Cairo University and a written informed consent was obtained from all participants.
Results: *Bcl-xL* expression did not differ according to the disease stages. Within group I, TLC and % basophils were significantly higher in patients with *Bcl-xL* positive than those with *Bcl-xL* negative (P=0.004 and 0.02 respectively). Hasford Score was available for 15 cases in group I and 15 in group II; it was significantly higher in group II (1383.16±1259.98 vs.1122.97±474.43, p value=0.01). Within group I, *Bcl-xL* showed a statistically significant higher expression in patients in high risk group according to Hasford score than patients in intermediate or low risk groups The difference was statistically significant in the total cohort (P=0.01) and in group II (P=0.046) but insignificant in group I. A statistically significant better outcome was observed in *Bcl-xL* negative patients as compared to *Bcl-xL* positive ones (P=0.05).
Summary / Conclusion: *Bcl-xL* is not involved in the mechanism(s) underlying the progression into accelerated phase or blastic crisis in CML. However it might serve as a prognostic parameter.

B1349

BCR-ABL KINASE DOMAIN MUTATION FREQUENCY IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS RESISTANT TO IMATINIB THERAPY

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Background: The major mechanism of resistance to imatinib and another inhibitors of tyrosine kinases of patients with chronic myeloid leukemia (CML) is the mutations in the locus between exon a3 and exon a11 in *ABL* gene, which present in *BCR-ABL* fusion tyrosine kinase and its mRNA. According to recommendations European Leukemia Net (ELN) sequencing of the *BCR-ABL* kinase domain is a necessary analysis for all patients with CML with primary reduced capacity of imatinib to inhibit kinase activity.
Aims: Analysis of incidence mutations frequency in the BCR-ABL kinase domain in patients with chronic myeloid leukemia (CML) with resistance to imatinib therapy.
Methods: Present study involves 846 CML patients with resistance to imatinib therapy in 68 hospitals of 53 cities of Russia during 85 months from January 2006 to February 2013. The patients had different disease stage. Efficiency of imatinib therapy was analyzed in GeneTechnology LLC Molecular Oncology and Hematology Lab in Moscow by RQ-PCR of *BCR-ABL* transcript according IS (International Scale). Identification of point mutations in the locus between exon a3 and exon a11 in mRNA *BCR-ABL* was performed by direct sequencing of the *BCR-ABL* kinase domain.

Fig. 1. Distribution among CML stages and frequency of mutations coursing resistance to 2nd generation ITKs.

Mutation	CML (CP), N=129	CML (AP), N=78	CML (BC),N=55	Resistance
T315I	5,4%	16%	27%	Imatinib, Nilotinib, Dasatinib
E255K/V F359V/C Y253H	6,2% 9,3% 5,4% } 20,9%	12,8% 7,7% 8,9% } 29,4%	16,4% 2,7% 7,3% } 26,4%	Imatinib, Nilotinib
F317L/I	4,6%	12,8%	16,4%	Imatinib, Dasatinib

Results: 31% (n=262) patients with CML and resistance to imatinib therapy had mutations in the *BCR-ABL* kinase domain, among them 59,9% men (n=157) and 40,1% women (n=105), median age – 50 (from 15 to 74). As well as5,7% patients (n=16) had two mutations, we were detected 278 mutations from 262 patients with CML. Total amount of mutations comprise 40 variations sorting by decrease: T315I or G250E (35/262 – 12%); T317L (33/262 –7,9%); M244V (21/262 –7,5%); F359V, H396R or Y253H (18/262 –5,6%); E255K (16/262 –5,7%); E255V, L248V or M315T (11/262 –3,9%); E355G (8/262 –2,8%); F359C (7/262 –2,5%); del ex7, Q252H or L387F (5/262 –1,8%); S348L (4/262 –1,4%); Ins 98-72 bp, F317I or E255D (3/262 –1,1%); E275K, E279A, K247R, L387M or V299A (2/262 – 0,7%); E292V, E334G, E450K, E459A, E459K, F359I, F486S, L383F, P441L, Q252M, Q491L, T305I, T345I, Y312C, T520S or G425Stop (1/262 – 0,3%). Double mutationswere associatedwith the variations in the P-loop domain. The median of detecting mutations was 27 months (from 3 to 83 months). Part of the mutations (include T315I) with resistance to nilotinib comprised 40,3%, to dasatinib – 21% (Table). 69% (n=584) patients with CML and resistance to imatinib therapy had not mutations in the *BCR-ABL* kinase domain, among them 46% men (n=268) and 54% women (n=316), median age – 51 (from 24 to 74).
Summary / Conclusion: Present study confirmed essential influence mutations in *BCR-ABL* kinase domain on resistance to imatinib therapy (for 31% patients). But in 2/3 cases resistance to inhibition of the tyrosine kinases associated with no *BCR-ABL* mutations. We also indicated that besides well know point substitutions took place another mutations that lead to essential alteration in protein structure and cause resistance. Patients during imatinib therapy had significant count mutations in *BCR-ABL* domain of leading to resistance to second-line inhibitors of tyrosine kinase. As the result it is necessary to identify *BCR-ABL* mutations for changing therapy to using dasatinib and nilotinib. Similar structure of imatinib and nilotinib explained that common sets of mutations of resistance to nilotinib therapy are prevailed over mutations of resistance for dasatinib. If dasatinib and nilotinib will move to first-line therapy the change of this situation is expected.

B1350

LOWER INCIDENCE OF CML IN O BLOOD GROUP AND SECRETOR INDIVIDUALS

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Background: ABO blood groups have been associated with many bacterial, fungal infections, and malignancies.
Aims: To examine such association and the possibility of explaining it, through studying (591) patients with hematological malignancies (HM); AML, ALL, CML, CLL, HL, NHL, and MM, compared to (196) blood donor controls
Methods: Standard conventional techniques were used for ABO; Rh grouping. Secretor status was determined by Lewis blood grouping and haem-agglutination inhibition test.Informed consents were obtained from both patients and controls according to medical ethics regulation.
Results: ABO & Rh blood groups and secretor status results showed no significant difference from controls, except in CML patients; where blood group A incidence was significantly (*P: 0.0007*) higher (55.0 %) than normal control (32.1%) and than other (HM) (31.3%) (*P: 0.0001*). Blood group O was significantly (*P: 0.0074*) lower in CML (21.7%) than normal (38.3%) and than other (HM) (41.4%) (*P: 0.0015*). No significant difference in the secretor status was found between different (HM) but a significant lower incidence was found in CML (60%) as compared to controls (74.0%)(*P: 0.0187*), and to other (HM) (73.6%)(*P: 0.0127*). Also there was a lower incidence of secretor status in CML patients with O blood group (45.5%) as compared to controls and to other (HM) patients (*P: 0.0197)(P: 0.0198*) respectively.
Summary / Conclusion: A significant lower frequency of O blood group was found in patients with CML. This means blood group O individual are protected from CML. This can be explained at least partially, by the higher frequency

of secretor status in these patients. Evidences, from other studies, have shown that ABO antigens can modulate cellular interactions making them more accessible for glycan-binding proteins through their sialic acid constituents, also have shown an increased EDF binding sites on normal blood group A1 RBCs (which are also present on other hematopoietic cells) as compared to group O and B individuals. Which means more ability to bind EDF and then after, more Tyrosine Kinase activity with it's known implicated role in the pathophysiology of cancer

B1351

BCR-ABL1 GENOMIC MOLECULAR CHARACTERIZATION IN CHILDREN WITH CML: DESCRIPTION AND INTEREST FOR MOLECULAR FOLLOW-UP

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Background: Chronic myeloid leukemia (CML), is a rare disease in children characterized by *BCR-ABL1* fusion. Molecular follow-up is a major factor for evaluation of treatment efficiency. However, BCR-ABL1 transcript fusion quantification is sometimes technically difficult due to RNA extraction and stability. Breakpoints genomic regions, never described in pediatric CML, are classically along a 3 Kb intronic region on *BCR*, and more than 140 kb intronic region in *ABL1*.
Aims: Characterize breakpoints regions in pediatric CML and quantify *BCR-ABL1* genomic fusion to design a molecular follow-up
Methods: 20 patients, between 10 months and 16 years old, are studied. They present CML with M-BCR transcript. After sequencing analysis of M-BCR transcript, genomic fusions are amplified by a long-range multiplex PCR, using 2 forward primers on *BCR* and 25 reverse primers on *ABL1* all along potential breackpoint region. Amplification product is sequenced and a patient specific PCR is designed among genomic fusion. A FISH based method, using 6 contiguous fosmides on *ABL1*, allows limiting the patient potential breackpoint region to about 30 kb. If failing, a LDI-PCR (Long Distance Inverse PCR) technique is used on *BCR* to amplify *BCR-ABL1* genomic fusion.
Results: Genomic fusion has been characterized for 17 patients (85%). Method used was long range multiplex PCR for 14 patients and LDI-PCR for 3 patients. FISH were used for 3 patients. *BCR* breackpoints are located all along M-BCR region. *ABL1* breackpoints are located along the 2 first intronic regions, and for 2 patients, before 5' end of the gene. There is no genomic particularity for breakpoints regions. Only 3 breakpoints on *BCR* and 6 on *ABL1* occurred in repeated sequences.
Summary / Conclusion: 70% of genomic fusions are characterized by multiplex long range PCR. With additional LDI-PCR and FISH techniques, 85% are characterized. These techniques are more difficult to use than transcript quantification by quantitative PCR, but DNA extraction is easier and stability much better. Molecular follow-up, with patient specific genomic quantitative PCR, could be more precise and reproducible. We think that genomic characterization of BCR-ABL1 fusion for CML patients is technically more fastidious than RQ-PCR, but possible in routine procedures, and could improve molecular follow-up for CML patients.

B1352

IDENTIFICATION OF IL-6-174G/C PROMOTER POLYMORPHISM IN CHRONIC MYELOID LEUKEMIA

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Background: Cytokines such as interleukin and interferon are immunomodulating agents that are secreted by active lymphocytes, macrophages, endothelial and epithelial cells in case of inflammation and immune reactions. Interleukin-6 (IL-6) is secreted by the T-cells and macrophages and serves both pro-inflammatory and anti-inflammatory effect in addition to B-cell growth. IL-6 has been shown to be associated with many diseases including hematological malignancies, being a positive growth factor. However, the highest levels of cytokines which are produced by immune cells vary between individuals due to genetic polymorphisms. It has been shown that these genetic polymorphisms influenced the total cytokine expression and release, being an important distinguishing factorfor risk of disease occurrence, course of illness and disease prevention.
Aims: In this study, promoter polymorphism of IL-6 (-174G/C) was identified and investigated in chronic myeloid leukemia (CML) patient group whether it is a risk factor for the occurrence of the disease.
Methods: Twenty five unrelated CML patients were selected for this study. Control blood samples were obtained from 30 unrelated, healthy volunteers. The consent of the local ethics committee was obtained for this study. Genotyping of -174G/C polymorphism was performed by polymerase chain reaction (PCR) and restriction fragment polymorphism analysis (RFLP) using specific primers and NlaIII restriction enzyme, respectively.
Results: Seventeen of 25 (68.0%) CML patients were found as homozygote for G/G genotype. Five of 25 (20.0%) were heterozygote and 3 of 25 (12.0%) were homozygote for C/C genotype. In the control group, 29 of 30 (96.7%) were homozygote for the G/G genotype and only 1 of 30 (3.3%) was heterozygote. Homozygote C/C genotype was not observed. The ratio of the C/C genotype in CML patient group was significantly higher than the control group ($P<0.05$).
Summary / Conclusion: IL-6 -174G/C polymorphism may be a risk factor for the occurrence of chronic myeloid leukemia.

B1353

CREATION OF A RESOURCE GROUP ON "GENOMICS IN THE CLINIC IN LOW RESOURCE SETTINGS": AN EXAMPLE USING A LONGITUDINAL STUDY OF A CML CASE

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Background: Genome sequencing has emerged recently as a technology that can be used to address questions regarding the clonal evolution of cancers such as CML and has potential to be translated into practical applications in a clinical setting. In order to lay the foundation for exploring the possible use of such technologies in a lower resource clinical setting, we have created a consortium involving basic experimental biologists, scientists with expertise in bio-informatics and structural biology along with clinicians. In the first such exercise, we have undertaken a whole exome sequence of a patient with CML, as its clinical outcome varies amongst patients during the progression of disease.
Aims: This study attempts to understand the variability of the clinical outcome to standard treatment from a genomics perspective. Our case study includes a patient who has been responding to Imatinib at 200 mg once a day or sometimes on alternate day because of symptomatic neutropenia for two years with no progression, both clinically and molecularly.
Methods: The exome sequencing of the bone marrow aspirate was performed at the time of diagnosis and after two years of treatment with Imatinib. Matched skin biopsy was used as a control. This study has been approved by the Institutional Ethical Review Board of St. John's Medical college and Hospital.
Results: We present the relevant clinical information and the initial analysis. The novel mutations are being mapped on genes to identify which functional domains could be affected and to provide a structural basis of such mutations. A bioinformatics pipeline has been established to map domain architecture and to perform homology modelling of genes of interest. These polymorphisms would allow us to use such readout as a screen across CML patients to identify cases with better disease prognosis.
Summary / Conclusion: In addition to adding insights on the evolution of CML and providing an opportunity to develop screens for better CML prognosis, this study also offers insights on organizing such studies in India, where genomics is still in the early stages. With the distinct population groups and diverse spectrum of diseases, we believe that our work lays the foundation for larger studies in both CML and other diseases requiring such approaches.

B1354

WHOLE EXOME SEQUENCING ANALYSIS IN CHRONIC MYELOID LEUKEMIA

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Background: Tyrosine kinase inhibitors (TKI) therapy of chronic myeloid leukemia (CML) has shown impressive results last decade. However, around 25% of patients on imatinib have failed to achieve optimal and 10% patients have lost the achieved response in 5 years. The resistance to TKI therapy remains in CML patients on TKI of second generation too. Exome analysis is now-a-days one of the most powerful tool in the search for the new prognostic markers and molecular targets for therapy.
Aims: We launched prospective study aiming to sequence exomes in CML patients to find out possible exomic differences of leukemic cells between

responders and non-responders as well as between leukemic and normal cells in the same patients.
Methods: Whole exome sequencing by NGS technology of 5 primary CML patients who achieved complete cytogenetic response in 6 months of TKI therapy were included in analysis.
Results: To the date there are 5 CML exomes sequenced with 26K variations found in average. About 8K of the found variants are non-synonymous. Preliminary analysis demonstrated that from 756 to 789 of these non-synonymous variants in each patient are not described in dbSNP. The comparison of the revealed non-synonymous variants between patients did not reveal identical SNP.
Summary / Conclusion: Preliminary findings shown that NGS analysis did not revealed identical non-synonymous variants in exomes of leukemic cells in CML patients with optimal response to TKI therapy. These and new data with deep bioinformatics analysis of comparison of leukemic and normal cells exomes will be discussed.

B1355
MESENCHYMAL STEM CELLS FROM CHRONIC MYELOGENOUS LEUKEMIA (CML) PATIENTS, A PROJECT TOWARDS EXPANDING AN AUTOGRAFT FOR TRANSPLANTATION
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Background: Human Mesenchymal stem cells (MSCs) are group of cells able to give rise to different types of stem cells and now that they do exist in bone marrow they can differentiate into hematopoietic stem cells.CML is a monoclonal myeloproliferative disorder characterized by Philadelphia chromosome t (9;22) and a fusion gene BCR-ABL at molecular level.
Aims: The goal of the study is to isolate MSCs from patients diagnosed with CML in chronic phase proven to be Philadelphia chromosome positive by means of Karyotype and fusion gene BCR-ABL positive by FISH, then to prove that MSCs are Philadelphia negative cells which enable us to differentiate them into hematopoietic stem cells in vitro.
Methods: The study was performed at the molecular cytogenetics section of Kenj cytogenetics laboratory in Damascus (SYRIA). Bone marrow samples were taken from 20 newly diagnosed patients with CML still in chronic phase. All samples were Philadelphia positive by karyotype and BCR-ABL fusion gene positive by FISH. Samples were cultured and Mesenchymal stem cells were isolated after proper passages , and were phenotyped by flowcytometer to reveal positive CD105 and negative CD14, CD34 and CD45 which meets the MSCs criteria.
Results: isolated MSCs, showed normal Karyotype, negative BCR-ABL fusion by FISH, and negative BCR-ABL cDNA by rt –PCR, out of 10^6 cells showing that MSCs completely isolated from the leukemic cells. MSCs reserved its osteogenic differentiation potential and we tried to trigger hematopoietic differentiation of those cells , and have got promising results. confirmed by increasing percentage of CD34 positive cells which were in turn BCR-ABL negative by rt-PCR.
Summary / Conclusion: Results showed that the bone marrow is an abundant source of MSCs and the genetic abnormality in CML is taking place in a certain phase post-hematopoeitic stem cell. Also expansion of CD34 population in vitro beginning from a MSC derived from the bone marrow opens a spectrum of ideas such as making an autologous stem cell transplantation in CML patients which seems like a dream nowadays.

B1356
CO-EXPRESSION OF P190 AND P210 TRANSCRIPT IN A PEDIATRIC CHRONIC MYELOID LEUKEMIA PATIENT AT THE TIME OF BLAST CRISIS
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Background: It has been suggested that the co-expression of p190 BCR-ABL transcript (p190) being detected frequently at a low level in adult p210 BCR-ABL transcript (p210) positive chronic myeloid leukemia (CML), but of no pathogenetic significance. On the contrary, the pathogenetic significance of this co-expression remains unclear in pediatric population.
Aims: Here we report a pediatric CML patient, who presented co-expression of the p190 and p210 at the time of blast crisis.
Methods: A fifteen years old boy, who had been diagnosed as CML in Iraq before one years of his referral to our service, had been receiving imatinib treatment for seven months until his first lymphoid blast crisis. Complete hematologic response had been achieved with two course of hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone (Hyper CVAD). He had been transmitted to our service with imatinib treatment after two months from his second course of Hyper CVAD, for the plan of hematopoietic stem cell transplantation. At the time of his admission he had fullnes and weakness complaints and found to have 4 cm palpable spleen below costal margin. Laboratory findings showed an anemia (10 g/dL) and leukocytosis (70x109/L). Bone marrow aspiration revealed diffuse L1 type lymphoblastic infiltration. Flow cytometry was positive for CD10, CD19 and he was diagnosed as second lymphoid blast crisis relapse of CML. Bone marrow karyotype revealed the presence of %50 Philadelphia chromosome in 100 interphase and RT-PCR revealed the presence of both the p210 and p190. He had failed the antileukemic remission induction therapy with ALL-REZ-BFM-2002 treatment protocol while the search was going on for match unreleated donor, because of not having a match releated donor. Bcr-Abl kinase domain mutational analysis was performed and revealed no mutations. He was given imatinib treatment with clofarabine, cyclolphosphamide and etoposide combination chemotherapy, He finished the induction phase of the combination therapy without a major complication. Complete cytogenetic response was achieved. RT-PCR control revealed negativity of the p190 but very weak persistence of the p210. After one month when he became clinically stable, a second course of the combination chemotherapy was given as consolidation. Despite not having Bcr-Abl kinase domain mutation, he was considered as having clinical resistance to imatinib and was given dasatinib treatment with the beginning of consolidation chemotherapy. Bone marrow analysis on the fourteenth day of the consolidation chemotherapy revealed complete response at the cytogenetic and molecular level, neither p210 nor p190 were detected on PCR analysis. He is now clinically stable, on dasatinib treatment and match unreleated donor search is going on.
Results: Although p210 is present in majority of pediatric CML patients, p190 is very rare.It is suggested that p190 may be a secondary event in at least some cases of childhood CML, suggesting an association with imatinib resistance and progression to a blastic crisis in these patients. In conclusion it is worthful of searching p210 and p190 with RT-PCR in Philadelphia chromosome positive pediatric CML patients both at the diagnosis and during the course of therapy, because co-expression of p210 and p190 may be associated with resistance to imatinib and progression to the blastic phase.
Summary / Conclusion:
This case shows that there is need to further molecular hematological researchs and gathering data with interpretation in pediatric CML.

B1357
ASSESMENT OF THE EFFICACY OF HESA-A ON THE PROLIFERATION AND APOPTOSIS OF CHRONIC MYELOGENOUS LEUKEMIA CELL LINE
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Background: Chronic myelogenous leukemia is characterized by philadelphia (ph0 chromosome, the presence of BCR-ABL fugene gene and constitutive activation og the ABL1 tyrosine kinase. Despite an excellent result of target therapy by imatinib, some patients develope resistance to imatinib. HESA-A has produced as an anti-cancer drug by Iranian scientists in order to reduce sid effects of conventional therapy.
Aims: In this study efficacy of HeSA-A on proliferation and apoptosis of K562 cell line was assessed.
Methods: In this study doubling time of K562 cell line was calculated.The cells were affected by various concentrations of HESA-A (1,2,4 and 8 mg/ml respectively). Cytotoxicity and IC50 dose of HESA-A were detected by MTT and trypan blue exclusion assay. Appoptosis was assessed by flowcytometry after 48 h cell treatment in the presence of IC50 dose.
Results: Doubling time of K562 was 24 hours. HESA-A reduced the number of viable K562 cells in a concemtration manner dependent. IC50 dose was 3.5 mg/ml. In flowcytometry analysis of apoptosis, 19.22% of the treated cells were located in the position of the necrotic cells.
Summary / Conclusion: The results of MTT and trypan blue exclusion assay suggest that HESA-A inhibits the growth of K562 cells in a concentration dependent manner and induce necrosis in K562 cells.

Chronic myeloid leukemia - Clinical

B1358

SECONDARY MALIGNANCIES IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS.

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Background: Tyrosine kinase inhibitor (TKIs) in CML has become such a success that it has given patients hope for a large disease free-survival. Preclinical studies with Imatinib in rats showed neoplastic changes in kidney, urinary bladder, urethra, preputial and clitoral gland, small intestine, parathyroid glands, adrenal glands and non glandular stomach. The question about the possibility of late effects if TKI treatment includes the possibility of developing other malignancies arises in patients with long disease free survival.
Aims: We analyzed our CML patient population (in 5 sites of Argentina) to investigate the frequency and characteristics of second malignancies among patients with CML treated with TKIs.
Methods: All patients with CML treated with TKI between November 2001 December 2012 were included in this analysis. All the patients had a medical history and physical examination, complete blood counts and blood chemistry before the beginning of therapy and every month for the first 3 months, then every 3 months until 12 months, from the beginning of the therapy and then every 6 months. Cytogenetic response was assessed in the BM and the molecular response was assessed by real-time PCR. The records of 383 patients with CML in chronic phase (CP) treated with TKI were reviewed. The patients with CML included in CP treated with Imatinib Mesylate (IM) after IFN failure, and patients treated with second generation TKI in front line therapy. Fifty patients (0,03%) developed 15 different second cancers. Of these 15 patients 8 were men. 14 patients received frontline therapy with IM for CML, and one patient with dasatinib. 8 patients received IM after IFN treatment failed. None of the 12 patients had received stem cell transplantation. The types of second cancer during TKI therapy were: gastrointestinal (GI cancer); colon: 4 patients; breast cancer: 1; lung cancer: 3 patients; genitourinary (GU cancer): urinary bladder, kidney , uterine, and penis 6: patients; and Non Hodgkin Lymphoma 1 patient. The most common second cancer were GU cancer representing 0,4 % of all the cancers. The median time from the start of TKI therapy to diagnosis of the initial second cancer was 46,1 months (range 2-22 months) After a median follow up of 21,2 months from diagnosis of a second cancer, 9 patients had died among the dead, 4 had died of CML related cause. The best response to TKI at the time of detection of the second cancer was HCR in all patients, and RCyC in 10 patients. The CMR was observed in 8 patients.
Results: In our analysis, the incidence rate of secondary malignancies inCML patients was 0,03%. The most common second malignancies was GU cancer. A Continuous long term monitoring of these patients, together with a report of the patients who developed a second cancer, is necessary
Summary / Conclusion: In our analysis, the incidence rate of secondary malignancies inCML patients was 0,03%. The most common second malignancies was GU cancer. A Continuous long term monitoring of these patients, together with a report of the patients who developed a second cancer, is necessary

B1359

SUCCESSFUL MANAGEMENT OF CML DURING PREGNANCY AND IN POST-PARTUM PERIOD

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Background: The occurrence of any leukemia during pregnancy is rare, with an estimated incidence of 1:100,000 pregnancies annually. Chronic myeloid leukemia (CML) covers less than 10% of leukemias during pregnancy. The median onset age of CML is in the sixth decade, however about 10-15% of cases occur in women in childbearing age. Possibility of pregnancy is therefore a current topic. The CML therapy during pregnancy is limited by teratogenic side effect risk. Except some case reports Pye at al. (2008) reported relevant cohort of these patients. Data on human fetal development in pregnant women and patient partners withCML are still limited, which led us to report data of our centre.
Aims: To verify a possibility of effective CML treatment during running pregnancy without fetal development hazard together with achievement of optimal response after delivery.
Methods: Between the years 2000-2012 we determined 12 pregnancies in 11 patients with CML and 9 pregnancies in 8 patients´ partners.
Four patients decided for artificial abortion. Remaining 8 pregnancies in 7 patients continued to delivery. Except one patient who recognized the pregnancy in 20th week, all the others were found out in the first trimester. Five pregnancies were confirmed in parallel with CML diagnoses without pretreatment. One patient was pretreated with imatinib (IM) for 18 months, one with interferon (IFN) for 24 months. One patient after successful delivery, who achieved major molecular remission (MMoR) on dasatinib (DA), discontinued treatment because of planning the second pregnancy.
In the first trimester, patients were either untreated or leukodepleted, in the second and third trimester they were treated with lekapheresis and/or interferon, or were without treatment. Seven male patients were treated with imatinib and one with dasatinib median 18 months (range 8-101) before conception. One patient became twice father during tyrosine kinase inhibitors consumption.
Results: All these 8 pregnancies (in 7 women) terminated with birth of healthy infants carried to term (4 girls and 4 boys). Current age of children is 1 month to 12 years. In 5 patients after the delivery IM treatment was initiated, in 4 of them followed with DA, one patient continued on IFN treatment. In all 6 patients complete cytogenetic response was achieved (median 18 months), in 5 subsequently MMoR as well. One patient has not been evaluated yet due to short time of treatment. One patient was treated with dasatinib with lasting MMoR prior pregnancy. At her request to have a baby (second child during CML therapy), the treatment was interrupted before conception. Although she lost MMoR, she did not need any treatment during pregnancy and she gave birth to healthy child. In 3 months after dasatinib return MMoR was restored. Pregnancies of CML patients´ partners were without complication terminated with birth of healthy children, only one child required surgical resolution of umbilical hernia. All women have good quality of life 8 to 172 months from CML diagnosis and 1 to 111 months from delivery.
Summary / Conclusion: All female patients who had decided to keep going their pregnancies gave birth to healthy children and subsequently achieved very good treatment response. The treatment of CML during pregnancy did not jeopardize fetal development and did not negatively influence long lasting results. Also pregnancies of CML patients´ partners seem to be mostly without any complications. However the treatment of pregnant CML patients requires close cooperation between experienced hematologist and obstetrician.

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B1360

BCR-ABL TRANSCRIPT LEVELS AT 3 AND AT 6 MONTHS ARE PREDICTIVE FOR SURVIVAL, TIME TO TRANSFORMATION AND TIME TO SECOND LINE TREATMENT ONSET IN CHRONIC MYELOID LEUKEMIA PATIENTS ON IMATINIB THERAPY

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Background: The assessment of molecular response at 3 and at 6 months of imatinib treatment has been shown to play important role in predicting achievement of deep molecular response and survival of patients with chronic phase CML. The result of early assessment of BCR-ABL transcript level might indicate the group of patients who need more intensive therapy. The influence of clinical factors before introduction of TKI therapy on early molecular response is largely unknown.
Aims: We investigated the influence of some clinical factors (time from diagnosis to TKI, previous IFN alfa therapy, EUTOS score) on early molecular response and the outcome of patients with BCR-ABL transcript levels of ≤ 1%, > 1% to ≤ 10%, and > 10% at 3 and 6 months
Methods: A total of 270 patients (pts) treated with imatinib were investigated. The median age was 52 years (range 22-92, 43% female). Median follow-up was 5.5 years (range 2-12). Transcript levels of BCR-ABL, and ABL were determined by standardized RQ-PCR method from samples taken at 3 and 6 months. Only patients with typical BCR-ABL transcripts (b2a2 and/or b3a2) were included. Disease progression was defined by the incidence of accelerated or blastic phase, or death from any reason. Time to transformation (TFS) was defined by the incidence of accelerated or blastic phase, time to second line therapy onset (TFSA) by the incidence of start of second line treatment due to a failure or suboptimal response to imatinib. Patients were grouped based on BCR-ABL transcript levels of ≤ 1%, > 1% to ≤ 10%, and > 10% at 3 and at 6 months. A Kaplan – Meier analysis was performed for progression free survival (PFS) overall survival (OS), time to transformation (TFA) and time to second line therapy onset (TFSA).
Results: The level of BCR-ABL transcript after 3 months of imatinib therapy was not predicted by EUTOS score. The reduction of BCR-ABL transcript at 3 months below 10% was observed in 74% of patients with onset of imatinib therapy within first 6 months from diagnosis (P=0,005). The presence of addition-

al cytogenetic aberrations at diagnosis diminished the chance for BCR-ABL transcript reduction below 10% at 3 months (P<0,001). The influence of previous IFN alfa therapy on RQ-PCR result at 3 months was not significant. The chance for optimal response (according to ELN recommendations) was significantly reduced in pts with >10% transcript level. The same pattern were observed when the possibility for achievement of stable molecular remission 4 (MR4) and MR4.5 was analyzed. At 6 months the reduction of BCR-ABL transcript to ≤1% was important for the chance for further achievement of stable MR4 (P<0,001). There was no significant differences between other groups. The possibility to achieve a stable MR4.5 was not significantly different in all 3 transcript level groups assessed at 6 months. At 3 months the Kaplan – Meier analysis showed that OS was significantly worse (P=0,04) in patients with transcript >10% (differences between groups levels ≤ 1%, and > 1% to ≤ 10% were not significant). PFS was not significantly different for group with BCR-ABL > 1% to ≤ 10% and >10% and was significantly better for patients with transcript level of ≤ 1% versus > 1% to ≤ 10% (P=0,05) and versus >10% (P=0,01). Patients with transcript level >10% had significantly shorter time to transformation (TFA) (P=0,007%). At 6 months Kaplan – Meier analysis showed the similar results as at 3 months as far as OS and PFS are concerned, TFA was significantly longer for pts with BCR-ABL ≤ 1% versus > 1% to ≤ 10% (P=0,01) and versus >10% (P=0,01) and were not different between groups with level of > 1% to ≤ 10% versus >10%. The time to second line therapy onset (TFSA) was strongly correlated with the BCR-ABL transcript level at 3 and at 6 months and was the longest for patients with reduction to ≤ 1% (differences between all 3 groups were statistically significant).

Summary / Conclusion: Time from diagnosis to introduction of imatinib therapy could influence the reduction dynamics of BCR-ABL transcript level. Neither previous treatment with IFN alfa nor EUTOS score did not have significant influence on the BCR-ABL transcript level assessed at 3 months. The absolute transcript level at 3 months can be used to predict survival, progression free – and transformation free survival as well as the chance for optimal response and time to the onset of second line therapy with TKIs.

B1361

APPLICATION OF THE EUTOS SCORE IN A SERIES OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) IN CHRONIC PHASE (CP) TREATED WITH TYROSIN KINASE INHIBITORS (TKI)

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Background: In patients diagnosed with CML in CP treated with TKI the applicability of the EUTOS score and its superiority compared to the Sokal score are controversial. According to this score, one third of high-risk patients do not achieve complete cytogenetic response (CCyR) at 18 months and this predicts a poor progression-free survival (PFS). On the other hand, BCR-ABL/ABL ratio <10% at three months is associated with a better overall survival (OS) and PFS.

Aims: The objective of this study was to evaluate the prognostic value of the EUTOS score and the molecular response (MR) at three months in a series of 182 patients with CML in CP treated with IM and second generation TKI.

Methods: 182 patients in whom EUTOS score plus CCyR at 18 months and or MR at three months was available were studied. EUTOS score (high, low) was calculated based on splenomegaly (cm) and percentage of basophils in peripheral blood. CCyR at 18 months, OS and PFS according to EUTOS score and OS and PFS according to MR (BCR-ABL/ABL ratio <10% *vs.* >10%) at three months were analysed.

EUTOS	Low (n=151)	High (n=30)	p value
CCyR at 18 months, n (%)	118/125 (94%)	20/25 (80%)	0.03
8 yr. OS (95%CI)	94% (89%-99%)	53% (18%-88%)	0.01
8 yr. PFS (95%CI)	90% (80%-100%)	63% (35%-91%)	0.01
BCR-ABL/ABL ratio < 10% at three months	52/83 (63%)	6/16 (38%)	0.095

Results: 108 (59%) of patients were males. Median (range) age was 53 (20-85) years. Median follow-up (range) was 4.3 years (0.3-11.8). 58 patients received second generation TKI and 8 patients received an allogeneic stem-cell transplantation. EUTOS score: low 151 (83%), high 30 (16%), not evaluable 1 (1%). CCyR at 18 months, OS and PFS were analysed according to EUTOS score (Table). There were statistical significant differences in OS according to MR at three months: 98% (95%CI (93%,100%)) for MR group and 86% (95%CI (75%,97%)) for non MR group (P=0.041).

Summary / Conclusion: In this series of patients with CML in CP treated with ITK, high EUTOS score predicted poor OS and PFS. There was a trend for a better MR at three months in patients with low EUTOS score. These data confirm the applicability and prospective value of EUTOS score in an unselected series of CML patients.

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B1362

USING OF TYROSINE KINASE INHIBITORS IN FEMALES WITH CHRONIC MYELOD LEUKEMIA DURING PREGNANCY AND CONSIDERATIONS FOR TRANSPLACENTAL PENETRARION OF LEUKEMIC CELLS AND DRUGS

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Background: Tyrosine kinase inhibitors (TKI) at pregnancy in females with chronic myeloid leukemia (CML) are very rarely implemented because TKI usage is known as unsafe for fetus due to teratogenic risk. On the other hand imatinib was reported in literature not able to cross placenta. The question of possibility to use TKI after appearing placenta barrier remains opened.

Aims: To provide an information about pregnancy outcomes and newborns characteristics in CML women who had to use TKI therapy during pregnancy, especially in 2nd-3rd trimester.

Results: We inform about **11** cases of using TKI during pregnancy, mostly in 2nd-3rd pregnancy trimester in females with CML in chronic phase. In 9 of 11 cases imatinib in dose 400 mg daily was used. In 2 of 11 cases nilotinib was used in dose 600 and 800 mg daily. The *reasons for TKI usage* during pregnancy were the following: newly diagnosed CML in 3 cases, hematologic relapse in **3** cases (1 on imatinib treatment, therefore switched to nilotinib); cytogenetic relapse in **2** patients (1 of them switched to nilotinib due to previous resistance for imatinib) and high levels of BCR-ABL expression corresponding usually with cytogenetic relapse (7.7 and 10% BCR-ABL IS) in **2** patients. In **1** case imatinib was used for whole pregnancy period by decision of physician and female in a patient with major molecular response before pregnancy. In 7 of 9 cases imatinib was used from 17th-34th week of pregnancy, in 2 of those 9 during whole pregnancy period. Nilotinib was taken from 25th week of pregnancy and from 10th week of pregnancy for mentioned above 2 females. *Pregnancy outcomes:* 2 of 11 pregnancies are ongoing at the moment of publication (28 and 35 gestation weeks), 1 on nilotinib, 1 on imatinib; no abnormalities in fetus development are being reported. 9 of 11 pregnancies resulted in delivery, in 1 case (on nilotinib) delivery was premature at 35th week, in other cases at 37th-40th week. *Characteristics of children:* The infants had no abnormalities at birth and good development during further observation (aged from 2 to 45 month now). The noticed feature was low weight in 5 of 9 newborns (2080-2500 g). The weight growth was rapidly gained during further artificial feeding as the lactation was suppressed. *Transbarrier penetration of leucemic cells and drugs* In 1 case the BCR-ABL expression measurement by cartridge-based GeneXpert system showed BCR-ABL 25% (IS) in female blood and 0% in umbilical blood. In 1 case tandem mass spectrometry showed that imatinib concentration in maternal blood, umbilical blood and breast milk was 813 ng/ml, 105 ng/ml and 647 ng/ml correspondingly.

Summary / Conclusion: In spite of successful pregnancy outcomes the risk of using TKI at pregnancy cannot be precisely predicted due to the fact that a small proportion of TKI probably can penetrate through placenta barrier. In particular cases TKI therapy possibly can be used to control considerable leukemic cell mass for strict indications. Generally pregnancy should be safely planned in CML women who achieved deep remission of disease with no need of TKI therapy at pregnancy.

B1363

VALIDATION OF A PROGNOSTIC SCORING SYSTEM IN PATIENTS TREATED WITH SECOND-GENERATION TYROSINE KINASE INHIBITORS FOR CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE AFTER IMATINIB FAILURE

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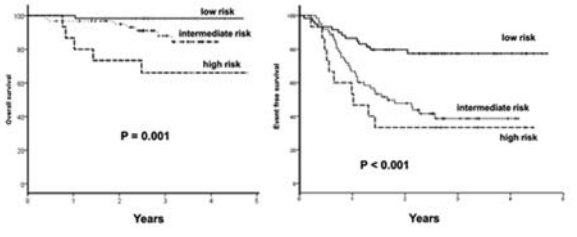
Background: Imatinib was the first tyrosine kinase inhibitor (TKI) to induce high rates of cytogenetic and molecular responses in patients with chronic myeloid leukemia (CML). Second-generation TKIs were introduced to treat patients with failure or intolerance to imatinib. A prognostic scoring system was recently proposed to stratify CML patients after imatinib failure into groups with different outcomes and cytogenetic responses (Jabbour E et al. Blood 2011;117:1822). Validation of this scoring system in an independent cohort was needed.
Aims: To validate the scoring system in an independent sample of CML patients treated with second-generation TKIs after imatinib failure.
Methods: 136 patients with chronic phase CML who switched to a second-generation TKI because of failure (N=134) or intolerance (N=2) to imatinib were included. All patients were treated at Hemorio (Rio de Janeiro, Brazil). The second generation TKI was nilotinib in 53% and dasatinib in 47% of the patients. Chronic phase CML was defined as blasts < 15%, blasts and promyelocytes < 30%, basophils <20%, and platelet count between 10^5 and 10^6/mm^3. Imatinib failure was defined according to European LeukemiaNet criteria. The prognostic scoring system was calculated by adding 1 point if performance status > zero and 1 point if there was a failure to achieve at least a minor cytogenetic response (Ph1 < 65%) during treatment with imatinib. Patients with score zero were defined as low risk, score 1 as intermediate risk and score 2 as high risk. Survival curves were determined from the start of the second-generation treatment, and were compared by the log-rank test. The probabilities of major cytogenetic response (MCyR) according to the risk groups were compared by the chi-square test.



Overall survival and event free survival according to the prognostic score system

Results: The median age at CML diagnosis was 45y (4-79), and 56% were male. Complete hematologic response was obtained in 91%. The best cytogenetic response during imatinib was: complete in 23.5%, major in 13%, minor in 9%, minimal in 23.5% and no response in 31%. The performance status at the start of the 2nd-generation TKI was zero in 87% of the patients. The prognostic scoring system was low risk in 59 patients (43%), intermediate risk in 62 patients (46%) and high risk in 15 patients (11%). Median follow-up was 33 months. Event-free survival (EFS) at 2 years was 75% in low risk patients, 45% in intermediate risk and 33% in high risk patients (P<0.001). Overall survival (OS) at 2 years was 98% in low risk patients, 95% in intermediate risk and 73% in high risk patients (P=0.001). The probability of attaining a MCyR at 12 months was 84% in low risk patients, 52% in intermediate risk and 40% in high risk patients (P<0.001).
Summary / Conclusion: The scoring system predicted the probability of OS, EFS and MCyR after a treatment switch from imatinib to second-generation TKIs. The information needed to generate the score was available in all patients, and the score was easy to calculate. Treatment outcomes were quite similar to thoseobserved in the original publication, indicating that treatment results are reproducible in the context of a public institution caring for underprivileged patients in a developing country. This scoring system is useful to inform patients about their prognosis and treatment choices, and can also identify a subgroup of patients in whom new treatment alternatives are needed.

B1364

CARTRIDGE-BASED AUTOMATED METHOD FOR BCR-ABL QUANTIFICATION IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH COMPLETE MOLECULAR RESPONSE ON TYROSINE KINASE INHIBITORS THERAPY

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Background: According to the current European LeukemiaNet recommendations molecular monitoring of chronic myeloid leukemia (CML) therapy has to be performed by quantitative RT-PCR with results expressed in % on international scale (%IS). Automated cartridge-based GeneXpert Dx System for detection of *BCR-ABL* transcript level was recently developed. High sensitivity and reproducibility of GeneXpert system was shown but there is lack of findings concerning the results concordance obtained by routine and automated methods
Aims: To compare the results of *BCR-ABL* gene expression analysis obtained by GeneXpert and manual standardized quantitativeRT-PCR in CML patients with complete molecular response (CMR).
Methods: 37 CML patients on tyrosine kinase inhibitors therapy with CMR were included in this study. Standardized quantitative RT-PCR and "Xpert BCR-ABL Monitor" assay aligned with the WHO reference standard allowed to express results in %IS were used.
Results: CMR$^{4.0\text{-}6.0}$(0.0001-0.01 %IS) was detected in 8/37 cases and 14/37 patients were negative by both methods. Concordance rate in those 22/37 patients was 59.5% (Pearson correlation r=0.6012, P=0.00008). Discrepant results were mainly RT-PCR-negative, but GeneXpert-positive: GeneXpert system revealed 8 extra cases negative by manual quantitative RT-PCR with appropriate sensitivity level. Median value of *BCR-ABL* in this discordant group was 0.004 %IS (range 0.00088-0.0074 %IS). It indicates that GeneXpert results were more sensitive than manual ones. 4/37 results showed to be within CMR^4 by standard RT-PCR and within major molecular response (MMR) by GeneXpert but the obtained *BCR-ABL* levels were expressed in very close values (0.005-0.009 %IS and 0.014-0.017 %IS, respectively). In 3/37 cases GeneXpert demonstrated less sensitive results than ones obtained by conventional method: negative results by GeneXpert were CMR$^{4.0\text{-}4.5}$ by quantitative RT-PCR. Probably it could berelated to samples' quality due to nonobservance of temperature conditions during transportation of blood samples.
Summary / Conclusion: The obtained results have demonstrated appropriate concordance rate of GeneXpert Dx System and conventional quantitative RT-PCR for *BCR-ABL* transcript quantification. GeneXpert allows detecting fusion gene transcript in higher percentage of cases. Cartridge-based PCR is a powerful and sensitive instrument to characterize the very low level of minimal residual disease in CML patients.

B1365

SKIN ADVERSE EFFECTS OF TYROSINE KINASE INHIBITORS IN THE THERAPY OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA – SINGLE CENTRE EXPERIENCE

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Background: Therapy with tyrosine kinase inhibitors (TKI) has significantly improved prognosis of patients with chronic myeloid leukemia (CML). Along its high efficacy, significant adverse events (AE) can occur during the therapy with TKI, among them skin AE are ones of the most frequent. Pathogenesis of skin AE remains to be elucidated and also the information about their incidence and severity in every-day clinical practice is insufficient.
Aims: To evaluate real prevalence of skin AE of TKI therapy in patients with CML treated in the large hemato-oncological centre and to provide detailed dermatologic investigation including skin biopsy.
Methods: Based on a prospective surveillance of skin AE prevalence in our institution, when 178 patients with CML treated with TKI were seen at the outpatient clinic during 3-months interval, real prevalence of skin AE of ≥ grade 2 according to CTCAEv0.4 was 3/128 (2%), 0/27 (0%), and 5/23 (22%) for patients treated with imatinib, dasatinib, and nilotinib, respectively. Thus, in further analysis we focused on skin effects of nilotinib. Except of detailed investigation by dermatologist including the photo-documentation, the patients with active skin manifestations underwent a skin biopsy with histological, histochemical and imunohistochemical investigation.
Results: In total, 47 patients with CML were treated with nilotinib in our centre in a period of 11/2006 – 2/2013; 24 females and 23 males. Median age at the time of nilotinib administration start was 58 years (range, 27-74). Forty three patients were diagnosed in chronic phase, 4 patients in accelerated phase. Except of 12 patients treated with nilotinib as the first-line therapy, other 35 patients were given nilotinib because of previous therapy resistance or intolerance. Six patients experienced skin AE during previous TKI therapy. Skin AE of nilotinib occurred in 13 (28%) patients in a median of 14 days (range, 1-40)

since the start of treatment. The nilotinib dose was 2 x 300 mg/day in 6 patients, 2 x 400 mg/day in 6 patients and 1 x 300 mg/day in 1 patient, respectively. Most frequent type of skin changes was exanthema maculopapulosum in follicular distribution (N=12), in 1 patients painful skin indurations were found. Regarding the severity of skin AE, grade 1 occurred in 8 patients, grade 2 in 2 patients and grade 3 in 3 patients, respectively. Nilotinib was interrupted in 4 patients for a median duration of 6 days. Complete resolution of skin findings was present in 9 patients after a median duration of 88 days (range, 4-269). Four patients experienced skin AE recurrence in a median of 12 days after the first episode resolution and the third episode occurred in 3 patients. No patient discontinued nilotinib therapy because of skin AE. Skin biopsy was performed in 5 patients and in all cases nonspecific folliculitis was found.

Summary / Conclusion: In every day clinical practice, we showed similar frequency of nilotinib skin AE as was reported in clinical trials. In most cases, skin findings were of mild to moderate intensity, easily manageable by nilotinib interruption or dose reduction. Histological investigation revealed unspecific findings of folliculitis. Thanks to nilotinib approval as a first-line therapy for newly diagnosed CML patients, the study will be extended to a larger group of patients with the aim of more detailed imunohistochemical, perhaps even proteomic analysis of skin biopsy specimens.

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B1366

CONTRIBUTION OF THE EPIDEMIOLOGICAL AND CLINICAL REGISTRIES TO XXI CENTURY MEDICINE. RESULTS FROM THE ANDALUSIAN REGISTRY OF CHRONIC MYELOID LEUKEMIA (RALMC)

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Background: *Hospital or clinical registry* is defined as a DATASET regarding all cases of a particular disease or other relevant health conditions, restricted to the scope of one or more hospitals or clinical care system within a defined population. Andalusia is a big region in the south of Spain with over than 8.5 million habitants in 2012, where the Andalusian Registry of CML (RALMC) was created in 2005 within the Andalusian CML working group. In 2012, the RALMC was remodeled with a new version of the website www.registroandaluzlmc.es which contains an updated CRF according to the current guidelines for CML treatment and monitoring from the ELN group published in 2009.

Aims: 1) To know about the CML burden in Andalusia: incidence, prevalence, mortality, overall survival, event-free survival and disease progression survival by age and sex. 2) To describe the characteristics of epidemiological, clinical, diagnostic and treatment of CML patients in the RALMC.

Methods: Autonomous, multicentric, epidemiological and observational registry for the study of more than 14 years old patients diagnosed of CML Ph+ in Andalusia, prior signed of the mandatory informed consent. The study complies with the Declaration of Helsinki and was approved by the Andalusian Ethics Committee. The BCR-ABL results are expressed in international scale. The patient visits are completed every 3 or 6 months according to each internal protocol. Patients are cataloged and monitored according to ELN guidelines, 2009. In February 2012, a cross-descriptive study was performed to analyze the current status of 165 patients treated with Imatinib in front line in terms of optimal response, suboptimal response, failure and intolerance to Imatinib.

Results: 467 patients from 35 hospitals. By the end of 2013 the registry is expected to collect data from 650 patients. Median age is 54 (19-86), gender distribution: 54% male and 46% female, distribution by Sokal groups: low-risk 43%, intermediate-risk 37% and high-risk 20%. Only 10cases were diagnosed in AP. 2GTKIs were used as first-line therapy in 30 patients outside of clinical trials. We performed a subanalysis on data from 165 patients classified following the 2009 ELN guidelines.

165 patients were treated in first line on Imatinib 400mg QD: 121 (73.4%) had optimal response [54 (44.6%) $MR^{5.0}$; 35 (29%) $MR^{4.5}$; 16 (13.2%) $MR^{4.0}$; 12 (10%) MMR; 3 (2.4%) CCyR; 1 (0.8%) CHR]. 19 (11.6%) had suboptimal response; 10 (6%) were failures and 15 (9%) intolerants.

43 patients were on 2nd line therapies: 27 on Dasatinib 100mg QD: 18 (66.7%) had optimal response (7 $MR^{5.0}$; 7 $MR^{4.5}$; 1 $MR^{4.0}$; 3 MMR); 2 had suboptimal response, 4 were failures and 3 intolerants. 16 on Nilotinib 400mg BID: 11 (68.7%) had optimal response (6 $MR^{5.0}$; 2 $MR^{4.5}$; 3 MMR); 2 had suboptimal response and 3 were intolerants.

On third-line, 2 patients were on Dasatinib ($MR^{4.5}$) and 3 on Nilotinib ($MR^{4.0}$).

Summary / Conclusion: The usefulness of the registries has been widely demonstrated in different matters such as health planning, analysis of the technology used, evaluation of the quality of health services in clinical research as well as in epidemiological studies and strong arguments to request its implementation. Clinical registries show a real overview of CML patients. They allow to be aware of the real incidence and prevalence of the disease in a determined area, without for example underestimating the true average age at diagnosis and controlling elderly patients which are excluded from clinical trials.

CURRENT STATUS OF 165 Ph+ CML PATIENTS OF THE RALMC ACCORDING 2009-ELN GUIDELINES			
IMATINB 400 QD ON FIRST LINE THERAPY: n = 165			
OPTIMAL RESP: 121 (73.4%)		54 MR5.0 (44.6%)	35 MR4.5 (29%)
		16 MR4.0 (13.2%)	12 MMR (10%)
		3 CCyR (2.4%)	1 CHR (0.8%)
SUBOPTIMAL RESPONSE: 19 (11.6%)			
FAILURES: 10 (6%)			
INTOLERANT: 15 (9%)			
SECOND LINE THERAPY: n = 43			
DASATINIB 100 QD: n = 27		NILOTINIB 400 BID: n = 16	
OPTIMAL RESP: 18 (66.7%)	7MR5.0(38.9%)	OPTIMAL RESP: 11 (68.7%)	6 MR5.0 (54.5%)
	7MR4.5(38.9%)		2 MR4.5 (18.2%)
	1 MR4.0(5.5%)		3 MMR (27.3%)
	3 MMR(16.7%)		
SUBOPTIMAL RESP: 2 (7.4%)		SUBOPTIMAL RESP: 2 (12.5%)	
FAILURES: 4 (14.8%)		FAILURES: -	
INTOLERANT: 3 (11.1%)		INTOLERANT: 3 (18.8%)	
THIRD LINE THERAPY: n = 5			
DASATINIB 100 QD: n = 2		NILOTINIB 400 BID: n = 3	
2 MR4.5 (100%)		3 MR4.0 (100%)	

B1367

COST-EFFECTIVENESS ANALYSIS IN NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA (CML) IN JAPAN

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Background: The new tyrosine kinase inhibitors (TKIs), nilotinib and dasatinib, have been approved to treat newly diagnosed chronic phase (CP) chronic myeloid leukemia (CML) in Japan since 2010. In addition to efficacy and safety, comparative cost –effectiveness may be considered when selecting medication.

Aims: This experimental analysis aimed to evaluate the cost-effectiveness of nilotinib versus dasatinb as a frontline therapy in newly diagnosed CML in Japan.

Methods: This cost-effectiveness analysis was performed from the perspective of the healthcare payer, and considered only direct Japanese drug costs as of 2013. The daily dose of each drug was based on the indicated dose in the package insert. A Markov model was used to estimate the risk of disease progression to accelerated phase (AP)/blast phase (BP) or death for newly diagnosed CML patients taking either dasatinib or nilotinib. Since there are no published head- to- head comparative clinical studies between the two drugs, rates of progression for the model were acquired from a published indirect comparison (Signorovithch et al.2011). The cost per life-year gained (LYG) was derived from the Markov model and incremental cost-effectiveness ratios (ICERs) were calculated for 1 year, 10 years and 30 years.

Results: The model predicted the incremental effectiveness of the nilotinib treatment arm at 1 year to be 0.022 LYG with an incremental cost of -11,439.21€ and at 10 years, 1.0997 LYG with an incremental cost of -62,663.95€. For patients with newly diagnosed CML, the model predicted that treatment with nilotinib was more effective and less costly than treatment with dasatinib at the point of 1 year and 10 years. At the point of 30 years, treatment with nilotinib extended 7.504 years of life compared with that of dasatinib , and the incremental cost was 57,773.75€, resulting in an ICER of7,698.81€per LYG for nilotinib.

Summary / Conclusion: Based on this experimental analysis, treatment of newly diagnosed CML patients with nilotinib is a cost-effective treatment option relative to dasatinib in Japan. Although limitations of this analysis include a lack of direct comparative data and considers only direct drug costs, this analysis suggests further investigation of the relative cost-effectiveness of these two treatment options is warranted.(exchange rate; 1Euro =124JPY)

B1368

PREGNANCIES IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH TYSOSINE KINASE INHIBITORS

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Background: Tyrosine kinase inhibitors (TKI) have completely revolutionized the management of chronic myeloid leukemia (CML), significantly improving the long term prognosis of the disease. The efficacy of these drugs combined with their ease of administration and a low level of toxicity has resulted in many patients leading relatively normal lives. For many patients, one of the clearest indications of normal life is the ability to conceive children and raise a family. We used to conduct a retrospective study to review the newly diagnosed CML patients administered in Shanghai from 2001 to 2006. A total of 615 cases entered study. High incidence was observed in the child-bearing age (20-50 years old), which accounted for 48.2%. The management of CML during pregnancy remains a great challenge for both physicians and patients.
Aims: Little is known about the effect of exposure to TKI during conception and pregnancy in human. In this report, we presented our experience accumulated to date in CML patients who conceived children and/or became pregnant while receiving TKI.
Methods: The records of all patients with CML treated with TKI were reviewed from Jan. 2003 to Sep. 2012 in our hospital. Data associated with pregnancy, delivery and neonate were collected retrospectively.
Results: We reported the experience on 22 pregnancies involving 22 patients (7 males and 15 females) who conceived while receiving TKI(21 imatinib and 1 nilotinib) for the treatment of CML. (1) Among male patients (6 imatinib and 1 nilotinib), 7 pregnancies resulted in the birth of 7 healthy babies. None of the patients interrupted therapy with TKI after the conception. Afterwards, 55 male patients of child-bearing age with imatinib therapy were tested with gonadal hormone level and were within the normal range. No significant relationship was confirmed between the hormone levels and imatinib treatment duration.5 patients who wished to have a baby in the near future agreed to do the semen examination. 2 were found oligospermia. (2) Among 8 female patients who diagnosed CML during pregnancy, 2 had spontaneous abortion; 5 had elective abortion; 1 continued pregnancy unevently and gave birth to a normal infant and started imatinib treatment after delivery. Among 7 female patients who became pregnant while they were receiving imatinib, 2 ended in elective abortion; 2 carried on pregnancy without imatinib interruption, following one intrauterine growth restriction and one spontaneous abortion; 3 other pregnancies carried to term with imatinib discontinuation, resulting in the birth of 4 healthy babies. (3) The median age of all 12 children at the date of this report is 23 months (range, 2 to 88 months) and they all have a normal growth and development. All patients remain in TKI therapy and in good response.
Summary / Conclusion: Patients who receive TKI at the time of conception or are treated with TKI during pregnancy can have normal pregnancies. There still remains an increased risk of spontaneous abortion or serious fetal malformaitons. Female patients are advised to practice adequate contraception and avoid breast-feeding while on therapy with TKI. In case of planned or unplanned pregnancy, risk/benefit evaluation must be carried out individually. No special precautions apply for male patients receiving TKI.

B1369

EARLY RESPONSE RATES TO THERAPY IN CHRONIC PHASE CML ARE HIGHER WITH NILOTINIB COMPARED WITH IMATINIB FRONTLINE THERAPY AND ARE PREDICTED BY THE EUTOS SCORE

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Background: Since the introduction of imatinib, a BCR-ABL tyrosine kinase inhibitor (TKI) a decade ago, a dramatic change in treatment of CP CML has occurred with great improvement of patients' outcome. The second generation TKI, nilotinib was designed to overcome imatinib resistance and is more potent and selective than imatinib. In the frontline therapy setting, nilotinib results in a more rapid achievement of CCyR and MMR compared with imatinib. Recently, early responses at the 3 months landmark were shown to predict long term outcomes including overall survival.
Aims: In this analysis we aim to compare the efficacy of imatinib versus nilotinib as first line treatment for CP CML in achieving an early 3 months response. We also studied whether the clinical prognostic scoring systems including Hasford, Sokal, and the Eutos score are predictive for the early response.
Methods: Clinical data was collected on all CP CML patients with frontline therapy imatinib or nilotinib. Prognostic scores were calculated based on presentation data. Results of RQ-PCR according to the IS, karyotype and FISH analysis in the first 18 months of therapy were extracted. An optimal 3 months response was defined as and RQ-PCR< 10% on the IS, >1 log reduction from baseline value in the absence of an IS result or FISH <10% in the absence of RQ result at this time point. Results of the 3 months response are presented.
Results: 77 CP CML patients with available response data at 3 months (64-RQ-PCR, 13-FISH), median age of 48 years (range 20-89), F-23 M-54 are included in this analysis; 56 (73%) treated with first line imatinib and 21 (27%) with first line nilotinib. The distribution of patients according to clinical prognostic scores was 61%, 30% and 9% for Sokal low, intermediate and high risk; 67%, 30% and 3% for Hasford low, intermediate and high; 91% and 9% for EUTOS low and high, respectively. An optimal 3 months response was achieved in 73% in the study cohort, 69% in imatinib and 90% in nilotinib treated patients, P=0.07. Optimal response rates at 3 months were significantly higher in EUTOS low (77%) compared to high risk (29%) patients in the entire cohort, P=0.01. Neither Sokal nor Hasford scores predicted responses at 3 months. Responses in subgroups according to TKI were not significantly different in EUTOS low compared to high risk score, probably due to low pt numbers in each subgroup.
Summary / Conclusion: Optimal response rates at 3 month were higher in patients treated with second generation TKI, nilotinib as reported in the literature. Response to treatment at 3 months was predicted by the EUTOS score, but not by Hasford or Sokal scores. This is in accordance with previous reports showing that predictive value of the EUTOS for TKI treated pts, but we show here for the first time its relevance to predict response as early as the first 3 months' time point. The EUTOS score may assist in clinical decision at diagnosis on which TKI to choose for newly diagnosed CP CML patients. Longer follow up of this pts cohort will be presented.

B1370

EXPRESSION OF DRUG TRANSPORTER GENES IN CHRONIC MYELOID LEUKEMIA WITH IMATINIB RESISTANCE TREATED BY SECOND GENERATION TYROSINE KINASE INHIBITORS

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Background: Second generation tyrosin kinase inhibitors (2nd TKIs) have demonstrated that about half of patients failing to respond to imatinib can be rescued.
Aims: This study investigated the treatment outcome of dasatinib or nilotinib in patients showing imatinib resistance. To elucidate the mechanism of their action, the expression of three drug transporters before and after imatinib treatment was evaluated.
Methods: Twenty adult patients with chronic myeloid leukemia (CML) (aged 34-72 years) who showed resistance to imatinib as a first line therapy were enrolled. After detecting imatinib resistance, twelve and eight patients received dasatinib and nilotinib as a salvage treatment, respectively. Bone marrow (BM) samples were obtained twice, before administering imatinib and at the time of detecting imatinib resistance and were analyzed for the mRNA expression of OCT-1, ABCG2 and ABCB1 genes.
Results: Using pair-wise analyses, OCT-1 expression was significantly reduced in the samples at the time of imatinib resistance compared with imatinib-naïve samples (P<0.001). In addition, ABCG2 expression also significantly decreased (P<0.001). ABCB1 expression was not different before and after imatinib (P=0.22). However, ABCB1 expression was significantly higher in patients with primary resistance than in those with secondary resistance to imatinib treatment (P=0.02). mRNA expression of OCT-1 was lower in secondary resistance than in primary resistance group, however, there was no statistical significance (P=0.06). With salvage therapy with 2nd TKIs, 65% and 40% of the patients with imatinib resistance could achieve complete cytogenetic response (CCyR) and major molecular response (MMR), respectively. Achieving CCyR (P<0.001) and MMR (P=0.07) were also associated with higher progression-free survival (PFS). In multivariate analysis, achieving CCyR was the most powerful prognostic factor in PFS. In 8 nilotinib-treated patients, ABCG2 expression after imatinib was lower in patients achieving MMR than in those without MMR (P=0.05). Patients with lower OCT1 expression after imatinib showed shorter PFS compared to those with higher OCT1 expression, however, there was no statistical significance (47.0 months vs. N.A., P=0.46).
Summary / Conclusion: In this study, dasatinib and nilotinib as a salvage treatment could achieve appropriate optimal responses in some patients failing to imatinib treatment. OCT-1 and ABCG2 mRNA expressions showed significant changes after imatinib treatment. However, the expression levels of OCT-1, ABCG2 and ABCB1 at the time of salvage treatment may not affect the treatment outcome of dasatinib or nilotinib.

B1371

THROMBOPOIETINE RECEPTOR AGONISTS ARE EFFECTIVE TO MANAGE THROMBOCYTOPENIA ON TKI-TREATMENT

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Background: The adoption of tyrosine kinase inhibitors (TKI) in chronic myeloid leukemia (CML) gave a possibility of long-term disease control. Continuous TKI treatment could bring various adverse events. Hematologic toxicity can occur in considerable part of patients and cause TKI therapy interruption. The use of erythropoiesis stimulating agents and granulocyte growth factor (G-CSF)

for the management of TKI-related cytopenia is considered as a treatment option for such patients. Management of immune thrombocytopenic purpura has been changed dramatically with introduction of thrombopoietin receptor agonists (TRA), which were highly effective. It is possible that administration of TRA will demonstrate treatment effects with respect to increasing platelet levels in CML patients.
Aims: The objective of our study was to assess TRA potential in the management of TKI-related thrombocytopenia.
Methods: TRA – Romiplostim (Rom) was administered as a subcutaneous injection weekly at dose of 1 mcg/kg in CML patients with severe thrombocytopenia which could lead to TKI interruption.
Results: Three adult CML patients on TKI treatment were prospectively treated with TRA. Patient#1, male, 52-years. CML chronic phase, first-line treatment with Imatinib 400 mg/day. There was no cytogenetic response with 49.249% of BCR-ABL in 6 months. Patient was switched to Nilotinib 800 mg/day, treatment was complicated with recurring neutropenia grade III and thrombocytopenia grade III-IV. Hematological toxicity led to temporary interruption and dose reduction of Nilotinib to 400 mg/day. Cytogenetic response was not achieved in 3 months of TKI therapy, but BCR-ABL level was decreased to 14.4%. Minor cytogenetic response (Ph+ 55%) was achieved in 6 months. Unfortunately, thrombocytopenia and neutropenia were not ameliorated. We have used G-CSF 5 mcg/kg and Rom 1 mcg/kg to manage hematologic toxicity. Two weekly administrations of G-CSF and TRA resulted in neutrophil recovery and rising of platelet level up to $61x10^9$/l. Nilotinib administration was continued with 400 mg daily dose without subsequent interruptions. At 11 months of Nilotinib therapy neutropenia and thrombocytopenia were only grade I and complete cytogenetic response was reached. Patient#2, male, 49-years: CML chronic phase with Nilotinib 600 mg/day as first-line. There was no cytogenetic response with 16.1% BCR-ABL level at 6 months. Nilotinib dosage was increased up to 800 mg/day. BCR-ABL level was 7.6% in 9 months, but recurrent neutropenia grade IV and thrombocytopenia grade IV put obstacles in the treatment. G-CSF 5mcg/kg and Rom 1 mcg/kg were used to correct toxicity. Complete neutrophil restoration and rising platelets up to $66x10^9$/l were achieved in a week after the injections. Patient#3, male, 64-years: CML chronic phase with Nilotinib 300 mg BID as first-line. There were no cytogenetic response and BCR-ABL level was 11.7% at 3 months of therapy. Nilotinib dosage was increased up to 800 mg/day. Recurrent neutropenia grade III-IV led to temporarily interruption of Nilotinib that needed G-CSF use. There was no cytogenetic response with 9.7% BCR-ABL level in 6 months of therapy. Patient was switched to Dasatinib 100 mg/day. Dasatinib-related adverse events were also hematologic, neutropenia grade III and thrombocytopenia grade III. To correct this toxicity G-CSF 5mcg/kg and Rom 1mcg/kg were used. Neutrophils normalized and platelet level increased up to $87x10^9$/l in a week.
Summary / Conclusion: These cases are proof of TRA role in management of thrombocytopenia in CML patients on TKI treatment.

B1372
SCREENING OF ABL KINASE DOMAIN MUTATIONS USING NEXT GENERATION SEQUENCING IN CHRONIC MYELOID LEUKAEMIA: IMPACT IN THE RESPONSE TO TREATMENT WITH TYROSINE KINASE INHIBITORS
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Background: Mutations in ABL kinase domain (KD) are associated with the resistance to tyrosine kinase inhibitors (TKIs) in CML patients. Sanger sequencing is the gold standard method for KD mutation detection. However, Next Generation Sequencing (NGS) approach presents a significantly better sensitivity (1%) when compared to conventional Sanger sequencing (c.a. 20%).
Aims: This study aimed to test NGS as a high sensitive method for screening ABL KD mutations in CML patients and to correlate them with TKI response.
Methods: The present study included 62 different CML patients followed quarterly at the Hematology Service of CHUC (Coimbra, Portugal). A total of 494 peripheral blood samples collected in Paxgene RNA blood tubes were analysed by 4 distinct overlapping HRM PCR reactions, using specific primers covering the entire ABL KD region. In a similar approach, four fragments where analysed using Roche 454 GS Junior NGS system.
Results: From routine nested-PCR and RT-qPCR BCR-ABL analysis, 13.3% of the samples were suggested from HRM profiles for sequencing. Using NGS, we confirmed known mutations in 20% of these samples in an frequency as low as 1.1%. Additionally, one patient presented a 72 bp deletion (nt 1450-1521; GenBank M14752.1) in3,58% of the analysed fusion transcripts.
Summary / Conclusion: Sequencing is an expensive and a time-consuming procedure to test for ABL KD mutations. Nevertheless, combining routinely HRM PCR strategy with NGS allows a higher sensitive method that should be considered for screening of ABL KD mutations in CML patients.

B1373
NEUROMUSCULAR TOXICITY OF TYROSINE KINASE INHIBITORS IN THE THERAPY OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA
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Background: Prognosis of patients with chronic myeloid leukemia (CML) has been fundamentally improved with highly effective treatment with tyrosine kinase inhibitors (TKI). There are, however, adverse events (AE) occuring during treatment with TKI, which can modify expected excellent quality of life of patients with CML. One of the most frequent AE is neuromuscular toxicity (muscular cramps, weakness or pain) with its unclear pathogenesis mostly after treatment with imatinib. Although muscular symptoms occur usually in lower or moderate grade, they disturb patients in every day life.
Aims: To find out prevalence of neuromuscular AE in patients with CML treated with TKI in one large centre, to reduce therapeutically symptoms and to provide detailed analysis including muscle biopsy.
Methods: Our pilot project consists of two parts – analysis of patients already on TKI therapy who developed muscular AE and prospective follow up study for newly diagnosed patients, who are examined by taking family history (myopathy in parents), history of medication (statins), symptoms (muscle cramps, myalgia) and tested for blood levels of creatinphosphokinase (CK), myoglobin (Mb), Ca, ionis. Ca (Ca i), Mg, P, TSH, fT4, parathormone, 24-h urinary ion excretions (Ca, P, Mg) and myodynamic test (knee-bend, stand up test) before treatment and after start of TKI in 1 month, 3 months and then each 3 months. By elevation of CK grade II (according to CTCAE v4.0) is indicated electromyography (EMG) or then muscular biopsy for analysis on molecular basis.
Results: There have been so far 20 patients with CML in chronic phase (CP) (10 females, 10 males) included in our pilot prospective study in a period of 3/2012 – 2/2013. Median age at the time of TKI administration start was 52 years (range, 26-92). As a first line treatment 13 patients received imatinib (ima) and 7 nilotinib (nilo). Muscular AE occurred in 3 (23%) patients treated by ima within the first 3 months of therapy, each one of three patients expierenced different symptom - muscular pain, weakness, cramps - all in grade I-II. CK was elevated in 3 (23%) patients treated by ima, all of them in grade I. None of the patients treated by nilo developed muscular AE nor elevation of CK within 3 months of follow up. TKI treatment also leads to significant hypophosphatemia, which seems to be more severe after therapy of imatinib.
Summary / Conclusion: Our preliminary results show trends of higher elevation of CK in patients treated with imatinib raising in time, significant hypophosphatemia in both groups patients with imatinib and nilotinib. It is also suggesting higher prevalence of muscular AE in patients with imatinib with mostly low and moderate grade. As the study is extending in number of patients and time check ups we are ready to perform more detailed analysis including EMG and muscular biopsy.

B1374
COMPARISON OF BCR-ABL1 GENE EXPRESSION ASSESSED BY GENEXPERT V1 AND V1-IMPROVED CARTRIDGES VERSUS ELN STANDARDIZED RQ-PCR METHOD IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH TKIS
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Background: The GeneXpertSystem was introduced to facilitate the molecular monitoring of therapy with tyrosine kinase inhibitors (TKI) of patients suffering from chronic myeloid leukemia (CML).
Aims: The aim of the study was to compare the expression of *BCR-ABL1* gene obtained by novel V1-improved PCR GeneXpert System cartridges and V1 cartridges with and without IS-correction-tool *versus* internationally standardized RQ-PCR method.
Methods: The comparison of the *BCR-ABL1* gene expression using GeneXpert System was performed in Molecular Diagnostics Laboratory in Krakow – national polish reference EUTOS laboratory for *BCR-ABL1* gene expression assessment. Patients with chronic phase chronic myeloid leukemia expressing e13a2 or e14a2 *BCR-ABL1* transcripts and treated with tyrosine kinase inhibitors were subdivided into three groups according to *BCR-ABL1* transcript level estimated by standardized RQ-PCR method. Group 1 consisted of patients with *BCR-ABL1/ABL1* ratio below 0,1% [IS], group 2 with *BCR-ABL1/ABL1* ratio between 0,1% and1,0% [IS], and group 3 with *BCR-ABL1/ABL1* ratio between1,0% and 10,0% [IS]. Samples obtained from 48 patients were analysed by V1 improved GeneXpert cartridge and results were compared with the results of RQ-PCR TaqMan methodology aligned to International Standard (IS).
Results: The comparable results were observed in (27/48) 56,2% of samples. The Wilcoxon test did not reveal statistically significant differences between the

results obtained by both techniques and (P<0,2871). The comparable results of both techniques in group1, 2 and 3 were noted in 42%, 68% and 100% respectively. The differences were not statistically significant in each evaluated group. Samples from 35 patients were analyzed by V1 GeneXpert System (Lot #05001) corrected with IS-correction-tool and the results were compared with standardized RQ-PCR methodology. The comparable results obtained by V1-corrected with IS-correction-tool *versus* standardized RQ-PCR *BCR-ABL1/ABL1* method were observed in (23/35) 65,7% of samples. The Wilcoxon test did not reveal statistically significant differences between the results of both techniques and (P<0,2529). The comparable results of both techniques in group1, 2 and 3 were noted in 46%, 50% and 75% respectively. The differences were not statistically significant in each evaluated group.

Summary / Conclusion: The GeneXpert system provides very easy to use and rapid diagnostic platform, which could be very useful in evaluating CML patients' response to TKI therapy. Either V1-improved system cartridges or use of V1 with IS-correction-tool increases the concordance of BCR-ABL1 expression analysis results by GeneXpert and results of internationally standardized RQ-PCR method in patients with CML monitored for the response to TKI therapy.

B1375

HEALTH-RELATED PROFILES INCLUDING QUALITY OF LIFE AFTER CESSATION OF IMATINIB IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS WITH UNDETECTABLE MOLECULAR RESIDUAL DISEASE

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Background: As the number of studies of imatinib discontinuation is increasing, the information on post-discontinuation quality of life is becoming of considerable important for patients with CML and physicians.
Aims: The purpose of this study was to investigate whether chronic myeloid leukemia (CML) patients who maintained undetectable molecular residual disease (UMRD) with long-term imatinib therapy show different health-related profiles after cessation of imatinib.
Methods: Thirty four patients with UMRD (> 2 yrs) discontinued imatinib and were given questionnaire for 3 times (before discontinuation, at 1 month and at 6 month post-discontinuation). The health surveys were modified SF-36 + FACT.leu composed of imatinib-related adverse events, laboratories, physical and mental health parameters.
Results: All blood forming cells, serum electrolytes, cholesterol, triglyceride, lipase, and lactate dehydrogenase returned to normal ranges quickly but fasting blood sugar, total bilirubin, arginine aminotransferase, amylase, and g-GT did not change after cessation. The changes were remarkable within 2 months. Anorexia, abdominal discomfort, skin and hair whitening, and muscle cramp constantly improved. Night sweat, skin rash showed delayed improvement whereas nausea, vomiting, indigestion, skin fragility, edema, sore throat, night spasm, cold intolerance, easy bruise, easy bleeding, and easy fatigue improved promptly. Enjoy life, well-being sense, vulnerable to illness, hope in the fight against the illness constantly improved. Satisfy to good QoL or treatment, limited daily and social activity, healthy as anyone I know, sleep well, do for fun, satisfaction with family communication, and worry about getting worse improved immediately and deteriorated late. Feel achievement and worry about dying improved early whereas sadness, accept the illness, and emotional instability improved slowly. Sense of isolation and nervousness deteriorated constantly after cessation.
Summary / Conclusion: Late disappearances of early improvements (sleep well, do for fun, satisfy to good QoL, satisfy to treatment, and worry about getting worse and so on) may be due to apprehension about healthy future. Patients who are planned to stop imatinib should be carefully monitored their emotional stresses at least for 6 months as well as immediate after cessation.

B1376

IDENTIFICATION AND CHARACTERISTICS OF CANDIDATES FOR CESSATION OF TYROSINE KINASE INHIBITORS AMONG PATIENTS WITH CHRONIC PHASE CHRONIC MYELOGENOUS LEUKEMIA SHOWING DURABLE COMPLETE MOLECULAR RESPONSE

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Background: Tyrosine kinase inhibitors (TKIs) are currently the standard of care in the front-line treatment of chronic phase chronic myelogenous leukemia (CP-CML). Side effects and cost of TKI compounds, along with some patients' complete molecular response (disappearance of BCR-ABL transcripts) have raised the question whether TKIs can be safely withdrawn from these patients. Results from relevant clinical trials, such as the STIM study, are encouraging, showing that among patients with a complete molecular response (CMR) maintained for at least two years, CMR was sustained in 39% of them after 24 months of discontinuation of the drug, while 83% of those who relapsed upon withdrawal managed to re-gain CMR upon re-introduction of the drug.
Aims: We sought to identify candidates for TKI cessation according to the criteria of the currently recruiting EURO-SKI trial (EUROpean Stop tKI). We studied their clinical characteristics and will follow their outcome after TKI discontinuation in the context of this trial.
Methods: The archived data of our alive CP-CML patients were examined. We identified non-transplanted patients with CP-CML having received TKI therapy for at least 3 years and demonstrating sustained CMR4 for a continuum of at least 12 months, with at least three PCR results with CMR4 within the last year (± 2 months) before study entry and no results > 0.01% during the same period. CMR4 was defined as either (i) detectable disease ≤0.01% BCR-ABL (IS) or (ii) undetectable disease in cDNA with ≥10,000 ABL or ≥ 24,000 GUS transcripts).
Results: A total of eight patients that fulfilled criteria for getting off TKIs were identified out of our fifty-numbered CP-CML patient pool. All patients currently receive IM, except one being on nilotinib on grounds of imatinib and dasatinib toxicity. All of the patients achieved their CMR being initially on imatinib. Median age of these patients at diagnosis was 54,5 years (range: 27 - 73 years) and sex ratio was M:F 0.6. Sokal score at diagnosis was low in 4 patients, intermediate in 3 patients, and high in 1 patient, while the relevant Hasford score ratio was 4:4:0. The median interval from start of treatment to CMR was 20 months (range, 6-40 months), while the median interval to CCyR and MMR was 9 and 14 months, respectively. The median duration of detected CMR was 67,2 months (range 27- 101 months), which corresponds to a median of 84,7 months (range, 66 – 121 months) of total TKI treatment. Interestingly those who demonstrated more rapid CMR where the ones with low Sokal score at diagnosis (median time 11 months), while the ones with intermediate/ high score took longer (26,4 months) to reach this goal.
Summary / Conclusion: Durable complete molecular response is frequent among CP-CML patients. In our centre it was identified at a percentage of 16%. Achievement of CMR is feasible even with adverse prognostic factors at diagnosis, although it may require a longer treatment duration in this case, as far as our sample permits us a conclusion. Trials of discontinuation of TKIs could well identify a subgroup of patients who could transiently or permanently stay off therapy, changing the landscape of treatment in CML.

B1377

EVALUATION OF SECOND-GENERATION TYROSINE KINASE INHIBITORS EFFICACY AND SAFETY IN NOVO CML-CP THERAPY. RESULTS OF 29 PATIENTS OUTSIDE OF CLINICAL TRIALS FROM THE CML ANDALUSIAN GROUP

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Background: Results of clinical trials ENESTnd and DASISION have shown

more efficacy and safety of Nilotinib and Dasatinib, respectively, compared to Imatinib. This allowed their authorization as first-line treatment in patients diagnosed with chronic myeloid leukemia (CML) in 2011. Patients treated with second-generation tyrosine kinase inhibitors (2GTKIs) achieve faster and deeper responses and a smaller amount of progressions to accelerated phase (AP) and blast crisis (BC), with a good profile of safety and tolerability. A recent paper by Marin *et al* (*JCO, 2012*) indicates that a single measurement of *BCR-ABL* transcript at 3 months is the most accurate way to identify the risk of progression and overall survival in patients with chronic-phase (CP) CML treated with TKIs. In this sense we have analyzed the efficacy and safety of the 2GTKIs on first-line treatment in patients with CP-CML focused the attention on the molecular response (MR) at the third month of treatment in CML Andalusian Group Registry (RALMC).
Aims: To evaluate the 2GTKIs efficacy and safety in first-line treatment and the MR at 3 and 12 months.
Methods: We have analyzed retrospectively 29 adult patients diagnosed with CP-CML. They started with Nilotinib or Dasatinib in first-line treatment in Andalusia between July 2011 and November 2012 outside of clinical trials. Clinical and analytical data were obtained from the CML Andalusian Registry. BCR-ABL values are expressed in international scale and patients have been cataloged and monitored according to the ESMO 2012-guidelines.
Results: 16 males and 13 females have been included in the study. Median age is 45 years (19-78), distributed by Sokal score in: 14 (48.3%) low risk, 7 (24.1%) intermediate risk and 8 (27.6%) high risk. Eutos score: low 23 (79.3%) and high 6 (20.7%). All patients were diagnosed in CP, 18 (62%) were treated with Nilotinib 300mg BID and 11 (38%) with Dasatinib 100mg QD. Median follow-up is 12 months (3-20). At month3, 29 (100%) patients evaluated for MR had BCR-ABL transcript less than 10% and 24 (82.7%) less than 1%. Cytogenetic response was evaluated at 3 months in 21 patients: CCyR 18 (85.7%) and PCyR 3 (14.3%) while at month6, 22 (100%) achieved CCyR, 6 (27%) MMR, 3 (13.6%) $MR^{4.0}$, 3 (13.6%) $MR^{4.5}$ and 5 (22.8%) $MR^{5.0}$ and 5 did not achieve MMR. 3 (23%) out of 13 patients evaluated in the year achieved MMR, 1 (7.7%) $MR^{4.0}$, 2 (15.4%) $MR^{4.5}$ and 5 (38.5%) $MR^{5.0}$. 1 patient without MMR had BCR-ABL > 1% at month 3 and other patient that lost MMR and CCyR due to T315I mutation, the only treatment failure of the entire series. All patients in $MR^{4.0}$, $MR^{4.5}$ and $MR^{5.0}$ at month 12 had BCR-ABL ≤ 0.1% at month 3. Patients in MMR at month 12, had BCR-ABL > 0.1% at month 3. There has not been any AP/BC progression, as well as any death, and there has been only an interruption of Dasatinib for pleural effusion and congestive heart failure.
Summary / Conclusion: Despite the small number of patients treated with 2GTKIs in first-line, all patients, regardless their prognosis stadium and age, achieved a rate of BCR-ABL< 10% at month 3 and 83% achieved BCR-ABL transcript < 1%. According to other investigations, it shows the effectiveness of Nilotinib and Dasatinib in our patients. We have observed in our data that patients who achieve MMR at month 3 of treatment, they achieve $MR^{4.0}$, $MR^{4.5}$ or $MR^{5.0}$ at month 12. In this way, safety of the 2GTKIs in our series is reflected. There have not been hematological alterations grade 3/4 and only one patient with Dasatinib has withdrawn treatment due to adverse events.

B1378
TYROSINE KINASE DOMAIN MUTATIONS AND CYP3A4-CYP3A5 GENE POLYMORPHISMS IN IMATINIB RESISTANT CHRONIC MYELOID LEUKEMIA
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Background: Imatinib Mesylate (IM) resistance in CML patients is most frequently associated with point mutations in tyrosine kinase domain (TKD), whereas metabolization of IM by isoenzymes CYP3A4 and CYP3A5 is also one of the important BCR/ABL independent mechanisms of resistance. We have recently designed a detailed study to determine mutations in TKD and regulatory regions of BCR/ABL and also CYP3A4 -CYP3A5 polymorphisms, and correlate it with drug resistance and disease progression in Indian CML patients. Understanding the mechanism of drug resistance is important, as the therapy protocols can be modified.
Aims: To identify tyrosine kinase domain mutations and CYP3A4-CYP3A5 gene polymorphisms in imatinib resistant chronic myeloid leukemia patients.
Methods: 75 IM resistant CML patients were screened for mutations in TKD and regulatory regions of BCR/ABL gene by direct sequencing, for BCR/ABL gene amplification by FISH and for CYP3A4*1B and CYP3A5*3 polymorphism.
Results: Among 75 IM resistant patients, 41.33% of patients showed mutations in the TKD while 8% of patients had mutations in the regulatory region (SH3-SH2). Three novel mutations were detected, 185bp ΔD363-W423, Y167 and K84E. Y253F/H mutation was predicted to be possibly most damaging with a score of1, according to Polymorphism Phenotyping v2. 42 IM resistant patients not harboring mutations were screened for BCR/ABL gene amplification and for CYP3A4*1B and CYP3A5*3 polymorphism. 23 long term IM responders were considered as controls. None of the patients showed BCR/ABL gene amplification. Of 42 IM resistant patients, only two patients (4.7%) showed CYP3A4*1B polymorphism, one heterozygote and other mutant homozygote, as compared to control population (17.4%). The occurrence frequency of CYP3A5*3 (non-expressor) and CYP2A5*1 (expressor) in 42 IM resistant patients was 20% and 80% respectively, whereas the frequency in case of controls was 37% in non-expressor and 63% in expressor group.
Summary / Conclusion: Our study identified three novel mutations. Also, suggested that CYP3A4*1B and CYP3A5*3 polymorphisms are not associated with IM response

B1379
APPLICATION OF D-FISH ON BONE MARROW AND PERIPHERAL BLOOD SAMPLES FOR THE EVALUATION OF CYTOGENETIC RESPONSE TO TKIS THERAPY IN CHRONIC MYELOID LEUKEMIA
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Background: Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder induced by the fusion of the *ABL* gene with the *BCR* gene.The hallmark of CML is the translocation (9;22)(q34;q11). The translocation is observed in up to 100% of metaphase cells at diagnosis. After the introduction of the tirosyn kinase inhibitors (TKIs), it has become more relevant to monitor cytogenetically the response to treatment. The achievement and the maintenance of a complete cytogenetic response (CCgR) is of particular importance because it is the most solid marker of progression-free survival and overall survival. The cytogenetic response by CBA requires marrow cells, which cannot be always sampled, and an adequate number of banded metaphases, which cannot be always obtained. Finally, cytogenetic analysis requires bone marrow harvesting, which prevents repetitive analyses. Fluorescence in situ hybridization (FISH) enables a rapid detection of chromosomal rearrangements, even on interphase cells, and thus, avoids the requirement of metaphase obtention and is applicable to a variety of cytological samples, including peripheral-blood samples (PB). Thus, FISH is used, with increasing frequency, as a substitute for CBA. Given the need for frequent monitoring in patients with CML, interest in non-invasive methods has increased over years.
Aims: In this study, we investigated the usefulness and accuracy of dual color double fusion FISH (D-FISH) on bone marrow and peripheral-blood specimens to evaluate the cytogenetic response in CML patients treated by TKIs.
Methods: We performed the comparison of cytogenetic analysis by CBA and D-FISH on bone marrow specimens and by D-FISH on peripheral blood samples, simultaneously collected paired sets, from this set of patients. The analysis was performed on 62 samples obtained from 44 patients with BCR-ABL positive CML, representative of the different cytogenetic response groups.
There were 62 triplicate patient collection: 4 samples were selected at diagnosis and prior of treatment, while all others were collected at the follow-up visit during the treatment. We performed cytogenetic analysis (20 metaphase for sample) and FISH analysis (330 nuclei counted for sample) on bone marrow samples, while only FISH analysis (330 nuclei counted for sample) on peripheral blood samples. To attempt the accuracy and the utility of FISH analysis, we have correlate the data obtained by the software Graph Pad Prism 4.
Results: In our series , we demonstrate a tight correlation in quantifying BCR-ABL burden between bone marrow cytogenetic, bone marrow D-FISH and peripheral blood D-FISH. In all three paired studies analized, the strength correlation between the variables is reflected in a p value of <0.0001 in all cases and a correlation coefficient of r^2= 0.8749 at worst (fig1,2 and 3). Moreover, the D-FISH results on BM and PB samples are in agreement with the results of CBA on BM samples, classifying the patients rightly on the basis of the different cytogenetic response classification. By D-FISH it was possible quantify the percentage of Ph+ cells too in 7 patients with uninformative BM cytogenetic analisys. These results indicate that peripheral blood D-FISH yields a clinically equivalent detection of BCR-ABL fusion to CBA, expecially in long term management of CML patients. Thus, cytogenetic response might reliably be monitored by analyzing interphase peripheral-blood cells in MRD.
Summary / Conclusion: This study confirms the results of other reports that suggested the feasibility of D-FISH on peripheral blood specimens to monitor cytogenetic response.

B1380
SIGNIFICANCE OF EUTOS AND HAMMERSMITH SCORE IN SECOND LINE TYROSINE KINASE INHIBITORS
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Background: Although Imatinib is very effective for CML CP patients but CCyR are not reachable for whole patients. Second line tyrosine kinase inhibitors could provide to achieve CCyR nearly in half of patients, who resistant or intolerant to Imatinib. But in cases of ineffectiveness of 2nd line TKI this patients

today must get allo-BMT for chance of long-term survival. It is very important to predict cytogenetic response in 2nd line TKI treatment.
Aims: The aim of our study was to estimate prognostic value of EUTOS risk score and Hammersmith scale for patients on 2nd line TKI therapy.
Methods: 62 patients with chronic phase of CML were included in our study. There were male/female ratio as 28/34 respectively. Median age at diagnosis was 47 years (range 24-71). Median of age at start of 2nd line TKI therapy was 51 years (range 25-72). Patients were treated with Nilotinib (n=37) or Dasatinib (n=25). Median time on previous Imatinib therapy was 15.4 months (range 1-64). Median time from diagnosis to 2nd line TKIs initiation was 27 months (range 3-190). The majority of patients were resistant to first line Imatinib (57/62). Follow up time from start of 2nd line TKIs was 33 months (2-90). EUTOS score was low in 32 (51%) and high in 12(18%) patients. It was unknown in 18 (29%) patients. Hammersmith score was low in 11 (17%), intermediate in 27 (43%), high in 6(9%) and unknown in 17 (27%) patients. Median time of 2nd line TKI therapy was 14 months (0.7-78). The therapy was discontinued in 34/62 (55%) patients, mainly due to resistance to TKIs (67%).
Results: Probability of CCyR was not significantly differing according EUTOS risk groups. It was 58% and 41% by 12 months respectively for low and high EUTOS risk groups (P>0.05). None of patients with high Hammersmith score had achieved CCyR. It was significantly higher in patients with low (63.6%) than intermediate (48.1%) Hammersmith score (P=0.042) (Fig.1). Probability of CCyR by 12 months was 78% and 40% in low and intermediate risk groups. Median time to CCyR in low and intermediate groups was 3 months and 6 months respectively. During follow up 15/62 (24%) dead (12 deaths were CML-related). 5-years estimated overall survival was 73%. Both EUTOS and Hammersmith scores were not predictable for survival in our study.
Summary / Conclusion: In our study EUTOS score calculated at diagnosis was not valid for predicting both CCyR and overall survival on 2nd line TKI treatment. It could be due to the fact of late switching to 2 TKI in this cohort of pts. The prognostic value of Hammersmith scale was high for forecast CCyR, but not for overall survival. Other prognostic models should be applied for overall survival in 2TKI treated pts.

B1381

BENEFITS/RISKS OF DASATINIB THERAPY IN PATIENTS WITH IMATINIB-RESISTANT OR -INTOLERANT CHRONIC MYELOID LEUKEMIA: PHYSICIAN'S AND PATIENT'S PERSPECTIVE

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Background: Comprehensive evaluation of benefits and risks of second-line therapy in patients in chronic phase chronic myeloid leukemia (CML-CP) is worthwhile to better define treatment outcomes in this patients' population.
Aims: We aimed to study clinical and patient-reported outcomes in patients with imatinib resistance or intolerance receiving dasatinib as the second-line therapy during the first year of treatment.
Methods: 62 CML-CP patients resistant or -intolerant to imatinib were enrolled in the study (mean age – 51.5 years old, SD 15.5; range – 22–84 years; male/female – 32/30). The median of disease duration was 5.8 years (0.75–17 years). 51 patients had resistance to imatinib; 11 patients were intolerant to imatinib; the median duration of imatinib treatment – 41 months (3–106 months). All the patients received dasatinib as the second-line therapy (100 mg daily). Median follow-up was 11.5 months. For quality of life (QoL) and symptom assessment patients filled out the SF-36 and Comprehensive Symptom Profile in Chronic Myeloid Leukemia Patients (CSP Leuk-CML), respectively, at baseline, in1,3, 6 and 12 months after treatment start. For comparisons Mann-Whitney test was used. Mean symptom severity and percentage of patients with moderate-to-severe (ratings ³5) symptoms was evaluated.
Results: At 12 months of treatment the majority of patients had high rates of hematologic (complete, 82%) and cytogenetic (major, 57%; complete, 25%) response. Dasatinib was well tolerated in the majority of cases. Four cases of pleural effusion events were registered: they were easily managed in 3 cases; one patient died at 1 month after treatment start due to accompanied infection complication. No severe hematological adverse effects were observed except two cases of grade III-IV neutropenia. Two patients were resistant to dasatinib. One patient died of disease progression at 6 months of follow-up. During dasatinib treatment QoL parameters were stable with a slight tendency of improvement in vitality, mental health and pain scales. At 12 months after the start of therapy QoL treatment response in terms of stabilization or improvement was registered in the majority of patients (81%). In nearly all the patients symptom severity became lower or did not change at different time-points of treatment as compared with base-line. Before treatment 75% of patients experienced at least one moderate-to-severe symptom; more than 40% had more than 7 moderate-to-severe symptoms. The majority of patients (96%) experienced fatigue; half of them suffered from moderate-to-severe fatigue. While treatment the number of patients with moderate-to-severe symptoms decreased. After 12 months of therapy only 25% of patients experienced moderate-to-severe fatigue. Remarkably, in the subgroup of patients (21%) with critical or severe QoL impairment at base-line dramatic QoL improvement was observed: QoL index increased 3.7 fold (P<0.05). In this group severity of common symptoms significantly decreased.
Summary / Conclusion: Thus, dasatinib as second-line therapy in CML-CP patients is effective both in terms of clinical outcomes and patient-reported outcomes, as well as exhibits good tolerability. Comprehensive evaluation of the outcomes of the second-line treatment of CML-CP allows to assess the benefits and risks of therapy both from physician's and patient's perspective.

B1382

STRUCTURE OF MORTALITY DURING INTERNATIONAL RESEARCH PROJECT THE EUROPEAN OUTCOME AND TREATMENT STUDY FOR CML, OUT STUDY PATIENTS (EUTOS OSP) IN RUSSIA OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Highly effective treatment by tyrosine kinase inhibitors (TKI) provides increased survival rates and good life quality to patients (pts) with chronic myeloid leukemia (CML). Therefore long-term treatment results, analysis of additional events not related to CML and analysis of the causes of death are very important.
Aims: To analyze structure of mortality in CML pts treated in routine clinical practice in Russian Federation.
Methods: The analyzed cohort consisted of 607 pts from 29 regions of Russia (OSP EUTOS): Ph'/bcr-abl-positive CML diagnosed in 2002- 2006, initiation of imatinib (IM) therapy ≤6 months (mo) after diagnosis. Median (Me) age was 48(18-82) years (y), M:F% ratio 47:53% pts. Pretreatment: hydroxyurea 454(76%) pts; chemotherapy 25(4%) pts, IFN-α 37(6%) pts. Me follow-up from starting of IM treatment was 60(1-112) mo. Chronic phase (CP) of CML was diagnosed in 557 (93%) pts, accelerated phase (AP) and blast crisis (BC) – in 38(6%) and 6(1%) pts respectively. Due to retrospective data collection we found that in first 3 y of observation the data were not described completely and we considered that fact.
Results: Mortality in a cohort of 607 pts was 15% (93 pts). Of these 93 pts, 48 (52%) pts death was the result of progression to AP or BP. 32 (34%) were reported dead in CP CML, 3 pts (3%) - after allogeneic stem cell transplantation (2 of them due to infection), in 10 (11%) cause of death was unknown. So, mortality due to progression CML in CP and AP+BC was in 58 (63%) of 93 pts. The cause of death in 32 pts in CP without progression to AP was reported as following: 10 pts with CML progression with no signs of advanced phases; 22 pts with reasons not related to CML. Among 22 (24%) CML not related causes of death there were: coronary artery disease/myocardial infarction/heart failure in 11 cases, a second malignancy in 5 cases (tumor pulmonis, esophageal cancer with metastases, stomach cancer, cancer of the sigmoid colon, metastasis of melanoma), blood stroke (acute ischemic stroke) in 2 cases, accident - 2 cases, cirrhosis of the liver in 1 pts, in 1 case as opposed to infection bilateral pneumonia (during virus epidemic). This subgroup of 22 pts was male and female in equal proportions with Me age 63.5 (47-76) y.
Summary / Conclusion: Retrospective data collection does not always allow to adequately evaluating the structure of mortality. Feature of presented cohort of Russian pts- mortality in 15% of 607 pts, with 3.6% (22pts) the cause of death was not associated with CML. Eventually the rate of death due to progression of CML in CP decreases and in AP+BC remains a leading cause. Mortality from cardiovascular causes and secondary tumors in pts with CML is a significant proportion (14 and 5%, respectively), and should be considered when analyzing the long-term results of therapy.

B1383

CO-EXPRESSION OF P190 AND P 210 BCR-ABL FUSION TRANSCRIPTS AND RESPONSE TO TKI THERAPY IN CHRONIC MYELOID LEUKEMIA: THE RET (RETE EMATOLOGICA TOSCANA) EXPERIENCE

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Background: The hallmark of chronic myeloid leukemia (CML) is the presence of the Philadelphia chromosome and its resultant fusion gene *BCR-ABL*, and fusion protein, p210. Occasionally patients with CML can have a smaller *BCR-ABL* fusion transcript possessing only the first exon of *BCR* fused to *ABL*, resulting in a p190 protein, more frequent in Philadelphia positive acute lymphoblastic leukaemia (ALL). A co-expression of both proteins, p190 and p210 has been described in CML, even in chronic phase; p190 mRNA usually is expressed at low level and may arise through alternative or missplicing. Its presence seemed to have no impact on prognosis in the pre-TKI era.
Aims: A co-expression of both proteins, p190 and p210 has been described in CML, even in chronic phase; p190 mRNA usually is expressed at low level and may arise through alternative or missplicing. Its presence seemed to have no impact on prognosis in the pre-TKI era.
Methods: 82 patients with new diagnosis of CML have been referred to our institution between 2008 and 2013, within the RET (Rete Ematologica Toscana) project. 13 cases (16%) expressed both transcripts. Median age was 55 years (range 31–83 years). At diagnosis 11 patients were in chronic phase (CP), 2 in accelerated phase (AP), none was in blastic phase (BP). Sokal risk was low in 2 patients, intermediate in 6 patients, and high in 3 patients. As frontline therapy, 8 patients received Imatinib 400 mg/day, 3 Nilotinib 600 mg/day, two patients in AP Dasatinib 100 mg/day and Imatinib 600 mg/day. The median follow-up was 22 months (range 3–57 months).
Results: Amount of p190 transcript was always low at the diagnosis (ratio *BCR-ABL/ABL* <1% in IS), becoming undetectable within 12 months of treatment in all patients; occasional, sporadic positivity at very low levels (ratio *BCR-ABL/ABL* ratio always in the range of MR4-MR.4.5 in IS) was frequently documented during the follow up , with no correlation with raise in p210 amount. One of two patients in AP obtained a CCyR (complete cytogenetic response) and a MMR (major molecular response) within 6 months of starting the treatment with Dasatinib, the other one was resistant to Imatinib and underwent allogeneic bone marrow transplant (HSCT) from an unrelated donor; 3 of 8 patients in CP treated with Imatinib obtained a MMR at 12 months, 3 patients were resistant and switched to second generation tyrosine kinase inhibitors, a case at 18 months showed still a ratio of 0,21, while BCR-ABL/ABL ratio of the last patient with only three months of follow up is not available. Nilotinib as first line therapy induced a MMR at six months in 3 patients.
Summary / Conclusion: Co-expression of p210 and p190 transcripts is not a common event in CML, especially in chronic phase. Presence of low levels of p190 transcripts at the diagnosis appears to be related to an increased Sokal risk and/or advanced phases, and to an apparent reduced probability of response to Imatinib. The majority of patients showed fluctuating, very low levels of p190 transcripts over the time, with no correlation with changes in p210 transcript amount.

B1384

FREQUENCY OF HYPOCALCEMIA IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA RECEIVING TYROSINE KINASE INHIBITOR THERAPY

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Background: The introduction of tyrosine kinase inhibitors (TKI) have revolutionised the treatment of chronic myeloid leukaemia (CML). Despite the wide range of signalling pathways inhibited by Imatinib mesylate, the drug is relatively well tolerated by the majority of patients. Hypocalcemia is one of the side effects that was suggested to occur very frequently in patients with GIST tumours receiving Imatinib therapy. We have noticed hypocalcaemia in a number of patients with CML receiving Imatinib and Dasatinib treatment.
Aims: We aimed to study calcium levels in patients with chronic phase-CML within the first 12 months of the introduction of TKI therapy. The need for relevant replacement therapy in the presence or absence of hypocalcaemia symptoms was also assessed.
Methods: A retrospective observational study was undertaken at Singleton hospital (Swansea, UK) of patients receiving TKI therapy for chronic phase-CML. Symptoms related to hypocalcaemia and need for calcium replacement therapy were recorded by examination of the clinical notes. Blood results were reviewed by comparing pre-treatment serum adjusted calcium (SAC) level (reference range: 2.15-2.60 mmol/l) to the mean post-treatment levels over the 12 month period following the commencement of TKI therapy.
Results: The records of 28 patients who received 1st line TKI therapy between Feb 2004-Aug 2012 were examined. The median age was 55 years (range 24-77 years) and 36% were males. All patients received Imatinib (400mg/day) except 3 patients who received Dasatinib (100mg/day). A post therapy fall in the mean SAC was noticed in 21 patients (75%). The median decrease in SAC was 6.2% (range 0.92% to 12.5%). The mean reduction of SAC was 5.6 % in the Imatinib cohort (range 1.75% to 11%) as compared to 5% (range, 0.92% to 12.5%) in patients who received Dasatinib. Hypocalcaemia, defined as the mean SAC during the 1st year post TKI therapy <2.15 mmol/l, developed in 10 patients (36%). Only three of these patients had documented symptoms of muscle aches and cramps. Ten patients (36%) received combined oral calcium and vitamin D replacement therapy. One patient on Imatinib did not tolerate calcium supplements but had a spontaneous improvement in SAC. In three patients with hypocalcaemia, the SAC did not normalise with daily supplements of calcium (1 gm) and vitamin D3 (Colcalciferol 20 mcg). Serum vitamin D levels were checked and found to be significantly low in these patients (median 11 ng/ml, range (10-12 ng/ml) and required the addition of a treatment dose of vitamin D, given as Ergocalciferol 1.25mg for 5-10 doses and achieved normocalcemia. No difference was found in cytogenetic and molecular responses between the hypocalcemic and normocalcemic cohorts of patients.
Summary / Conclusion: In our study population of patients with chronic phase-CML, the reduction in SAC was a frequent laboratory but asymptomatic finding following introduction of Imatinib therapy. This was reported in 75% of all patients. Hypocalcaemia was seen in 36% of patients and responded to conventional dose calcium and vitamin D replacement therapy in the majority of these patients. However, in three of these patients, normal SAC was achieved only after addition of high dose vitamin D therapy. These three patients had significantly low serum vitamin D levels. This suggests that vitamin D deficiency may potentiate the hypocalcemic effect of TKI therapy. Clinicians need to be aware of this frequent side effect that may need treatment. Serum vitamin D levels should be checked in those patients who remain hypocalcemic despite conventional dose supplements. Further studies looking at effects of long-term TKI therapy on bone and calcium dysregulation are warranted.

B1385

EVALUATION OF TWO THERAPEUTIC STRATEGIES IN PATIENTS WITH CHRONIC MYELOID LEUKAEMIA IN SUBOPTIMAL RESPONSE TO IMATINIB

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Background: There is no consensus about the optimal treatment regimen in patients with Chronic-Phase Chronic Myeloid Leukaemia (CP-CML) presenting a suboptimal response to first-line imatinib.
Aims: The aim of our study was to evaluate the efficacy and safety of two therapeutic strategies in the management of CP-CML patients in suboptimal response to first-line imatinib.
Methods: Methods: All CP-CML patients treated in our teaching hospital with imatinib as first-line for CP-CML since the availability of the drug were eligible. Among them, those presenting a suboptimal response (according to the 2009 European LeukemiaNet criteria) to imatinib 400 mg once daily were included in this retrospective study. Patients were assigned in two groups regarding the treatment modification: group1, increase in imatinib dose (600 to 800 mg daily); group2, imatinib discontinuation and initiation of a Second-Generation Tyrosine Kinase Inhibitor (SG-TKI; nilotinib 800 mg daily, or dasatinib 100-140 mg daily). Cytogenetic response (CyR) was assessed 12 months after treatment modification and molecular response (MolR) was assessed every 3 months. Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for AE v 3.0.
Results: Out of the 150 CP-CML patients receiving imatinib 400 mg daily, 27 patients (18%) presented a suboptimal response. Twenty-two and five patients were assigned in group 1 and2, respectively. At 12 months, all patients except one in group 1 were in complete CyR. At 12 and 18 months, the rate of complete MolR was 23% and 36% in group 1 *vs.* 60% and 60% in group 2 (P=.13 and P=.37, respectively). The rate of treatment discontinuation was 40% in group 2 vs 0% in group 1. Two patients receiving nilotinib were hospitalized for one grade 4 cardiac AE, and one for grade 3 pain and deterioration in general condition. In group1, 50% of the patients developed at least one grade 1 or 2 AE; the most frequent imatinib-related AEs were muscle cramps, edema (eye, orbital, periorbital), and digestive disorders.
Summary / Conclusion: In CP-CML patients in suboptimal response to imatinib 400 mg daily, the use of a SG-TKI led to a higher non significant complete MolR rate but more severe toxicities at 18 months compared to an increase of the imatinib daily dose.

B1386

EXTREME THROMBOCYTOSIS IN CHRONIC MYELOID LEUKEMIA(CML) IN THE ERA OF TYROSINE KINASE INHIBITORS (TKIS)

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Background: Thrombocytosis is a common feature in chronic myeloproliferative diseases disorders. The incidence of thrombocytosis in CML is reported to be around 30 to 50% . Extreme thrombocytosis defined as a platelet count > $1.000x10^9$/l is uncommon in CML as well as isolated thrombocytosis. According to the WHO classification criteria, persistent thrombocytosis (> 1000 × 10^9/L) unresponsive to therapy defines accelerated phase of CML while this is not the case when standard criteria for the definition of CML staging in imatinib and second generation inhibitors of tyrosine kinase trials are adopted.
Aims: The aim of our study was to review the behavior of CML with extreme thrombocytosis at our center analyzing the problems associated to the clinical management of haemorrhagic and thrombotic risks and the therapeutic response in the era of tyrosine kinase inhibitors
Methods: From November 1997 to February 2013 we observed and treated one hundred consecutive patients affected by CML in chronic phase . All

patients were studied for JAK2 V617F mutation, and coagulation cascade.
Results: Only 11 patients (11%) presented at diagnosis an extreme thrombocytosis. There were 8 females and 3 males with a median age of 42 years. At diagnosis, median hemoglobin level was 12.2 g/dl, median white blood cell count 19.240/mmc and platelets count 1.160x109/l. The Sokal score was high in5, intermediate in 3 and low in 3 patients. In all cases PCR analysis showed the presence of p 210 and absence of JAK2 V617F mutation. Bleeding time (Ivy test) was prolonged with a median of 10.68 minutes. aPTT was within the normal range in all but two patients. Iron levels were normal in all but one female patient. Only one patient developed thrombosis of the caephalic vein in the left forearm at diagnosis while no patient reported history of bleeding. All but three patients received initial treatment with hydroxyurea and allopurinol. One patient underwent plateletpheresis. Platelet count was largely unresponsive to initial treatment. Low dose aspirin (100mg/day) was administered in 5 out of 11 patients. Upfront treatment with TKIs included imatinib in 8 patients and nilotinib in 3 patients. Platelet count normalization was rapidly achieved after introduction of TKIs. Haematological response according to ELN criteria was reached at a median of 1 month, complete cytogenetic response was achieved after a median of 3 months and major molecular response was achieved in 9 out of 11 patients after a median of 9 months. One patient was in suboptimal molecular response at 18 months of imatinib according to ELN recommendations and he was shifted to dasatinib. He achieved MMR on dasatinib and is now at 37 months on second line treatment. One patient lost MMR after 5 years of imatinib and she was shifted to nilotinib achieving MMR and she is now at 25 months on second line treatment . All patients are alive and in optimal response at a median follow up of 67 months from CML diagnosis.
Summary / Conclusion: Extreme thrombocytosis in CML is unfrequent. Prolonged bleeding time was detected in all patients although it was not accompanied by bleeding diathesis. Cytoreduction with hydroxiurea was not able to achieve normalization of platelet count that was easily and rapidly achieved by tyrosine kinase inhibitors. Response to treatment according to ELN criteria was optimal in all but two patients requiring second line TKIs respectively for suboptimal molecular response and secondary resistance to imatinib.

B1387

BLAST CRISIS OF CHRONIC MYELOID LEUKEMIA: LABORATORY, IMMUNOPHENOTYPC, AND CYTOGENETIC HALLMARKS AND RESPONSE TO THERAPY

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Background: Whereas the chances for cure have been improved in chronic phase (CP) and occasionally even in the accelerated phase (AP) of chronic myeloid leukemia (CML) by using tyrosine kinase inhibitors and hematopoietic stem cell transplantation, the blast crisis (BC) is extremely rarely curable and is usually a fatal phase of CML.
Aims: To evaluate clinical, laboratory, immunophenotypic and cytogenetic features of the BC-CML and response to therapy
Methods: We studied 48 patients in BC-CML treated at the Clinic of Hematology between 2004 and 2012. The physical examination of the patients included the estimation of organomegaly, lymphadenopathy and hemorrhagic manifestations. The type of BC was established by cytology and flow immunocytometry/immunohistochemistry of the bone marrow aspirates. The HD technique of chromosome banding and the RT-PCR procedure were applied in genetic studies.
Results: The median age of patients was 52 years (range:20 – 78), the M/F ratio was 27/21. The median of duration of CP was 57 months (spread: 0 – 96). The Sokal score was estimated in 40/48 patients: 9/48 were low risk, 20/40 were intermediate, and 11/40 were high risk patients. Nine patients with CP-CML were treated with imatinib mesylate, 20 patients with hydroxyurea, 13 patients with interferon-α with or without cytosine arabinoside, three patients with busulfan, and three patients with cytosine arabinoside alone. The following cytological types of the BC-CML were demonstrated: myeloblastic in 32/48, lymphoblastic in 8/48, megakaryoblastic in 3/48 and biphenotypic in 5/48 patients. Cytogenetic analyses in BC-CML demonstrated the sole presence of Ph chromosome in 20/48; double Ph chromosome in 4/48, additional chromosome aberrations in 6 patients [trisomy 8 in two patients and t(2;11); t(4;11); i(17)(q10); add 21; i 4q in a single patient each]. The complex karyotype was evidenced in five patients. Adequate mitoses were not obtained in 7 patients. Thirty patients received intensive combined chemotherapy (cytosine arabinoside+ daunoblastin: "3+7" or "2+5" and Flag-Ida, HyperCVAD, and CHOP protocols) relevant to cytological type of the BC-CML. Palliative cytoreduction was conducted in ten patients (busulfan, hydroxurea). Eight patients received only supportive therapy due to a poor performance status or an advanced age. The overall median survival in BC-CML was 3 months (range, 0.5-31). The median survival was longer in patients treated with high-dose chemotherapy,8,8 months, compared to palliation, 2 months (P=0,043). Out of ten patients who survived longer than 10 months, 7 patients were treated with Imatinib mesylate for their CP-CML. The median duration of CP was significantly longer in patients treated with Imatinib, 58 months (range: 38-96, P=0,02). The patients with an inferior (low and intermediate) Sokal score had a longer CP (P=0,025). The cytogenetic changes were not significantly related to survival in BC-CML.
Summary / Conclusion: This study provides yet another confirmation of the severity of BC-CML and a disappointing outcome of therapy. Only intensive chemotherapy lends a certain hope in prolonging the survival of patients in BC-CML. Hopefully, novel therapies of CP-CML shall have devalued BC-CML in the future to an exceptionally uncommon and unfamiliar clinical incident.

B1388

PROGNOSTIC VALUES EUTOS AND SOKAL SCORES IN NEWLY DIAGNOSED CML CP PATIENTS

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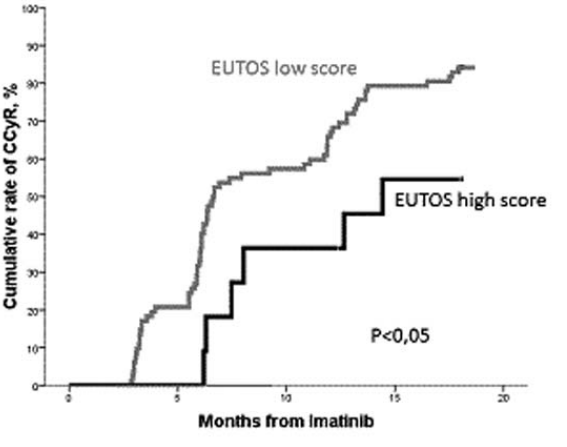
Background: Imatinib is highly effective in newly diagnosed patients with chronic myeloid leukemia (CML) in chronic phase (CP). Nevertheless some patients never obtained CCyR or loss it later. It seems that EUTOS score is predictable for CCyR at 18 months, but it's prognostic value for survival is questionable. In some studies SOKAL risk groups influenced on Imatinib efficacy.
Aims: The purpose of our study was to assess prognostic significance of EUTOS and SOKAL scores in newly diagnosed CML CP patients.
Methods: In our study 137 (male 67, female 70) newly diagnosed patients with CML CP treated with Imatinib as first line were included. Median age at diagnosis was 50 years (range 18-86). Median time from diagnosis to Imatinib therapywas 1.2 months (range 0-6). The median duration of imatinib therapy was 37 months (range 1.6-107). EUTOS score was low in 118 (86%) and high only in 11 (8%) patients. It was unknown in 8 (6%) patients. SOKAL score was low in 68 (50%), intermediate in 44 (32%), high in 17 (12%) and unknown in 8 (6%) patients.
Results: In our retrospective study 119/137 (87%) patients were evaluable for cytogenetic response. The best cytogenetic responses cumulative rates were: CCyR in 93/119 (78%), PCyR in 9/119 (8%), minor CyR in 4/119 (3%), minimal CyR in 2/119 (2%), non CyR 11/119 (9%). CCyR was obtained in 79/99 (80%) and 7/13 (53%) cases with EUTOS low and high scores respectively. Cumulative rates of CCyR by 12 months and 18 months were 73/115 (63%) and 79/96 (86%) respectively. By 18 months CCyR was obtained in 70/82 (85%) and 7/11 (64%) patients with EUTOS low and high scores (P<0.05) respectively. Median time to CCyR was shorter in low (6.7 months) risk group more than two times than in high (14 months) risk IFig. 1). There were no differences in the rate of CCyR (overall or by fixed time-points) between Socal risk groups. Only 14/137 (10%) of patients dead during follow up time. 9-years estimated overall survival was 86%. In our study both EUTOS and SOKAL scales were not predictable for overall survival.
Summary / Conclusion: In our study EUTOS score had high prognostic value for CCyR. Both SOKAL and EUTOS scores were not predictable for overall survival. Perhaps it was because of small number of patients in high SOKAL and EUTOS risk groups.



B1389

IMATINIB THERAPY RESPONSE IN CML PATIENTS WITH DIFFERENT BCR/ABL P210 TRANSCRIPTS

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Background: Multiple clinical trials showed extremely high efficiency of tyrosine kinase inhibitors (TKI) in the therapy of chronic myeloid leukemia (CML). However, despite the achieved progress, there is a group of patients who have almost no response to the therapy, or lose response at different stages of treatment. Mechanisms underlying the resistance to TKI have not been clarified yet. The main pathogenetic event which leads to the development of CML is the translocation t(9;22)(q34;q11.2) in stem cells which results into formation of a chimeric fusion gene *BCR/ABL*. Study of genetic mechanisms of TKI therapy resistance development and genetic prognostic markers of treatment response determines success of TKI therapy in patients with CML.
Aims: The ***aim*** of this research was to describe *BCR/ABL* transcripts pattern and to study prognostic value of different type *BCR/ABL* transcripts for imatinib therapy response in patient with CML.
Methods: Expression of *BCR/ABL* gene was studied by qualitative nested reverse transcriptase polymerase chain reaction (RT-PCR) in 900 patients with CML prior to the start of imatinib therapy. Patients' age ranged from 20 to 70 years old at the time of disease. Therapy response was assessed in 110 CML patients at 6 and 12 months of imatinib therapy (dose of imatinib 400 mg/day) by the level of Ph-positive metaphases in bone marrow. For this purpose results of bone marrow cells karyotyping by G-banding were used. Response was considered optimal if a major cytogenetic response (reduction of Ph-positive metaphase plates in the bone marrow to less than 35%) was achieved at 6 months and complete cytogenetic response (complete absence of Ph-negative metaphase plates in the bone marrow) - after 12 months of imatinib therapy.
Results: It was found that in 511 patients (56,8%) b3a2 *BCR/ABL* transcript was expressed, in 305 patients (33,9%) expression of b2a2 transcript was revealed. Three patients (0.3%) had only e1a2 transcript and in the 54 patients (6%) coexpression of several *BCR/ABL* transcripts took place. 27 patients (3%) had expression of houskeeping gene without expression of the examined *BCR/ABL* transcript. Such cases were considered as variant transcripts because primers only for widespread *BCR/ABL* transcripts b3a2, b2a2 and e1a2 were used.Assessment of the prognostic value of b2a2 and b3a2 transcripts for the imatinib therapy response showed that the number of patients who achieved optimal and complete cytogenetic response at 6 month of therapy was equal in both groups. However, among patients with the partial cytogenetic response (level of Ph-positive metaphases 5 - 35 %) at 6 month therapy more often achieved complete cytogenetic response at 12 month of therapy those who had transcript b2a2, while most part of patients with b3a2 transcript saved partial cytogenetic response. Thus, patients with b2a2 transcript respond to the standard dose of imatinib (400 mg/day) significantly earlier.
Summary / Conclusion: Accumulation of knowledge about molecular features of disease is the way to further improve CML treatment outcomes.Obtained results indicate that groups of patients with CML are not uniform. Patients with atypical forms of *BCR/ABL*transcripts require in-depth study and expansion of surveillance. Patients with expression of b2a2 *BCR/ABL* transcript have more stable and earlier cytogenetic remission with imatinib therapy than those with b3a2 transcript. To summarize, the type of transcript does have prognostic value for response to imatinib therapy.

B1390

A FRET-BASED DRUG SENSITIVITY TEST PREDICTS A CLINICAL OUTCOME OF CML PATIENTS AT THE DIAGNOSIS

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Background: The tyrosine kinase inhibitors (TKIs), imatinib, nilotinib and dasatinib are used as the first-line therapy for newly diagnosed chronic myeloid leukemia (CML). To predict a clinical outcome, Sokal, Hasford and EUTOS scores are widely used. But at this moment, we don't have a good tool to predict which TKI is most effective at the diagnosis. We have developed a FRET(fluorescence resonance image transfer)-based method to evaluate sensitivity of CML cells to TKIs (Mizutani et al. Clin Cancer Res. 2010).
Aims: We designed this study to evaluate a clinical utility of FRET-based drug sensitivity test.
Methods: Inter-MIchinoku DAsatinib Study (IMIDAS) group conducted a Phase II clinical study to evaluate the efficacy and safety of dasatinib. Newly diagnosed CML-CP patients were enrolled into this study after obtaining a written informed consent. In this multicenter phase II clinical trial, dasatinib (100 mg QD) was administered orally for one year and the molecular responses were monitored. FRET analysis was also conducted as an accompanying study. Bone marrow mononuclear cells at the diagnosis were isolated and transfected with FRET probe, Pickles. After 18 to 24 hours of transfection, the cells were treated with 0.1 microM dasatinib and then subjected to microscopic analysis to determine FRET efficiency. RQ-PCR evaluation of BCR-ABL mRNA was performed to monitor the clinical efficacy of dasatinib. Totally 45 patients were analyzed here, since they were subjected to FRET analysis and followed longer than three months.
Results: The samples form 45 patients were subjected to FRET analysis. Among them, 9 patients were defined as dasatinib-sensitive and 36 patients were categorized into dasatinib-insensitive. At 3 months analysis, MMR rate was 44% in the sensitive group (n=9) and 39% in the insensitive group (n=36). At 6 months analysis, MMR rate was 89% in the sensitive group (n=9) and 62% in the insensitive group (n=29). At 9 months analysis, MMR rate was 88% in the sensitive group (n=8) and 83% in the insensitive group (n=18). At 12 months analysis, MMR rate was 100% in the sensitive group (n=3) and 85% in the insensitive group (n=13). One treatment failure and one transformation to BC were observed in the insensitive group.
Summary / Conclusion: The FRET analysis to determine dasatinib-sensitivity in vitro could predict the clinical efficacy of treatment with dasatinib. Our method will add the new tool to stratify the patients with CML-CP.

B1391

FOUR CASES OF CML BLAST PHASE AS INITIAL PRESENTATION DIAGNOSED BY FLUORESCENCE IN SITU HYBRIDIZATION SHOWING BCR-ABL1 SIGNALS IN MATURE NEUTROPHILS

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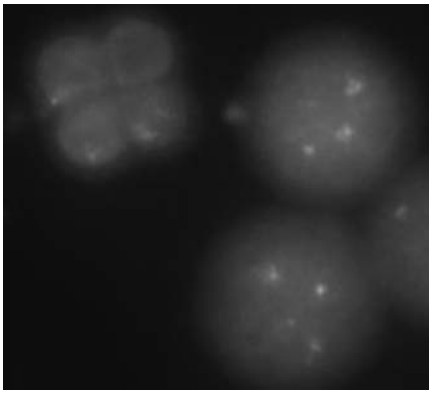
Background: Leukemia with t(9;22) and granulocytic hyperplasia at initial presentation can be diagnosed as acute leukemia with t(9;22) or chronic myelogenous leukemia – blast phase (CML-BP). These two entities have clinical, pathologic, immunophenotypic, and genetic similarities. However, CML is multilineage disease involved by the Philadelphia (Ph) chromosome and Ph+ acute leukemia is blast-restricted.
Aims: We experienced two adult and two pediatric patients with CML-BP as initial presentation who were initially suspected as having Ph+ acute leukemia. Identification of *BCR-ABL1* gene in mature neutrophils by fluorescence in situ hybridization (FISH) analysis led us to diagnose CML-BP.
Methods: All our patients showed peripheral leukocytosis due to blasts and granulocytic hyperplasia with or without absolute basophilia. We performed complete blood cell count (CBC), bone marrow study, immunophenotyping study, molecular study, and cytogenetic study. Fluorescence in situ hybridization (FISH) analysis was done to distinguish CML presenting in BP from Ph+ acute leukemia.
Results: We had initially suspected Ph+ acute leukemia based on molecular study and high proportion of blasts in the bone marrow, but the peripheral granulocytic hyperplasia with shift to the left of maturation pattern led us to finally find the *BCR-ABL1* fusion signals in mature neutrophils by FISH analysisindicating CML-BP. Three cases had mixed phenotype blasts and one case had lymphoblasts on the immunophenotyping study. Three patients had been treated with induction chemotherapy alone before adding tyrosine kinase inhibitor (TKI). At follow-up, high proportion of Ph+ cells by cytogenetic study and/or high level of the BCR-ABL1 transcript by Real-time quantitative PCR (RQ-PCR) were still detected, although the proportion of the blasts was much lower than before in the bone marrow. This supported the diagnosis of CML presenting in blast phase.
Summary / Conclusion: While interpreting FISH results for leukemia with t(9;22) and peripheral granulocytic hyperplasia as initial presentation, care should be taken to find the *BCR-ABL1* fusion signals in mature neutrophils to diagnose CML-BP. The exact diagnosis is needed to pool rare cases of Ph+ acute leukemia and CML-BP as initial presentation correctly and helps patients to be involved in proper clinical trials.



B1392

A POPULATION STUDY OF OUTCOMES IN CHRONIC MYELOID LEUKAEMIA IN N.IRELAND FROM 2000-PRESENT.

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Background: Imatinib mesylate given orally at a dose of 400my once daily is the standard of care as initial therapy of patients with chronic myeloid leukaemia in chronic phase (CML-CP). In 2009 the European LeukaemiaNet (ELN) released reviewed guidelines which are the current standard of care of patients with CML-CP.
Aims: Reviewing our CML-CP population, diagnosed and treated in the imatinib mesylate era, for response to therapy using current guidelines.
Methods: All patients aged 16 years or more with newly diagnosed CML-CP from 2000-present are included in this study. Considerable effort was taken to include all CML cases, from the regional cytogenetic laboratory, case note review and specific enquiry of clinical haematologists. Response rates were defined as per the 2009 ELN recomendations.
Results: 77 cases of CML-CP were diagnosed pre-2009. 52 (67.5%) achieved complete haematological response (CHR) and subsequent complete cytogenetic response (CCyR) as per the 2009 ELN recommendations. 35 (68.7%) of the 51 continued to a major molecular response (MMolR) by the recommended milestone. 68 were imatinib mesylate-treated, with 50 (73.5%) in CCyR at 12 months, and 35 (51.5%) having MMolR at 18 months.
20 (77%) of the 26 assessable cases achieved CCyR within 12 months in the CML-CP patients diagnosed from 2009 onwards. 17 (71%) of the 24 cases assessable achieved MMolR at 18 months.
Summary / Conclusion: Lucas et al, showed 40% of all patients to be in complete cytogenetic response equivalence (CCRe) at twelve months, and 41% for imatinib mesylate-treated patients. The outcome of our population-based study are markedly superior to similar studies outside of a trial setting. Compliance appears to be an issue in some patients failing to meet the recommended milestones of response.

B1393

ASSOCIATION OF GENE HLA-DRB1 POLYMORPHISM WITH THE OUTCOME OF THERAPY OF CHRONIC MYELOID LEUKEMIA WITH IMATINIB

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Background: The outcome of chronic myeloid leukemia (CML) therapy with imatinib mesylate depends on a number of the proved factors: risk group, mutation status, presence of chromosomal aberration. In some patients with CML imatinib therapy at the chronic phase with the dose 400-600 mg gives quick and deep response, the other part of the patients is resistant to treatment. This group of patients needs earlier transition to the second line and, what is more important, to begin treatment CML in those patients without administration of imatinib (it is preferable).
Aims: The search of immunogenetic markers, making it possible depending on the presence of the certain specificity of the HLA-DRB1 gene to choose the individual set of therapy, including the first line group medicine.
Methods: HLA-typing (PCR-SSP) of the 50 patients with CML under chronic phase treated with imatinib during 24 month has been carried out. Average age 50,3±15,0, correlation of males and females in the research was 1:1. The dose of imatinib was increased more that 400 mg per day in 35(70%) patients. The results of genotyping of the 94 donors (L.V. Saroyants) were used as the control data. All the patients with CML and the donors referred to the group of eastern-European Slavs. Statistical processing has been carried out with the use of x^2. The index of the association RR strength relative risk, etiologic fraction – EF, preventive fraction – PF. Critical level of the significance under control of the statistical hypotheses in the given research was considered equal to 0,05.
Results: It is found that in the patients with CML, having reached complete cytogenetic response (CCyR) in 24 month of imatinib therapy there noted the increase more than 4 times, specificity rate DRB1*16(02), (20% versus5,3%; RR-4,37, EF-0,154). Rate increase of the gene HLA DRB1*14(06) in comparison with the control was 12% versus 0% (RR-29,4, EF-0,116, P<0,01) in the patients with CML who have not got CCyR in 24 month of therapy. The rate of DRB1*15(02) in this group in comparison with the control was decreased, i. e. 8% versus 28,7% (RR-0,26, PF-0,188, P<0,025). Under comparative analysis of the gene profile HLA DRB1 in the patients with CML at the CCR and with the absence of CCyR in 24 month of therapy negative associations with the specificity if DRB1*11(20% versus 48%; RR-0,29, PF-0,363, P<0,025); DRB1*12(0% versus 8%; RR-0,18, P<0,05); DRB1*14(0% versus 14%; RR-0,13, p,<0,025) have been found. The increase of the rate of HLA-DRB1 (in comparison with the control) 14(06) –8,8% versus 0% (RR-21, EF-0,084, P<0,025) and the decrease of HLA-DRB1*15(02)-8,8% versus 28,7% (RR-0,27, PF-0,195, P<0,01) have been noted in the patients with CML, who have not got complete molecular response (CMR) in 24 month of therapy.
Summary / Conclusion: The marker of the unfavorable prognosis of CML without CCyR and CMR in 24 month of therapy with imatinib in the specificity of DRB1*14(06); the markers of the unfavorable prognosis of CML without CCyR in 24 month of therapy with imatinib are the specificities of DRB1*11(05), DRB1*12(05). It is more advisable to begin therapy of the patients with CML, having specificity DRB1*14(06), 11(05), 12(05), with the inhibitors of tyrosinkinases of the second line. The marker of the favorable prognoses having CCyR and CMR in 24 month of therapy with imatinib is the specificity of DRB1*16(02). Specificity of DRB1*15(02) possesses protective property. Under the presence of the specificities HLA DRB1*16(02), DRB1*15(02) in the genotype of the CML patient it is possible to use imatinib as the medicine of the first line.

B1394

ACCELERATED PHASE OF CHRONIC MYELOID LEUKEMIA: THE IMPACT OF THE DIFFERENT CRITERIA ON TREATMENT RESPONSE AND SURVIVAL

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Background: Chronic Myeloid Leukemia (CML) is a clonal myeloproliferative disease which progresses from an indolent to a more aggressive phase, the accelerated phase (CML-AP). Unless effective therapy is instituted, it transforms to a blastic phase (CML-BP). Even though CML-AP is well recognized, the criteria that define this phase vary in the literature. Thus, different definitions of AP have been described and the heterogeneity among patient outcomes and response is marked.
Aims: This study was designed to analyze the several existing criteria and correlate them with treatment response and prognosis in a CML-AP Brazilian population of a referral center.
Methods: This is a retrospective study from January 2000 to November 2011 in which data from the chart of 143 patients with CML-AP (according to any of aforermentioned criteria) treated with imatinib, were selected from BMT Center database of Hospital de Clínicas of Universidade Federal do Paraná, Brazil. Survival data was analysed by Kaplan-Meier method. P level of signifcance was defined as 0,05. Univariated and Multivariated analysis were performed using STATA program version 8.0. Primary endpoints were minor cytogenetic response, survival and death.
We evaluated as risk factors all variables included in previous reported classification of accelerated phase CML (blasts >10% in peripheal blood, basophils >10% in peripheral blood, spleen >10 cm from left costal margin, hemoglobin ‹10 g/dl, platelets >1.000.000 and <100.000, clonal evolution) as well as the development of hematologic toxicity after imatinib.
Results: 43.4% of the patients were female and 56.6% were male. Median age was 45 years. Multivariate predictors of poor outcome were hematologic toxicity (P=0.003) hazard ratio (HR) of 2.53; 95% confidence interval (95%CI), 1.36-4.70, blasts (P=0.003) (HR of 2.99; 95% CI, 1.45-6.16, WBC (P=0.008) HR of 3.80; 95% IC, 1.41-10.22) and anemia (P=0.005), HR of 2.53; 95%CI 1.32-4.84)
Summary / Conclusion: These data indicate that patients with the above risk factors have a worse prognosis than some other also classified as AP patients. This information can guide therapy to be instituted. Prospective multicenter studies are necessary to achieve a classification that can be used universally in the era of TK inhibitors.

B1395

COMPARISON OF THE SOKAL AND EUTOS PROGNOSTIC SCORES IN PATIENTS WITH CML: ONE CENTER'S EXPERIENCE

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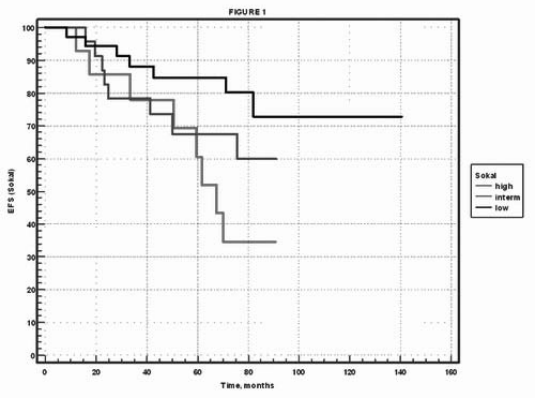
Background: European Treatment and Outcome Study (EUTOS) score is the new prognostic system developed by the European LeukemiaNet for newly diagnosed chronic myeloid leukemia (CML) patients in chronic phase (CP) treated with imatinib (IM). Its prognostic value has been discussed by several study groups with some discrepancies in their results.
Aims: This retrospective study was intended to compare EUTOS score against conventionally used Sokal score in CML-CP patients treated in our institution with imatinib either frontline or after different pretreatments.
Methods: 72 CML patients treated with imatinib were analyzed. In 23 of them IM was administered frontline; in 25 – after interferon failure; in remaining 24 – after long term treatment with hydroxyurea. Risk subgroups were determined using Sokal and EUTOS criteria at disease diagnosis. Each subgroup was evaluated for the rate of complete cytogenetic response (CCyR) at3,6, 9, 12, 18 and 24 months of IM treatment, as well as for event-free survival (EFS), measured

from the start of IM until one of the following events: death from any reason, acceleration phase, blastic phase, loss of complete hematologic response or loss of CCyR.
Results: High risk criteria were found in 14 patients (19,4%) according to the Sokal and 8 (11,1%) - according to EUTOS systems. Low risk subgroup accounted for 35 (48,6%) and 64 (88,9%) patients according to Sokal and EUTOS respectively; 23 (32%) patients were considered as Sokal intermediate risk subgroup. No significant difference in CCyR was found between all risk groups at 3 months of imatinib therapy. Beginning from 6 months CCyR was clearly lower in high risk patients according to both scores as compared to low EUTOS or low and intermediate Sokal risk patients. Importantly, no difference was revealed between low and intermediate Sokal risk groups in terms of rate of CCyR and time to its achievement. As to the EFS, no significant difference was revealed for all risk groups according to both scores at 12, 24 and 36 months. Starting from 48 months EFS was clearly better in low risk patients than in high risk following Sokal and EUTOS. Notably, no difference was detected between intermediate and high risk Sokal patients at this point. However, at 5 years further decrease of EFS in high risk patients was detected resulting in discrimination between high and intermediate Sokal risk groups, which grew even more prominent at 72 and 80 months (Fig.1). Difference between EFS of the two EUTOS risk groups appeared to be less apparent.
Summary / Conclusion: Analyzing collected data we conclude that for our group of patients both Sokal and EUTOS scoring systems effectively prognosed cumulative rate of CCyR at different time points dividing the investigated group into 2 prognostic subgroups with intermediate Sokal risk group losing its prognostic importance. As to the EFS, however, Sokal score seemed to discriminate more distinctly than EUTOS between low risk and high risk patients distinguishing also prognostically valuable intermediate risk group. These results however may be significantly influenced by the heterogeneity of the investigated sample in terms of their pretreatment.



B1396
DYNAMICS OF T315I MUTATION IN PHILADELPHIA POSITIVE PATIENTS ON TYROSINE KINASE INHIBITOR THERAPY
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Background: The discovery of imatinib, as a first smart drug, brought revolution into treatment of patients with Philadelphia positive chronic myeloid (CML) or acute lymphoblastic (ALL) leukemia. Development of ABL kinase domain mutations is still one of the major reasons for resistance to imatinib therapy. Second generation tyrosine kinase inhibitors (TKI) have the power to overcome all ABL mutations except T315I mutation which still represents one of the biggest challenges for treatment and indicates in most cases very low survival rate.
Aims: The aim of the study was to investigate the dynamics of T315I mutation development and bcr-abl1 transcript level kinetics in Philadelphia positive CML and ALL patients.
Methods: Ten patients with T315I mutation (6 women and 4 men), median age of 49.5 years at the time of diagnosis (range 34-62 years) were included in the study. Nine of them had CML with major bcr-abl1 transcript (7 with b3a2 type and 2 with b2a2 type) and one had ALL with minor bcr-abl1 transcript (type e1a2). T315I mutation was detected by allele specific oligonucleotide PCR (Kang et al, Haematologica, 2006;91:659) with declared sensitivity of 0.001%. Bcr-abl1 transcript level was measured by real time quantitative PCR and results were expressed according to International Scale (IS - CML patients only). Bcr-abl1 transcript quantification and T315I mutation detection were done in 8 diagnostic samples and in all follow-up RNA samples.
Results: Three patients who were diagnosed with CML before 2001 were treated with interferon-alpha, busulfan or hydroxyurea while the ALL patient was on chemotherapy before switching to imatinib. Other six patients had imatinib as a first line of therapy. After imatinib failure in 3 patients, therapy was switched to second generation TKI. Eight patients progressed to blast crisis (5 ALL and 3 AML) and 2 to accelerated phase. Eight patients died and 2 patients in whom T315I mutation was detected in August of 2012 and January of 2013 are still alive, one is in blast crisis and the other on chemotherapy after relapse and is waiting for hematopoietic cell transplantation. None of the investigated patients had T315I mutation at the time of diagnosis. Median time of the T315I occurrence after diagnosis was 30.5 months (1-121 months). Retrospective analysis of T315I mutation showed that in 3 patients mutation could be detected in earlier follow-up samples that correlated with bcr-abl1 transcript level elevation. Three patients became T315I positive after therapy with dasatinib, which can be the effect of dasatinib on other existing mutations that allow progression of T315I mutation clone. CML patients (except 1 unavailable patient's RNA) had median of bcr-abl1 transcript level 28.2% IS (range 16.0-41.3) at the time of detecting T315I while bcr-abl1 transcript level in ALL patient was 32.1%. Two patients developed T315I in less than 3 months from the time of diagnosis. Although other 4 patients had good response to therapy (3 patients were in MMR for 4-48 months), bcr-abl1 transcript level escalated because of T315I. Last 4 patients had constantly high bcr-abl1 transcript levels during follow-up, similar to diagnostic ones despite the fact that T315I mutation was detected 39-113 months after diagnosis.
Summary / Conclusion: In conclusion, the use of sufficiently sensitive molecular method in patients with bcr-abl1 transcript level elevation is important because early detection of small numbers of mutated clone, particularly for T315I, can prevent inadequate treatment with TKI as well as blast crisis, allowing more time to search for hematopoietic cell donor.

B1397
LOSS OF PREVIOUS COMPLETE CYTOGENETIC RESPONSE AND ABRUPT BLAST TRANSFORMATION DURING LONG TERM FOLLOW UP OF CHRONIC MYELOID LEUKEMIA PATIENTS IN CHRONIC PHASE TREATED WITH IMATINIB
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Background: Most of patients with CML in chronic phase achieving complete cytogenetic response (CCgR) have excellent long term results. But in small proportion of patients the events, like loss of previous good response or sudden transformation can influence treatment outcome.
Aims: To analyze occurrence of two events: loss of previous achieved CCgR (LCCgR) and occurrence of abrupt blast transformation (BC) of CML and factors associated with those events.
Methods: Cohort analysis in 151 patient with CML in chronic phase treated with frontline imatinib (IM, Gleevec®) and subsequently with other TKI (nilotinib and dasatinib) in 8 year period (2004- Sept 2012) in single academic medical center.
Results: We identified 11 patients with LCCgR and 3 patients with sudden BC in observed period. Patients without previous CCgR were not included.
Abrupt BC was seen in 3 Ph+ patients. At diagnosis, all Sokal score groups were present (one patient each) and two patients were younger than 40y. In all patients, BC occurred within two years of treatment, in 5th, 8th and 14th month. In two patients with BC (at 14th and 8th mo) previous cytogenetic follow up revealed good therapeutic response to imatinib (twice CCgR and 5% Ph+), without blasts in corresponding bone marrow. One patient was treated with dasatinib, achieving again CCgR but relapsed after SCT. Two young patients were treated with chemotherapy, one died within several days, and second one achieved remission but later died due to complications in SCT. Loss of CCgR occurred 12 to 76 months of IM treatment (mean 45 mth). Seven patients had intermediate and two had low or high risk Sokal score respectively. All patients had low EUTOS score, mean 41 (range 11 to 82). 8 of 11 pts achieved CCgR up to 6 mth, and 3/11 up to 1 year. In time of LCCgR mean age was 44 years. None of patients lost CHR, and LCCgR was detected only by regular cytogenetic follow up according to guidelines. In time of relapse, mean number of Ph+ cells was 43%, ranging from 5-90%, and 4 of 11 patients had additional cytogenetic aberrations /-Y in two, -8, and t(5;6;12)/. Bone marrow was corresponding to CML in most of cases, but in two cytological features of CML were almost absent. Six pts were treated with IM 800mg and 4 of them achieved second CCgR. One failed and other one was transferred to nilotinib with achievement of second CCgR on nilotinib. Four pts are treated with nilotinib only, and all of them achieved second CCgR. One patient progressed to BC and subsequently died. Additional cytogenetic aberrations had no influence on treatment outcome in patients treated with both TKIs. From 11 patients with LCCgR, 9 of them are still alive and are in second CCgR.
Summary / Conclusion: Occurrence of sudden BC of CML is rare but deleterious event in course of CML, and unfortunately it could not be predicted. LCCgR is also not frequent event, occurring in 7% of patients in our cohort, or less than 1% a year. Successful treatment result can be gained by second generation TKI (5 of 5 pts) but also in some patients with IM escalation to 800mg (4/6). Regular molecular follow-up can identify patients who are at risk for LCCgR.

B1398

CYTOGENETIC AND MOLECULAR RESPONSE AT 3 MONTHS AFTER SECOND-TKI THERAPY AND CORRELATION WITH LONG-TERM OUTCOMES FOR CML CHRONIC PHASE PATIENTS. EXPERIENCE IN ONE CENTER

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Background: The use of tirosin kinase inhibitors (TKIs) are the major advance in the therapeutic of Chronic Myeloid Leukemia in chronic phase (CML-CP). Imatinib was the first TKI and the results was encouraging but about 30% of patients will be discontinue it in the first 5 years by resistance or intolerance. Monitoring response to TKI therapy is a critical component of managing CML, and molecular response seems to be the most important milestone for predicting long-term outcomes. Marin et al (JCO 2011 38.6565) created a new score for predict long-term outcomes in patients treated with TKI's in first-line, but for second-line therapy, this still remain blinded, recently the MD Anderson group (Clin Lymphoma Myeloma Leuk: S2152-2650(12)00291-1) published that the achievement of a complete cytogenetic response (CCyR) at 3 months after switch to a second TKI predict a 98% of event free survival (EFS) for patients in chronic phase CML (CML-CP)
Aims: To describe characteristics of CML patients who were switched to a second-TKI after Imatinib failure/intolerance in our institution and applied the monitorization at 3th months after second-TKI therapy and correlated with outcomes
Methods: A review of clinical records of patient treated with second generation TKI since May 2005 to February 2013 was performed. Data on clinical characteristics, date of diagnosis, first ITK therapy and date of switched to second-TKI and outcomes were registry
Results: 19 CML patients have been treated with a second-TKI in this period, all in chronic phase. Median age: 60.68 years (38-80); 11:8 male/female ratio. Mean causes of change was resistance (9/47.8%): lost of response (8/42.1%), suboptimal molecular response (1); adverse effects to imatinib (10/52.2%). Dasatinib was the second choice for 16 patients and Nilotinib was for 3 patients. The time between diagnosis and use of ITK was variable and not have improved outcomes, on the other hand the presence of mutations have been negative impact in Overall Survive (OS), one patients developing T315I mutation and progress to blast crisis, 2 patient present M244V and 1 present E355G all of them lost response to imatinib, but had response to dasatinib. Overall response: at the moment of change 6 patients were in non-cytogenetic response (NCyR), two in partial cytogenetic response (PCyR), 4 in MCyR and 7 in CCR/MMR. At 3th month molecular assessment (MA) 6 patients remain without response (1 without mutation identified and switched to imatinib by failure, one for M244V mutation and one for T315I mutation without response), 3 in MMR and 6 in CMR, for 4 patients 3 months MA were unavailable at the moment of redaction. Actual status: 2/6 patients classified as high risk at 3 months MA shows a suboptimal molecular response (one of them only achieved a PCyR), three achieved a MMR and for 1 actual status was unavailable for missing control, of the rest of patients (13) achieved a MMR, 9 are in CMR. A second change to another TKI were registered for 4 patients, of them 1 were for the presence of M244V mutation who were switched from Nilotinib to Dasatinib, another for pleural effusion who were changed to Nilotinib, but later the patient requested to change to Dasatinib for digestive intolerance; the third-one was switched from Nilotinib to Dasatinib for dermatologic toxicity and the last-one after a suboptimal response to imatinib, nilotinib and dasatinib was recently switched to bosutinib.
Summary / Conclusion: Cytogenetic and molecular response at 3 months after switched to a second-TKI in patients with CML-CP could be an unvalued tool for an appropriated follow-up and predict long-term outcomes. More studies on this field are warranted

B1399

INCIDENTAL FINDING AND EVOLUTION OF PH+ CLONE IN TWO PATIENTS WITH KIDNEY/PANCREAS TRANSPLANT AND DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Detection of the Philadelphia (Ph) chromosome, a derivative of the reciprocal translocation t(9;22)(q34;q11.2) is a recognized clinical hallmark for chronic myeloid leukemia (CML) diagnosis. CML is often suspected on the basis of peripheral blood (PB) leukocytosis with "left shift", eosinophilia and basophilia. At the time of diagnosis, hematopoiesis results mostly from Ph+ clone, which is quickly debulked by target therapy with tyrosin kinase inhibitors (TKI).
Aims: Here we update the clinical course of two previously described patients incidentally diagnosed with CML, followed with cytogenetic and molecular monitoring and subsequently treated with imatinib after Ph+ clone expansion.
Results: A 65 years old woman was diagnosed with stage IVA Diffuse large B-cell lymphoma, in april 2011. Unexpectedly, bone marrow (BM) cytogenetic analysis showed 22% of 25 metaphases carrying the t(9;22) as sole alteration; RT-PCR identified b3a2 variant of BCR-ABL transcript. CBC count was normal and no splenomegaly was present. The patient underwent six courses of R-CHOP with achievement of complete remission. BM evaluation after the completion of chemotherapy (October 2011) did not show evidence of lymphoma or leukemia; conventional cytogenetic analysis was not evaluable but FISH analysis detected BCR-ABL fusion signal in 1.6% of 300 interphase nuclei; PB RT-PCR showed 1.5% IS of BCR-ABL transcript. Subsequent molecular evaluations showed a progressive increase of PB BCR-ABL transcript (5.9%IS on February and 22.7% IS on November 2012) and concomitant cytogenetic analysis revealed expansion of Ph+ clonal hematopoiesis (50% Ph+ in 14 metaphases). Therefore, TKI therapy was started and, after 3 months, a major molecular response was achieved. A 51-year-old man with type one diabetes mellitus who underwent kidney/pancreas transplantation in June 2005. Shortly after transplant, he developed stable mild leukocytosis with neutrophilia, which was not furthly investigated as it was ascribed to steroid therapy. However, an unscheduled quantitative RT-PCR analysis performed in October 2011 revealed the presence of 1.01% IS BCR-ABL transcripts (both b3a2 and b2a2 variants). Bone marrow evaluation showed normal cellularity but cytogenetics confirmed peripheral blood findings disclosing 12% of metaphases with t(9;22) translocation and 11%IS BCR-ABL transcript. As concomitant immunosuppression was considered a risk factor for disease progression, imatinib therapy was started and rapid reduction of BCR-ABL transcript was achieved (0.5%IS after 45 days). Unfortunately, renal function worsened, leading to TKI discontinuation. While off-therapy, the patient was monitored with PB RT-PCR analysis every three months, but a further increase of the BCR-ABL transcript was observed (on August 2012 PB RT-PCR 6.23%IS). In addition, cytogenetic analysis demonstrated an increase from 13% to 30% of 30 metaphases carrying the t(9;22), therefore TKI therapy was resumed, with rapid achievement of major molecular remission after 3 months of treatment and no side effects were reported.
Summary / Conclusion: The follow-up of these patients demonstrated a significant raise of BCR-ABL transcript in both cases within few months. As progression to overt CML seems to be ineluctable because of Ph+ clone growth advantage over normal hematopoiesis, we consider a reasonable approach starting TKI treatment even in the absence of classical hematological hallmarks of disease.

B1400

EXPRESSION PATTERN OF CANCER-TESTIS ANTIGENS SP17, GAGE1, HAGE, NY-ESO1, MAGE1, PASD1, SCP, SEMG, SLLP1, SPANXA, SSX1 AND PRAME IN CML.

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Background: This study was performed to evaluate mRNA expression of cancer-testis (CT) antigens SP17, GAGE1, HAGE, NY-ESO1, MAGE1, PASD1, SCP, SEMG, SLLP1, SPANXA, SSX1 and PRAME in peripheral blood of CML pts.
Aims: We sought to analyze cancer-testis genes expression profile in peripheral blood cells of CML pts and its association with distinct stages of the disease (CP and AP&BC).
Methods: In this study we used RQ PCR to analyze and compare mRNA expression of SP17, GAGE1, HAGE, NYESO1, MAGE1, PASD1, SCP, SEMG, SLLP1, SPANXA, SSX1 and PRAME. Quantitative expression analysis of cancer-testis antigens was carried out relatively using the expression of a housekeeping gene ABL as endogenous control to compensate for irregular cell numbers.
Results: In blood of CML chronic phase pts (N=36) we observed HAGE, SLLP1, SPANXA and PRAME gene expression with frequencies of 61,11% (22/36), 36,11% (13/36),2,78% (1/36) and8,33% (3/36), respectively. In the case of AP&BC of CML we observed not only HAGE (30,77%, 4/13), SLLP1 (15,38%, 2/13), SPANXA (23,08%, 3/13) and PRAME (38,46%, 5/13) gene expression, but also antigens SP17 (7,69%, 1/13), GAGE (7,69%, 1/13) and MAGE1 (23,08%, 3/13). In contrast to CP, expression frequency of some genes in AP&BC was lower (HAGE 30,77% against 61,11% in CP and SLLP1 15,38% against 36,11% in CP) and some higher (SPANXA 23,08% against2,78% in CP and PRAME 38,46% against8,33% in CP). Expression level of genes found in CP did not differ significantly to compare with AP&BC. The following antigens showed no mRNA expression both in CP and AP&BC of CML: NY-ESO1, PASD1, SCP1, SEMG and SSX1.
Summary / Conclusion: While the CT-gene expression profile was rather similar both in CP and AP&BC, there is a clear trend for some gene expression incidence to decrease or to increase during progression of CML from CP to AP&BC. Interestingly, the former group of genes corresponds to those known to be active in the later stages of spermatogenesis and the latter group corresponds to earlier stages of this process. It seems that progression of CML depends at

least partially on the activation of molecular mechanisms responsible for an early stage of spermatogenesis.

B1401
THE STUDY OF THE INFLUENCE OF PRIOR THERAPY ON RESPONSE TO TYROSINE KINASE INHIBITORS THERAPY IN CML PATIENTS
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Background: Despite the high efficiency of the treatment of tyrosine kinase inhibitors (TKI) part of patients with chronic myeloid leukemia (CML) in the chronic phase (CP) , and the most patients in phase of acceleration (AP) and blastic phase (BP) are initially insensitive to TKI or lose the response during the treatment. There are many causes that lead to treatment failure to TKI. Moreover, the mechanisms of primary and acquired resistance to TKI still is not completely clear. One possible factor of resistanse is the impact of prior therapy of various drugs on the response to TKI.
Aims: To study the effects of previous therapy of busulfan and hydrea on response to imatinib therapy in CML patients.
Methods: We observed 87 patients with CP-CML pretreated by busulfan and 387 patients with CP-CML pretreated by hydrea. Diagnostics and monitoring of CML were perfomed on the basis of cytogenetic examination of bone marrow cells by G-banding, and also molecular-genetic research of bone marrow and peripheral blood.
Results: The efficiency of TKI application was evaluated after 12, 18, 24, 30, 36 monthsof therapy. The complete cytogenetic response (CCyR) in patients pretreated by busulfan was received approximately in 17 % of patients, and more than 80% of patients had the failure to achieve a complete response. The effect of the duration of previous busulfan therapy to the achievement of response to imatinib in CML patients has also been studied. The patients who reached CCyR had been taking the prior busulfan therapy no more than 22 months. The failure to Imatinib treatment was observed after 43 months of busulfan therapy. Studying the impact of the previous therapy of hydrea on the effectiveness of TKI therapy showed that the optimal response achieved 5.5 times more frequently in patients who previously had received hydrea not more than 6 months comparing with the group of patients receiving this drug for more than 6 month (71.6%±3.9% and 13.0%±2.1% respectively, P<0.05).
Summary / Conclusion: The previous use in patients with CML hydrea which lasts more than 6 months and busulfan significantly deteriorates results of TKI therapy.

B1402
FAST PROGRESS OF A CML PATIENT WITH UNUSUAL GENETIC ABNORMALITIES – A CASE STUDY
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Background: Tyrosine kinase inhibitors have revolutionized therapy of CML (chronic myeloid leukemia) in the last decade. However, different genetic events may influence therapy success and survival. Here we present a CML patient with a *BCR-ABL1* minor breakpoint rearrangement uncommon in CML and a mosaic of two different isodicentric Philadelphia chromosomes.
Aims: As rare genetic alterations may influence outcome of CML patients, we wanted to characterize these events in more detail.
Methods: Thirty metaphases were analyzed by conventional cytogenetics using Giemsa-banding. FISH was performed using Abbott *BCR-ABL* dual color dual fusion probes. *BCR-ABL1* PCR was done according to the EACR protocol (Gabert et al). Bidirectional sequencing of *BCR-ABL1* cDNA covered the amino acids 239 to 415 of the *ABL1* gene. For SNP array, Affymetrix Cytoscan HD platform was used according to the manufacturer.
Results: Conventional cytogenetics showed a t(9;22)(q34;q11) in all metaphases. Two subclones with a small (23%) and a large (27%) isodicentric Philadelphia chromosome were detected. *BCR-ABL1* FISH confirmed a *BCR-ABL1* gain in these two subclones with three and about seven fusion signals, respectively. PCR detected a *BCR-ABL1* minor breakpoint rearrangement rarely described in CML. Neither a *BCR-ABL1* nor a *JAK2* (V617F) mutation could be found. SNP array indicated a gain of 9q34.12q34.3 and 22q11.1q11.23 with a copy number state of 3. Interestingly, a small segment on 22q11.22 showed a copy number state of4, including micro RNA 650. Alterations of this miRNA seem to influence aggressiveness in colorectal and gastric carcinomas, melanoma and chronic lymphocytic leukemia.
Summary / Conclusion: We describe a CML patient with rare genetic events and death by insult few days after initial diagnosis. Beside a mosaic of two different isodicentric Philadelphia chromosomes resulting in *BCR-ABL1* gain, we could detect a possible involvement of miRNA 650, which is suspected to alter aggressiveness in different cancer types.

B1403
THE ALTERATION OF CPK IN CML PATIENTS TREATED WITH IMATINIB HAS IMPACT ON RESPONSE AND TOXICITY?
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Background: The observation of increased CPK in some CML patients treated with imatinib, has raised the question how to affect this change in patients managment.
Aims: The aims of this study was to evaluate wheter ther are differences in treatment response and toxicity betwen the two groups of patients. From 2003 to now we followed 47 consecutive new patients with chronc myeloid leukemia. 44 was evaluable. 57% of these, showed an increase of CPK (cpk+), and 43 % did not show this increase (CPK-).
Methods: We observed that there is no differences between the two groups of patients in terms of OS, PFS and EFS. In CPK+ group, 11/25 (44%) are in COMPLETE MOLECULAR RESPONSE, 10/ 25 (36%) in MAJOR MOLECULAR RESPONSE , whereas in CPK- group, 7/19 (37%) patients were in CMR, 6/19 (32%) in MMR, and 6/19 (31%) in suboptimal response. The median months for the major molecular response in these cohorts of patients was 12 and 17 months in CPK+ and CPK- groups respectively. In CPK+ group 76% patients suffered of muscle pain and fatigue of grade I-II (sec CTC), correlated with the values of CPK. In CPK- group only 5% suffered of muscle pain. No patiens discontinued treatment for toxicity in both group.
Results: 5/44 (11%) patients changed TKI, two for progression (1 CPK+ and 1 CPK-), three for suboptimal response (0 CPK+ and three CPK-)
Summary / Conclusion: In conclusion although the number of patients is low, the alteration of CPK in patient with CML treated with imatinib seems to correlate with increased muscle toxicity. More rapid and intense achievement of major molecular respnse with significant difference (80% vs 69%). Such observation may be considered CPK as a prognostic factor for response and compliance treatment and may affect the managment of early shift therapy in these patients to other TKI.

B1404
CHRONIC MYELOID LEUKEMIA ASSOCIATED WITH OTHER HEMATOLOGIC AND NON-HEMATOLOGIC MALINANCIES: REPORT OF FOUR CASES
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Background: Chronic Myeloid Leukemia (CML) accounts for approximately 15 to 20 percent of leukemias in adults with an annual incidence of 1 to 2 cases per 100,000, and a slight male predominance. The median age at presentation is approximately 50 years for patients enrolled on clinical studies, but around 60 years in real life. CML secondary to other malignancies has been described in medical literature, in patients that received previous chemotherapy ou radiotherapy and at least one study showed increased risk of secondary leukemia up to 25 years after the first diagnosis. Less frequently has CML been diagnosed concomitant to other hematologic or non-hematologic malignacies. The unregulated growth of predominantly myleoid cells in the bone marrow seen in CML is caused by chromosomal translocation t(9;22)(q34;q11) - Philadelphia chromosome - generating the Bcr/Abl fusion kinase. This leukemogenic tyrosine kinase has a key role in the pathogenesis of this desease which promotes the survival and proliferation of CML cells. Other cellular pathways, including the process of angiogenesis, a targeted therapy in solid tumors, has been proposed as a potential role in leukemogenesis.
Aims: This presentation reviews the association of CML with other hematologic or non-hematologic malignancies, ocurring after or concomitant with these diseases. While chemotherapy ou radiotherapy for previous neoplasias may be implicated in the pathogenesis of CML in some patients, the concomitant diagnosis of CML and a hematologic or non-hematologic malignancy is a rarer condition that requires a new insight concerning the biologic, cytogenetic and biomolecular aspects of the problem.
Methods: We describe four cases of patients with CML treated at Hospital Federal da Lagoa and at Clínicas Oncológicas Integradas (COI) since 2003. Two of them have had the diagnosis of CML at least two years after the previous neoplasia (breast cancer and plasmocytoma) and the other two had the leukemia concomitant to other diagnosis (breast cancer and MALT non-Hodgkin´s lymphoma). We discuss the difficulties in the diagnosis and in the treatment of these patients. The cytogenetic, biomarkers of each condition and the biomolecular (Bcr/Abl level) evolution in each patient are described when available.
Results: The patients that presented with CML after the first malignancy have had good response to treatment with imatinib with major molecular responses. The patient with MALT non-Hodgkin´s lymphoma received rituximab based chemotherapy treatment which brought his leucocyte count to normal values during the treatment. The fourth patient that had breast cancer and CML diagnosed at the same time developed pancytopenia secondary to myelodisplasia and became transfusion dependent during imatinib (and later dasatinib) and

letrozole concomitant treatment. All patients are alive from two to ten years of follow up.
Summary / Conclusion: The coexistence of two different malignancies in the same patient is a very rare condition. Secondary malignancies in survivors of cancer after chemotherapy or radiotherapy, especially in breast cancer and lymphoma can be seen since the life expectancy in these disorders is long. The drugs or the radiation these patients receive during their treatment could provide the etiopathological basis for development of secondary cancers. However, other cytogenetic abnormalities besides the development of Ph cromossome, the production of certain cytokines and even angiogenesis may be implicated in the concomitant appearence of CML and other malignancies leading to new therapeutic possibilities.

B1405
LMO2 EXPRESSION DOES NOT EFFECT CYTOGENETIC AND MOLECULAR RESPONSE IN CML PATIENTS TREATED WITH IMATINIB
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Background: Chronic myeloid leukemia (CML) is a stem cell disease characterized with proliferation of mature and progenitor myeloid cells. The Philadelphia chromosome (Ph), resulting in the formation of the *BCR–ABL* fusion oncogene, is a specific cytogenetic abnormality in CML. Ph encodes different proteins, usually p210 in CML, with dysregulated tyrosine kinase activity causing leukemogenesis. Imatinib mesylate (IM) is the first tyrosine kinase inhibitor that specifically inhibit the activity of the BCR-ABL protein. Cytogenetic and molecular responses to IM treatment in patients with CML vary among users. A lot of factors have been accused that affect the treatment response. LIM domain only-2 (LMO2) is a transcript factor and its gene is localized in chromosome 11p13. LMO2 has a function of regulating erythropoiesis, embryogenesis, angiogenesis, and hematopoietic stem cell development. LMO2 protein is secreted from precursor of myeloid and red blood cells in the bone marrow. It is not detected in mature cells. LMO2 was first studied in patients with acute T-cell leukemia and has been typically recognized as a T-cell oncogene. LMO2 was also reported as aberrantly expressed in acute myeloid leukemia, CML, B-ALL and some non-Hodgkin B cell lymphomas. Though it is discussible LMO2 has suggested having prognostic significances on those diseases. In one study, it was shown that LMO2 protein expression was correlated with improved hematologic remission and overall survival in the CML patients treated with IM.
Aims: The aim of this study was to investigate the relationship between LMO2 protein expression, and cytogenetic and molecular response in the CML patients receiving IM, which has not been studied before.
Methods: This study included 32 patients diagnosed as CML between May 2007 and January 2011 in Ondokuz Mayıs University, Hematology Department. Imatinib 400 mg/day was started for all patients after diagnosis. According to European Leukemia.net (ELN) criteria's, the optimal responses to imatinib were evaluated at$_{3,6,}$ 12 and 18 months of the treatment. Hematological response was used for evaluation of the response at 3 months of the treatment, cytogenetic response for 6 and 12 months, and molecular response for 18 months.
LMO2 protein was studied with immunohistochemical methods in bone marrow biopsy materials at the time of diagnosis. Staining in greater than 30% of myeloid lineage cells was assigned as positive.
Results: All 32 patients were in chronic phase at the time of diagnosis. Average follow-up period was 29.4 (12-58) months. The Sokal risk scores of the patients were determined. Of 32 patients, 10 (31%) patients were in low risk group, and 17 (53%) patients in intermediate risk group and 5 (16%) patients in high risk group. LMO2 was stained as positive in 20 (62.5%) of patients and negative in 12 (37.5%). LMO2 staining did not significantly differ among Sokal risk groups (P=0.54). There were not statistically significant differences in LMO2 staining between patients who had optimal responses or not according to the ELN criteria's at$_{3,6,}$12 and 18 months of the treatment (P>0.05).
Summary / Conclusion: In our study, we did not find any relationship between LMO2 protein expression, and cytogenetic and molecular response in the CML patients receiving IM.

B1406
EIGHT YEAR RESULTS OF THE THERAPY OF CML TYROSINE KINASE INHIBITOR IN A LARGE INDUSTRIAL CENTER OF SIBERIA
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Background: The use of tyrosine kinase inhibitors (TKI) has significantly changed prognosis in patients with chronic myelogenous leukemia (CML). Thanks to a wide range of currently available approaches to the treatment of CML, modern cytogenetic and molecular techniques for the diagnosis and monitoring of treatment efficacy tyrosine kinase inhibitors has become possible to effectively control the previously incurable disease.
Aims: To evaluate eight year results of therapy of chronic myelogenous leukemia treatment with tyrosine kinase inhibitors.
Methods: For the period of January 2004 and up until now the 74 CML patients have been observed at the municipal hematology center of Novosibirsk – capital of Siberia, specifically: 65 patients in the chronic phase (CP), 7 patients in the accelerated phase (AP), and 2 patients with blast crisis. Those 74 patients included 29 men (38.2%) and 47 women (61.8%), with age ranging from 16 to 78 years, and the mean value being 44.7±15.1years. 82.9% patients was included in the analysis (63 patients): 56 people in the chronic phase, 5 - in the acceleration phase and 2 in blast crisis phase. All patients in the chronic phase started taking the TKI in the first 6 months after diagnosis of CML. Patients in accelerated phase and blast crisis were diagnosed before 2003, these patients were significantly pre-treated with various cytotoxic medications and interferons. All patients are receiving treatment by various tyrosine kinase inhibitors (imatinib at a dose of 400-800 mg per day, 3 patients - nilotinib at 800 mg per day, 2 of them - as first-line treatment, dasatinib 100-140 mg per day). In Russia, imatinib therapy became available only in 2004, as part of the charity program GIPAP (on treatment 10% of patients). All the patients treatment became available in 2005. Today in Russia are using generic medications Philachromin® and Genfatinib®, instead of using original medication Glivec® , their effectiveness requires further observation. 13 patients have died: 8 patients in chronic phase, for reasons not related to hematological malignancies, 2 patients in accelerated phase, and 3 patients in blast crisis phase because of the progression of the underlying disease.
Results: Administration of a TKI as a single agent was followed by a complete clinical and hematological response in 94.6% patients, complete cytogenetic response (CCyR) – in 80%, major molecular response (MMO) – in 66,1%. In 4 patients AP - clinical and hematological response, 3 patients in AP and patients in blast crisis - the stabilization process. Survival was analyzed in patients administered TKI, as compared to patients not administered TKI (data based on the retrospective review of medical records of CML patients observed at Novosibirsk municipal hematology center for the period of 1999 – 2004). A statistical method of calculating the cumulative fraction of survivals (Kaplan-Meier) was used to evaluate survival, with P<0,05 established as the reliability criterion. No medial survival was established in the group administered TKI, the 8-year survival - 82.9%, the estimated 10-year survival was 70%. In the group treated with other cytotoxic agents median survival was 4.1 years, the estimated 10-year survival rate - 9%, P<0,000001. Overall event-free survival rate was 56.6%, in the chronic phase - 68.3%.
Summary / Conclusion: TKI are considered an effective and safe method of treatment for CML in CP, associated with a high MCyR rate in the chronic phase, which leads to a significant increase in the overall and event-free survival.

B1407
DASATINIB IS EFFECTIVE IN ETV6-ABL1 CML LIKE DISORDER WITH COMPLEX TRANSLOCATION INVOLVING CHROMOSOME 9, 12 AND 14
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Background: A rare group of myeloproliferative disorders has been described associated with gene rearrangements producing novel tyrosine kinases other than BCR/ABL. A rearrangement involving the ETV6 and ABL1 genes, associated with t(9;12)(q34;p13) translocation, has been detected in Ph-negative chronic myeloproliferative disorders and has been demonstrated to have tyrosine kinase activity in signal transduction pathways similar to the BCR/ABL fusion protein. The ETV6 gene is a member of the E26 transformation-specific family (ETS) of transcription factors located at 12p13. It has been implicated in the rearrangement of over 48 different chromosome bands, ultimately playing a role in leukemogenesis. Thus far, only eleven BCRABL1-negative CML patients with a variant ABL1 gene (9q34) rearrangement, involving fusion to ETV6 aka TEL (12p13) have been reported. ETV6-ABL1 rearrangement have also been implicated in other hematologic diseases including 6 acute B lymphoid leukemia, 1 acute T lymphoid leukemia, 3 acute myeloid leukemia, 1 myelodysplastic syndrome, and 3 chronic myeloproliferative disorders.
Aims: We present a new patient with ETV6-ABL1 rearrangement and check the efficiency of second generation TKI.
Methods: He is a 62 year old male who sought medical attention for acute coronary syndrome with bone pain and asthenia in May 2011. Physical exam showed a splenomegaly. He was found to have hemoglobin of 9.9g/dL, WBC 90G/L and platelets 105G/L. The peripheral blood differential count revealed 67% segmented neutrophils, 2% eosinophils, 0 basophils, 3% monocyte, 28% immature stages and 5% lymphocytes per 100 white blood cells. Bone marrow biopsy revealed myeloid hyperplasia suggestive of a myeloproliferative disorder. The genetic characteristic of this patient is to present an exceptional chromosomal complex rearrangement according to Pellestor classification, involving 3 chromosomes 9, 14, 12 with five breakpoints: 9q, 12p, 12q, and two different on 14q. More precisely, it probably compounds of a three way translocation t(9;12;14)(q34,p13,q?) associated with a independent translocation between both long arms of chromosomes 12 and 14. FISH analysis using BCR/ABL TC DFusion (Kreatech) shows involvement of ABL1 probe in a translocation with short arm of chromosome 12 and no evidence of rearrangement of BCR gene suggesting the possibility of juxtaposition of ABL1 gene with a gene different than the BCR gene. Moreover whole chromosome 14 painting

confirms various involvements of chromosome 14. Molecular biology confirmed the suspected ETV6-ABL1 fusion gene and allows us to follow minimal residual disease. It should be noted that a t(9;12;14)(q34;p13;q22) has already been described as a variant of the t(9;12)(q34;p13) with complex insertions of ETV6 into ABL1. Other chromosome than the 14 has known to be implicated in t(9;12) variant as chromosome 17.
First, he was treated with imatinib with progression despite the addition of hydroxyurea. Treatment was switched at 3 months for dasatinib
Results: At 3 months, ETV6-ABL1/ABL1 is 0.32% and at 6 months he obtains a Major Molecular Response (defined as MRD under 0.1% like in BCR-ABL measurement). After 18 months, he remains in $MR_{4.5}$.
Summary / Conclusion: In conclusion, we present a new case of ETV6-ABL1 myeloproliferative disorder due to complex rearragement which experiments good response to dasatinib suggesting that second generation TKI are a good option for treatment of such disease.

B1408

IMMUNOLOGICALLY DETECTION OF BCR/ABL FUSION PROTEIN WITH FLOW CYTOMETRY IN K562 CHRONIC MYELOID LEUKEMIA CELLS AND COMPARISON WITH RT-PCR RESULTS

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Background: Chronic myeloid leukemia (CML) is a clonal myeloproliferative disease arising from a neoplastic transformation of hematopoietic progenitor cells, usually characterized with a specific chromosomal abnormality called Philadelphia (Ph) chromosome. Ph chromosome occurs with reciprocal translocation of BCR and ABL genes located on chromosomes 9 and 22, respectively. Follow up of minimal residual disease in CML patients is based on the detection of BCR/ABL mRNA by RT-PCR. Showing BCR/ABL oncogene by RT-PCR is a difficult and technician dependent method. Development and use of novel methods for diagnosis and follow up of CML patients are of importance in routine practice due to the difficulties in RT-PCR technique and its standardization.
Aims: In this study, we aimed to standardize detection of BCR/ABL protein using a flow cytometric method in K562 cells, and to compare the results with the molecular method which is the gold standard.
Methods: The study consisted of two arms. In the first arm, RT-PCR and flow cytometry methods were compared using nine different cell groups with gradually increasing amounts ($1x10^3$, $1x10^4$, $5x10^4$, $7.5x10^4$, $1x10^5$, $5x10^5$, $1x10^6$, $1.5x10^6$, $2x10^6$) in K562 cells. In the second arm, $1x10^7$ K562 cells were incubated for 72 hours with each of the 6 groups(1 control group and 5 different concentration of imatinib applied groups). Afterwards, cell viability analysis was performed and the results of the RT-PCR and flow cytometric analyses were compared.
Results: While BCR-ABL/ABL ratio was found to be positive in all groups with RT-PCR in the first arm of the study, BCR/ABL fusion protein could not be detected by flow cytometry in groups with 1.000 and 10.000 cells. BCR/ABL protein was found to be positive in the groups that contain 50.000 or more cells ($P<0.05$). Specifity and sensitivity were 100% when flow cytometry results of the groups thatcontain 50.000 or more cells as compared with RT-PCR. RT-PCR and flow cytometry results were positive in all groups in the second arm. The mean fluorescence intensity was observed to decrease in flow cytometry methods in those groups with reduced viability. However, RT-PCR and BCR-ABL/ABL ratios were not affected by the viability.
Summary / Conclusion: In conclusion, BCR/ABL fusion protein can be detected by using flow cytometry method, when a minimum of 50.000 viable cells are studied.

B1409

COMPARISON OF FLUORESCENCE IN SITU HYBRIDIZATION AND CHROMOSOME BANDING ANALYSIS FOR THE DEFINITION OF COMPLETE CYTOGENETIC RESPONSE IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH GLEEVEC.

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Background: Background: Chronic Myeloid Leukemia (CML) is a clonal disease characterized by balanced translocation between chromosomes 9 and 22 (Philadelphia chromosome). Imatinib mesylate (Gleevec) is the most successful among a new generation of specific inhibitors of signal transduction. It inhibits the BCR/ABL tyrosine kinase activity by competing with ATP at the ATP binding site of BCR/ABL. The BCR/ABL rearrangement is an important tumor classification marker and a useful prognostic factor allowing an adequate therapy management. Clinical trials of imatinib mesylate have shown promising results in chronic phase of CML.
Aims: The aim of our study is to determine the effectiveness of Gleevec in the treatment of patients with chronic myeloid leukemia (CML) during the chronic phase through cytogenetic and molecular responses and to compare these results
Methods: Methods: From March 2009 to September 2012, we analyzed 28 CML patients aged 18 -70 years old at chronic phase of disease in parallel with CA and interphase FISH. All patients were diagnosed at the Hematological Service in University Hospital Center ¨Mother Theresa¨ in Tirana. Eligible patients should not have received treatment for CML before and were randomly assigned to receive imatinib as the initial therapeutic treatment ambulatory at an effective oral (300-600 mg/day). Results were determined by analysis of peripheral blood, bone marrow aspiration, chromosome banding analysis (GAG banding) at 0,3, 9, 12 months and FISH (fluorescence in situ hybridization) at 12 months.
Results: Results: Fish results and the results of banding methods were directly compared. Based on the analyses of > 200 nuclei per patient, FISH correlated closely with CA. After 12 months therapy, 22 of these patients (78%) had cytogenetic responses, of which 9 patients or (32%) achieved complete cytogenetic responses and 2 of these patients (7%) had partial cytogenetic responses evaluated by chromosome banding analysis. In 3 cases with no Ph^+ metaphases in CA, interphase FISH detected 2 to10% BCR-ABL^+ rearrangements and 19 of 28 patients (67%) had complete molecular cytogenetic responses.
Summary / Conclusion: Conclusions: CA and interphase FISH give reliable results but in our study we confirmed that FISH is a sensitive technique for the evaluation of cytogenetic response in patients with CML. Furthermore, FISH probe detected BCR/ABL rearrangement, which were not visible by CA. We conclude FISH reliably detects standard Ph chromosomes as well as its variant translocations and accurately quantifies BCR/ABL rearrangements prior and during cancer treatment, in daily routine tumor cytogenetic diagnostics.
Continuation of the study with a larger number of patients is needed to confirm these preliminary observations.

Hodgkin lymphoma - Biology

B1410

B-CELL CLONES IN HODGKIN'S LYMPHOMA DETECTED BY NEW GENERATION SEQUENCING

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Background: In Hodgkin lymphoma few tumor Hodgkin/Reed-Sternberg (HRS) cells are scattered among the reactive cellular environment. B-cell nature of the tumor has been proven by single-cell PCR of immunoglobulin (Ig) genes from HRS cells isolated from histology sections [Küppers R et al, 1994]. It has been shown that in most cases Ig gene rearrangements result in functional alleles that carry significant amount of VH mutations [Marafioti T, et al 2000]. However approximately 25% of Hodgkin's lymphomas carry the nonfunctional rearranged IgH genes with "crippling" mutations [Kanzler H et al, 1996]. The presence of VH mutations indicates germinal or post-germinal nature of HRS cells. In a small percentage of cases (2 of 25 patients), subclonal differences in HRS has been reported [Marafioti T et al, 2000]. Despite the fact that 60% of patients carry functionally rearranged both IgH and IgK genes in HRS cells, Ig are not expressed on the cell surface, presumably due to the transcription errors [Re D et al, 2001; Jundt F et al, 2002; Theil J et al, 2001]. We set out to detect B-cell clonality in Hodgkin's lymphoma and subclonal variations in HRS cells using next generation sequencing (NGS) circumventing single cell PCR.
Aims: To study Ig genes, B-cell clonality and subclonal variations in Hodgkin's lymphoma by NGS.
Methods: The study included biopsy samples (5 FFPE and 2 frozen tissues) from 7 patients with classical Hodgkin lymphoma, verified by immunohistology (nodular sclerosis subtype, EBV-negative). Patients were 20 to 40 years old (mean 29 years), 6 women and 1 man. In 4 of the patients clear signs of monoclonality were obtained previously in IgH genes (FR1, FR2, FR3) using Biomed-2 concerted action primer sets [van Dongen JJ et al, 2003] and GeneScan analysis (ABI 3130). One patient had oligoclonal GeneScan profile and 2 patients were polyclonal. PCR amplified (35 cycles IgH FR1+10 cycles IgH FR2) DNA fragments were analyzed on GS Juniour (Roche) sequencing platform. Data analysis was performed with GSMapper (Roche).
Results: We obtained 7437 to 48673 sequence reads (average 17850) for each patient. More than 95% of the sequences were in 260-320 bp range as expected. Clusters of reads with more than 0.2% representation were analyzed. We observed 3 to 16 clones (mean 9.9) in the samples from patients previously shown to be clonal by GeneScan analysis. "Monoclonal" patients had one dominant clone with 7% to 35% abundance, with the length (+/- 1-2 nucleotides) matching that obtained by GeneScan. The only "oligoclonal" patient carried 4 clones with 11.8%, 11.6%,6,7% and 4.67% representation. Finally, two "polyclonal" patients also revealed low abundance clones (one patient - 3.1% and 2.7%, the other - 3.4%). Most dominant clones were productive. We also found subclones of dominant clones with minor nucleotide substitutions and deletions. It should be noted that some clones from different patients, being unrelated by nucleotide sequence, were translated to CDR3s wich were almost identical in amino acid sequence. It may indicate the possibility of antigen-driven clonal selection.
Summary / Conclusion: NGS allows to sequence rearranged Ig genes in Hodgkin's lymphoma without microdissection. Intraclonal variations occur in HRS cells. Possible signs of antigen selection also observed.

B1411

IDO IS FREQUENTLY EXPRESSED IN STROMAL CELLS OF HODGKIN LYMPHOMA AND IS ASSOCIATED WITH ADVERSE CLINICAL FEATURES

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Background: Hodgkin lymphoma(HL) is characterized by relatively fewer numbers of tumor cells and excess of reactive cellular components. Regulation of tumor microenvironment is thought to be involved in the prognosis of HL. Indoleamine2,3-dioxygenase(IDO), is an immune modulator acting through increase of regulatory T-cells and induction of T-cell anergy.
Aims: Purpose of the study is to investigate role of IDO in the microenvironment of HL.
Methods: A total of 124 cases of HL were enrolled to do immunohistochemistry for IDO, CD163, CD68 and FoxP3. Positivity was evaluated from area fractions of positive cells using automated image analyzer. The results were compared between the subtypes of HL and with various clinicopathologic parameters.
Results: IDO were variably expressed in histiocytes, dendritic cells, endothelial cells, but not in tumor cells. IDO positive cells were more frequently found in mixed cellularity type than nodular sclerosis, cases with EBV+, high Ann Arbor stages, presence of B symptoms, and high IPS(all P<0.05). Patients showing high IDO expression revealed significantly poorer overall survival(P<0.001).
Summary / Conclusion: IDO might be actively involved in microenvironment of HL and is related to adverse clinical outcomes of HL.

B1412

PROGNOSTIC SIGNIFICANCE OF CD68, CD20, FOXP3 EXPRESSION; PRESENCE OF EPSTEIN-BARR VIRUS AND MAST CELL INFILTRATION IN CLASSICAL HODGKIN LYMPHOMA PATIENTS

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Background: Classical Hodgkin Lymphoma (cHL) is a highly curable B cell lymphoma characterized by the presence of Reed-Sternberg (RS) cells in a background of mixed inflammatory cells and stromal reaction. The disease is unique in that the malignant RS cells constitute only the minority of the total tumor mass. Recently, emphasis is given to the contribution of the non-tumoral cells to disease outcome.
Aims: The aim of this study was to find out if there is a relationship between the composition of the immune infiltrate and clinical outcome in our cases with cHL.
Methods: Patients (pts) were retrospectively collected from 2000 to 2010 in one center (Cerrahpasa Medical Faculty, Istanbul). From approximately 250 adult cHL pts diagnosed at our center during this time period, tissue and data regarding clinical outcome were available for 57 pts (all HIV negative). The diagnosis derived from lymph nodes in all pts except one (spleen). Immunohistochemical analysis was performed on histological sections of formalin-fixed paraffin-embedded tissue samples from previously untreated in 51 (90%), and relapsed in 6 (10%) of the cases. The slides were stained for CD68 (Dako, KP-1), CD20 (Dako), FOXP3 (Leika), LMP1 (Dako) and tryptase (Dako). CD68 was scored 1: CD68+ cells <5%, 2: CD68+ cells from 5% to 25%, 3: CD68+ cells >25% relative to overall cellularity. Using cutoffs of <10%, 10% to 50% and >50% relative to overall cellularity, 3 scores were defined for FOXP3. RS cell positivity of CD20 was scored 0: when no staining, 1: <5%, 2: from 5% to 25%, 3: >25% relative to a total of RS cells. CD20 positivity of the surrounding reactive small B cells and presence of B cell follicles were also scored. Mast cell infiltration was evaluated by counting the cells and was scored 1: 0 to 10, 2: 11 to 25, 3: >26 cells. Expression of LMP1 on RS cells showed EBV infection. Clinical and laboratory data available on presentation and follow up were recorded. Sex ratio was 1.6, median age was 38 (range 13-77) years. Histologic subtypes according to the WHO classification system were nodular sclerosis in 30 pts (53%), mixed cellularity in 23 (40%), lymphocyte-depleted in 2 (3.5%) and unclassified in 2 (3.5%). 61% of the pts had Ann Arbor stage I-II and 39% had stage III-IV disease. 26 pts (46%) had B symptoms, 8 (15%) presented with bulky disease ($\geq$10 cm). Extranodal involvement was seen in 23 pts (40%). Among pts with advanced stage disease 71% had an IPS >2. Treatment protocol was ABVD in 55 pts (96.5%). Additional radiotherapy was performed in 20 (35%) pts. Median follow up was 59 (range 9-144) months. 1 patient included in the study had a follow up time less than 24 months (9 mo). Statistical analysis was made with STATA software (version 11). The study was approved by the institutional ethics committee.
Results: CD20 expression of both RS cells and reactive B cells, mast cell infiltration in the microenvironment and EBV positivity were not predictive of outcome. 18 pts (32%) were EBV positive. EBV status did not influence expression of any of the biomarkers analyzed. No significant association of mast cell infiltration with existence of B symptoms was found. CD68 expression and absence of lymphoid follicles were predictive of inferior overall survival. Overall survival was improved for patients with increased FOXP3 cell density.
Summary / Conclusion: Microenvironment composition is of prognostic value in cHL patients. For obtaining more accurate and reliable results we are intending to double the number of pts until the time of the congress of EHA.

B1413

PROGNOSTIC IMPACT OF PERIPHERAL BLOOD LYMPHOCYTE IMMUNOPHENOTYPES IN CLASSICAL HODGKIN'S LYMPHOMAS

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Background: Classical Hodgkin's lymphoma (cHL) is characterized by the presence of tumoral cells in a rich background of T and B cells, macrophages, and other inflammatory cells. The contribution of these non tumoral cells to the pathogenesis of HL is still poorly understood.
Aims: In our study we evaluated the prognostic significance of peripheral blood B,T, NK cells at diagnosis in 118 immunocompetent patients (pts) with cHL treated at our institution between January 2006 and December 2010.
Methods: 54 (46%) were male and 64 (54%) female. Median age at diagnosis was 33 years (range 15-82), 71 pts (60%) presented advanced stage (IIB-IV), 54 (46%) a bulky disease, 55 (47%) presented B symptoms.

Results: At the end of treatment, 94 patients (80%) were in complete remission (CR), while 24 in partial remission. 18 patients (15%) relapsed after a median follow-up of 54 months. The variables that had a negative impact on PFS in univariate analysis were advanced stage, bone marrow involvement, IPI score 3-5, PET2 positive, NK cells<200/mcl, CD19<85/mcl, CD3/CD19 ratio>13, CD4/CD19 ratio>10 ; by multivariate analysis, advanced stage, PET2 positive, CD4/CD19>10 were independent prognostic factors of PFS.
Summary / Conclusion: New biological markers could be predictive of response to treatment and survival in cHL. Pts with a CD4/CD19 ratio >10 seem to be associated with worse outcome.

B1414
PROCALCITONIN (PCT) LEVELS IN NEWLY DIAGNOSED PATIENTS WITH HODGKIN LYMPHOMA (HL) PRESENTING WITH FEVER AND/OR ELEVATED C-REACTIVE PROTEIN (CRP)
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Background: CRP (and other inflammatory biomarkers) is elevated in 70-75% of newly diagnosed HL patients and nearly always in the 30-40% of them who present with fever and/or other systemic B-symptoms, in which the presence of infection must be ruled out. Serum PCT levels, which are potentially useful for this purpose, have not been studied in HL.
Aims: To assess PCT levels in patients with HL at diagnosis in correlation with conventional inflammatory biomarkers.
Methods: Serum PCT levels were determined in 69HL patients with "inflammatory activity" at diagnosis(fever and/or elevated CRP>5 mg/L).PCT values >0.5ng/mlwereconsideredtobeabnormal. PatientswithnormalPCTconcentration were further divided into those with undetectable PCT (<0.1 ng/mL) and those with measurable PCT(0.1-0.5 ng/ml).
Results: 1/69 patients had very high PCT levels (15 ng/mL) in the setting of bacteremia. Four additional patients had measurable but normal PCT levels (range, 0.10-0.24 ng/mL) in the presence of urinary tract infection (n=2), probable respiratory tract infection and C. dificillae colitis. Thus, the main analysis was restricted to the 64 patients with no evidence of infection [median age 32 years (16-82), 55% males, 47% stage III/IV, median CRP 45.65 mg/L (5.92-227.0), CRP≥100 in 19%]: Elevated PCT levels were found in 1/64 (1.6%). Serum PCT concentration was 1.6 ng/ml, without clinical evidence of infection, and returned to normal one week after the start of chemotherapy without antibiotic administration.49/64 patients(77%) had undetectablePCT(<0.10 ng/ml) and 14 (21%) exhibited measurable but normal PCT(0.10-0.50 ng/ml). A strong association between PCT and CRP was observed: The median CRP of patients with undetectable PCT levels was 36.2mg/L versus 89.4mg/Lin those with measurable but normal PCT(P=0.008). The percentage of patients with CRP>100 mg/L in the above groups were 10% versus 47% (P=0.005) respectively. CRP levels strongly correlated with ESR, ferritin, haptoglobin and the a_2-globulins (Spearman's rho 0.634-0.791, P<0.001) and moderately with gamma globulins (Spearman's rho 0.419, P=0.001) and platelets (Spearman's rho 0.342, P=0.007).Instead, PCT levels correlated moderately only with ESR (Spearman's rho 0.350, P=0.007), a_2-globulins (Spearman's rho 0.323, P=0.014) and serum haptoglobin levels (Spearman's rho 0.268, P=0.04). Marginal associations were observed with serum ferritin levels and platelet counts. Prognosis did not differ according to PCT levels: The 2-year Freedom From Progression was 74% versus 81% for patients with undetectable and measurable levels (P=0.74).
Summary / Conclusion: PCT is normal in 98% of patients with newly diagnosed HL that present with elevated CRP and/or fever without obvious infection, even when CRP levels are extremely high. Thestrongassociationbetween-PCTandCRPsuggestsacommoncytokine-regulated initialpathwayfortheirproduction. However, the development of abnormal PCT levels occurs only in the case of infection. These observations support the use of PCT as a marker for the exclusion of infection in newly diagnosed patients with HL with fever and/or elevated CRP. These findings might also apply to relapsed/refractory patients, where the possibility of a concomitant infection is much higher, although this needs further confirmation.

Hodgkin lymphoma - Clinical

B1415
CHEMOTHERAPY ALONE VERSUS CHEMOTHERAPY PLUS RADIOTHERAPY FOR ADVANCE STAGE HODGKIN'S LYMPHOMA: A SYSTEMATIC REVIEW
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Background: The role of radiation therapy in the management of patients with advanced stage Hodgkin's lymphoma (HL) remains controversial and some study groups recommend chemotherapy only for the management for this stage.
Aims: The purpose of this meta-analysis is to examine the effect of added radiation therapy to chemotherapy (CMT) compared with chemotherapy-alone (CT) regimen on the prognosis of patients with advanced stage HL with respect to overall survival (OS) and tumor control.
Methods: This is a systematic review with meta-analysis of randomized clinical trials comparing chemotherapy alone versus chemotherapy plus radiation in the treatment of advanced stage HL. Databases were searched from inception through October 2012. Studies included patients with advanced HL were selected.
Results: Pooled results of 12 trials with2,059 participants showed improvement in overall survival of patient receiving CMT compared to CT alone [Hazard ratio [HR] 0.76, 95% confidence interval (CI) 0.69-0.85, *P*<0.0001]. It also did not improve tumor control significantly [HR 0.89; 95 CI% 0.78-1.02, *p* <0.09]. CMT was associated with higher incidence of secondary cancers, especially solid tumors [Relative risk [RR] 1.81; 95%CI 1.1-2.98, *P*=0.02]. CMT was also associated with slight increase in the incidence of hematological toxicities mainly leukopenia and thrombocytopenia.
Summary / Conclusion: The addition of radiation to chemotherapy in the management of advanced stage HL is associated with an improvement in the overall survival but with modest effect on tumor control including progression-free survival and event-free survival. However, CMT is associated with an increased incident of secondary cancers.

B1416
SURVEILLANCE IMAGING OF HODGKIN'S LYMPHOMA – IS THERE ANY ADVANTAGE?
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Background: Post-treatment surveillance of lymphoma patients in complete remission (CR) usually relies on routine imaging, in an attempt to detect early asymptomatic relapse. Hodgkin's lymphoma (HL) patients undergo surveillance computed tomography (CT) imaging for several years after treatment. Although this practice seems reasonable, there is no proof of benefit in terms of overall survival (OS) or duration of second remission (CR2) and the resulting costs and radiation exposure are undeniable.
Aims: To determine the contribution of surveillance CT to the detection of disease relapse, as compared with clinical findings, and its correlation with CR2 and OS.
Methods: From 06/1998 to 06/2012, 58 first relapses of HL were detected or referred to our center (45 after treatment with Stanford V regimen, 13 after ABVD). We retrospectively reviewed the data from these patients to determine whether the suspicion of disease recurrence was based on imaging or clinical features. Correlation was made to relapse site and extension, time to relapse detection and OS.
Results: Median duration of first CR was 17 months (range 2 to 192); 58% (34) of relapses were localized, 77% (45) in a previously affected site and 67% (39) were histologicaly confirmed. From the 58 relapses, 42 (72%) were detected based on symptoms or physical examination findings and only 16 (26%) were detected by imaging. Among the 25 relapses within the first year after therapy (during which most relapses occur), only 4 (16%) were detected by imaging. CR2 rate was 82% and post-relapse OS was 62% at 5 years (22 deaths, 17 from disease progression, 2 from a second cancer and 3 from other causes), both regardless of the method of recurrence detection.
Summary / Conclusion: The majority of HL relapses were detected on clinical changes. The detection method had no influence on CR2 rate or post-relapse OS. These findings, in agreement with previous similar studies, strongly suggest that there is no advantage in routine CT surveillance imaging for patients in CR from HL. Neither the radiation exposure nor the financial and psychological costs are worth it. Therefore, routine CT surveillance imaging for detection of disease recurrence should not be performed.

B1417

RESULTS OF ABVD AND BEACOPP CHEMOTHERAPY IN ADULT PATIENTS WITH EARLY STAGE HODGKIN`S LYMPHOMA- A SINGLE CENTER RETROSPECTIVE STADY

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Background: More than 85% of patients (pts.) with early-stage Hodgkin's lymphoma (HL) may achieve a durable disease-free survival. Despite the excellent initial remission rates obtained with ABVD chemotherapy (CT) and radiotherapy (RT), approximately 15% of pts. in early unfavorable stages relapse within 5 years and about another 5% of pts. have a primary refractory disease. The optimal CT regimen, the number of CT cycles, the field sizes and the dose of radiation within these fields are the subjects of number studies.
Aims: To compare the efficacy and toxicity of two CT regimens, ABVD and bBEACOPP in early stage HL patients.
Methods: A retrospective study was performed on a cohort of 125 pts., ages 18 years and older, with newly diagnosed, histology-proven HL in clinical (CS) stage II in the period between 1998. to 2009. Patients with CS II HL were stratified into favorable and unfavorable risk cohorts on the basis of the presence of any of the following criteria: age ³ 50, elevated erythrocyte sedimentation rate ³50mm without or ³ 30mm with B symptoms, large mediastinal mass, lymphocyte depletion and mixed cellularity histology, and/or more than three lymph node areas involved. Of the 125 pts., 84 pts were considered with unfavorable risk and received 4-6 cycles ABVD (38 pts.) or 4-6 cycles bBEACOPP (46 pts.) regimens, whereas favorable risk pts. received 2-4 cycles ABVD (28 pts.) or 2-3 cycles bBEACOPP (13 pts.) therapy. Involved field RT (30-36Gy) was applied in 97% of pts. No difference in pts. characteristics were observed among different treatment group.
Results: Median follow-up for all pts. was 64 months. The 5-year overall survival (OS) and 5-year event-free survival (EFS) for 125 pts. was 90.5% (95% CI, 89-91.5) and 80.5% (95% CI, 79-81.5), respectively. We observed a significant difference in OS (100% vs. 85.5%; P< 0.05) and EFS (89.5% vs. 76.0%; P<0.05) at 5 years between favorable and unfavorable cohort. In a subset analyses according pts. stratified into the unfavorable cohort, no differences in complete remission rate (86.8% vs. 94.6%; P=0.40), 5-year OS (82.5 vs. 88.0; P=0.87) and 5-year EFS (70% vs. 81%; P=0.55) in relation to the chemotherapy (ABVD vs bBEACOPP) was seen. Toxicity was mild and comparable in two treatment arms. There are no secondary malignancies to date
Summary / Conclusion: This retrospective study demonstrated the efficacy of both chemotherapy in early-stage HL patients. At present, no statistically significant differences have emerged in favor of bBEACOPP over ABVD chemotherapy.

B1418

BENDAMUSTINE-BASED THERAPY IS EFFECTIVE AND HAS A FAVOURABLE TOXICITY PROFILE IN THE TREATMENT OF REFRACTORY AND RELAPSED HODGKIN LYMPHOMA.

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Background: The management of patients with refractory or relapsed Hodgkin lymphoma (HL), especially after stem cell transplantation (SCT) remains controversial.
Bendamustine has demonstrated efficacy in several lymphoproliferative disorders but limited data are available regarding the schedule in patients with HL, in particular its dosage and the possibile combinations for a synergic effect.
Aims: The objective of this phase II study was to evaluate efficacy and safety of bendamustine combined with other cytotoxic drugs in patients with refractory and/or relapsed HL. Two different schedules of bendamustine-based regimens were evaluated (high dose *vs.* standard dose).
Methods: From May 2011 to December 2012, 9 patients (6M/3F) with a median age of 25,5 years (range 18-34) received bendamustine as salvage treatment. Patients were divided by chance into 2 groups of treatment: 5 patients received standard intensity treatment (standard-B; bendamustine 90 mg/m^2 days 1-2 and Ara-C 0,5-0,75 g/m^2 day 1) and 4 patients received high intensity treatment (high-B; bendamustine 150 mg/m^2 days 1-2 combined with Ara-C 1-2 g/m^2 day 1 or modified BEACOPP$_{escaleted}$ regimen without adriamycin). Each cycle was repeated every 28 days and growth factor support was sistematically administered, in association with antimicrobial prophylaxis. The treatment efficacy in both group was evaluated according to Revised Response Criteria for Malignant Lymphoma. Any adverse event occurred was recorded and classified for type and grade using NCI-CTCAE criteria (v 4.0).
Results: The median number of previous chemotherapy lines was 3 for both groups(first line therapy was ABVD for all patients). Three patients had failed prior autologous-SCT in the high-B group and 2 in the standard-B group, 2 patients were primary refractory to ABVD in each group. A total of 26 cycles was administered (median3,5; range 1-6) according the above reported schedules. In the high-B group, 3 (75%) patients achieved complete remission (CR) and then underwent to SCT (two autologous and one haploidentical-SCT) and are in complete remission while one patient died for progressive disease (PD). By contrast, among the 5 patients who received standard-B, three (60%) were in stable disease (SD), one was in PD and one patient obtained partial remission (20%) and underwent to autologous-SCT. When bendamustine was used at standard doses, it didn't exert grade 3-4 side effects except for transient transaminase increase observed in only one patients. Grade 3-4 treatment related adverse events (AEs) reported in the high-B group were thrombocytopenia (75%) and anemia (50%).
Summary / Conclusion: Bendamustine used at high dose and in combination with Ara-C or a modified BEACOPP$_{escalated}$ regimen, seems to be effective in heavily pretreated patients with HL, suggesting a possible non cross-resistance with other agents. Its safety profile is acceptable and adverse events manageable also at this high dosage.

B1419

RISK FACTORS IN AUTOLOGOUS STEM CELL TRANSPLANTATION IN HODGKIN LYMPHOMA : A SINGLE-CENTRE EXPERIENCE

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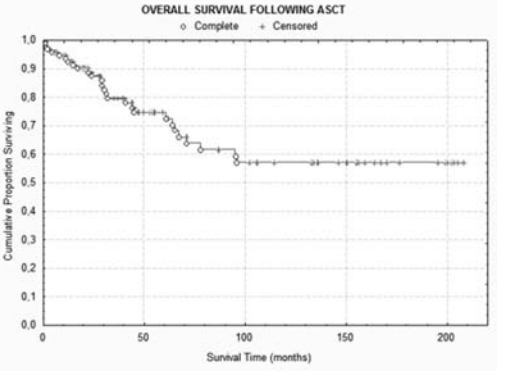
Background: High dose therapy followed by autologous stem cell transplantation (ASCT) is used in the setting of refractory or relapsed Hodgkin lymphoma (HL). Several independent risk factors associated with poor outcome following ASCT were indentified such as clinical stage (III, IV), anemia and early relapse (≤ 1 year) (Josting et al., 2002.)
Aims: The aim of this retrospective study was to show single centre experience in treating refractory/relapsed Hodgkin lymphoma by high dose chemotherapy followed by ASCT and identify possible risk factors associated with poorer disease free survival (DFS) and overall survival (OS).
Methods: 98 consecutive patients (41 males, 57 females, median age at ASCT; 30 years, median time to ASCT after diagnosis: 18 months) with HL who underwent BEAM myeloablative conditioning followed by ASCT were included in analysis in the period from November 1995 to November 2012 . OS and DFS were analyzed in the context of several features during first relapse (clinical stage, extranodal disease, presence of B symptoms, early relapse (≤12 months) and anemia), time to transplantation, age at transplantation, type of harvesting and clinical factors at the diagnosis.



Results: Median time from the diagnosis to the first relapse was 15 months. The most common harvesting therapy used was miniBEAM (64%). Following ASCT, clinical CR rate was 75.5% and PR rate was 16.5%. 27 patients died during the observation period. Median for OS and DFS has not been reached at the maximum observation time of 208 months. Estimated OS at 96 months was 0.574 (S.E. 0.06). However as shown on the graph1, there is plateau after 96 months extended to the maximal observation time. Out of 74 patients, who achieved CR, DFS at 167 months was 0.66 (S.E=0.09). Median OS for patients not achieving CR was 41 months (95% CI 17-64 months). None of the parameters at the diagnosis (age, sex, clinical stage, extranodal disease, bulky disease, presence of B symptoms, German Hodgkin Study Group stage, International Prognostic Score) were associated with OS. Poorer OS was significantly associated with the presence of anemia during relapse (defined as Hb<105 g/l for females and <120 g/L for males) (P=0.048) and B symptoms (P=0.047), while other factors (early relapse, extranodal disease, advanced stage, type of harvesting, time to transplantation, age at transplantation) were not significant. We have not been able to identify any factors associated with poor DFS. Also, there was no significant differences in OS and DFS between group period from 1995. to 2003. (N=41) and period from 2004. to 2012. (N=57) when adjusted for the factors present at the diagnosis.
Summary / Conclusion: We have demonstrated successful treatment of refractory/relapsed HL by miniBEAM harvesting followed by BEAM/ ASCT with high remission rates. As seen in graph1, the observed plateau indicates prob-

able cure for this subset of patients. Anemia and B symptoms, present at relapse, were related with poorer outcome, while some previously described risk factors have not been confirmed probably due to small number of events. In line with previous studies, our results imply that ASCT represents adequate approach towards relapsed/refractoty HL.

B1420

BRENTUXIMAB VEDOTIN IS AN EFFECTIVE TREATMENT OF CLASSICAL HODGKIN LYMPHOMA TYPE POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD)

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Background: Posttransplant lymphoproliferative disorders (PTLDs) occurring as serious complication after solid organ or allogeneic stem transplantation embrace a heterogeneous group of lymphoproliferative diseases. The optimal treatment is not well defined and rely on the histological subtype and stage. In advanced stage of Hodgkin lymphoma chemotherapy with AVBD is suggested. As previously reported brentuximab vedotin, is an antibody-drug conjugate that combines an anti-CD30 antibody with monomethylauristatin E, a potent antimicrotubule agent able to induce responses in CD30-positive lymphomas with mild toxic effects.
Aims: The goal was to achieve remission of the PTLD without compromising the graft.
Methods: We report on a 50-year-old male patient with blastic plasmacytoid dendritic cell neoplasm (BPDCN) undergoing umbilical cord blood transplantation in 1st CR employing a reduced conditioning regimen (cyclophosphamide: 50mg/kg, fludarabine: 150mg/m^2 and fractionated total body irradiation: 400cGy) comprising anti-thymocyte globulin as graft versus host prophylaxis in May 2011. The post-transplant course was uneventful and in the absence of graft versus host disease Cyclosporin A was stopped after 6 months. Eleven months after transplantation the patient presented with abdominal pain and a CT scan revealed a bowel tumour with multiple enlarged mesenterial lymph nodes and a single liver lesion. An extended right hemicolectomy and a liver wedge resection were performed revealing a classical Hodgkin lymphoma type post-transplant lymphoproliferative disorder (PTLD) with typical immunphenotype (CD30+, CD15+ in the majority of the HRS-cells and and EBV+). After completion of wound healing the patient received 6 cycles of brentuximab vedotin at a dose of 1.8 mg/kg BW every 3 weeks on a compassionate use basis.
Results: Eleven months after diagnosis of the PTLD the patient is in good clinical condition and experiencing a continuous complete remission as demonstrated by multiple PET-CT scans. Side effects of Brentuximab were mild and did not compromise the graft.
Summary / Conclusion: Targeted therapy with brentuximab vedotin seems to be an effective treatment in classical Hodgkin lymphoma type PTLD even in advanced stage.

B1421

THE ROLE OF POSITRON EMISSION TOMOGRAPHY (PET) IMAGING IN THE TREATMENT STRATIFICATION OF STAGE I-II HODGKIN LYMPHOMA

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Background: PET imaging has become integral to staging and assessment of treatment response in Hodgkin lymphoma (HL). Minimising long term treatment side effects is of direct clinical importance, given the high remission and cure rates in localised disease. Our centre has adopted an approach of omitting radiotherapy (RT) when post-chemotherapy PET is negative, which is also the focus of on-going phase III trials.
Aims: To retrospectively determine the relapse rates of stage I-II HL patients whom underwent PET evaluation in comparison with those monitored by CT only.
Methods: 67 Ann-Arbour stage I-II patients were identified from hospital records that were treated at our centre from 1998 onwards. Traditionally patients received consolidative RT on completion of chemotherapy, with response to treatment being monitored by CT. Overall survival (OS), relapse free survival (RFS) and site of relapse in this group of patients were compared with patients who did or did not receive RT based on post-chemotherapy PET evaluation. Patients with HIV (n=5) or lymphocyte predominant disease (n=2) were excluded from analysis.
Results: The median age at diagnosis was 33 (range 16-85). 28 were male and 39 were female. 58% of patients had stage 2a disease, 22%, 18%, and 1% had 2b, 1a, and 1b disease respectively. The average length of follow-up was 64 months (range 2-181 months). 64 patients had chemotherapy of which 62 received ABVD as first line therapy, median of 4 cycles (range 2-8). The median age was 84 for those who did not receive chemotherapy.
46 patients had PET assessment post-chemotherapy. Of these 38 patients had complete response (CR) post chemotherapy and 8 had partial response (PR). All those with PR received RT and three patients with CR also received RT. Patients imaged via PET had an average length of follow-up of 53months (range 2-145). 21 patients were imaged with CT of which 9 were deemed to have achieved a CR. 15 of the 21 patients imaged with CT received radiotherapy post-chemotherapy. 8 patients in total relapsed during the follow-up period, at an average at 37 months (range 7-98months). None of the 38 patients in CR on PET scanning post-chemotherapy relapsed. However, three of the nine patients in CR by CT criteria post-chemotherapy suffered a relapse which was significantly higher (P=0.01 Fisher's exact two tailed test). However, one patient with a positive PET scan post-chemotherapy did relapse 14 months later but at a site outside the radiotherapy field. There was no difference in overall survival (Chi-squared P=0.624) or relapse free survival (Chi-squared P=0.127) between those who did or did not receive radiotherapy irrespective of imaging modality used. Three patients relapsed within a previous radiotherapy field, two of these were during a second relapse. One patient relapsed both inside and outside the radiotherapy field at their first relapse. In total three patients died, two of whom were elderly (aged 79 and 84) and received no RT or chemotherapy. The other patient died during salvage chemotherapy prior to a planned BEAM autograft.
Summary / Conclusion: Our data indicate that a negative post-chemotherapy PET following HL diagnosis is a strong predictor of sustained complete remission. These results support an approach of omitting involved field radiotherapy in this cohort of patients, which will spare this group of patients the long term morbidity and mortality associated with this treatment.

B1422

EVALUATION OF THE MANAGEMENT OF HODGKIN'S DISEASE IN BLIDA, ALGERIA

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Background: Considerable progress has been made in the management of Hodgkin's disease in order to improve treatment outcomes but also reduce long-term side effects.We present a retrospective study over a period from 1998 to 2009 during which patients with Hodgkin's disease have been supported
Aims: to evaluate outcome of our patients treated with conventional therapy.
Methods: Patients were included after additional tests: lymph node excision biopsy for histology , chest X-ray, thoracic abdominal and pelvic computed tomography, bone marrow biopsy, inflammatory balance. The therapeutic approach consisted of chemotherapy combined or not with radiotherapy according to clinical stage.
Results: During this period, 372 patients were included, 178 men and 194 women (sex ratio 0.92), mean age 31.47 (men 34.77 years, women 28.17 years). The histological type was specified in 350 cases (94 %): type 1: 15 cases (4.2%), type 2: 222 cases (63.4%), type 3: 102 cases (29.1%), and type 4: 11 cases (3.1%). Classification: favorable stage I: 15 cases (4%), unfavorable stage I: 13 cases (3.5%), unfavorable stage II: 28 (7.5%), favorable stage II: 118 (31.7%), stage IIIA: 17 (4.5%), Stage IIIB: 80 (21.5%) stage IV 101 (27.1%). Chemotherapy regimens: 367 patients were treated : 277 (74.4%) patients were treated with ABVD protocol, 68 with cisplatine + ABVD, 22 with MOPP / ABV, radiotherapy was associated in 219 patients (58.8%). Among these patients, failure was observed in 52 cases (14 %) with 40 (77 %) in disseminated stages. Evolution : 232 (62.3%) patients are still alive in Complete Response (CR) or Incomplete Response (IR) ; relapse: 32 patients (8.6%); deaths: 61 patients (16.4%), however 43 patients (11,6) were lost to CR or IR. Complications: Pulmonary fibrosis: 16 patients the cause of recurrent chest infections, cardiac toxicity: 6 patients, endocrine disorders: 14 patients (hypothyroidism, early menopause, impotence), neuropathy : 9 patients, herpes zoster : 5 patients, ENT neoplasia : 2 patients, Myelodysplasia: 4 patients.
Summary / Conclusion: The adaptation of therapeutic indications stage prognosis will improve our results, especially in adverse localized stages and disseminated stages, which represent the majority of our patients. ASCT will represent a treatment of choice for these patients who relapse after initial chemotherapy or for those primary refractory to first chemotherapy. Unfortunatly, this methode of treatment is not yet feasible in our country.

B1423

PROGNOSTIC VALUE OF 18F-FDG PET/CT IN HODGKIN LYMPHOMA

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Background: Hodgkin Lymphoma (HL) has been converted from a uniformly fatal disease to one that is curable in about75 % of patients.
Toxicity related with treatment and second malignancy being considered as comorbidities to long term follow-up in survivors. The radiation dose and field, chemotherapy agents and doses administered, gender and patient age at treat-

ment are considered as common risk factors.
The combination of 18F-FDG positron emission tomography and computed tomography scans (18F-FDG PET/CT) is considered as a good tool for diagnosis and follow-up of the patients with HL in addition 18F-FDG PET/CT, provides prognostic information.
Aims: To determine the value of 18F-FDG PET/CT after the second and sixth cycle of first line therapy with ABVD (doxorrubicin, bleomicyn, vinblastine, dacarbazine) or BEACOPP (bleomicyn, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine and prednisone) in the outcome of HL patients.
Methods: Observational and retrospective study performed in HL patients diagnosed in our centre between January 2007-December2012. All patients were treated with ABVD or BEACOPP according subtype and Ann Arbor stage and were evaluated with 18F-FDG PET/CT after second and sixth cycle of treatment. 18F-FDG PET/CT were considered positive when the SUV≥ 2'5. Response, overall survival, progression free survival (PFS) and comorbidities were considered.
Results: Seventy nine patients have been included, M/F: 52 (66%) /27 (34%). Mean age 39 years (range: 17-86). Histologic classical subtype: 56 nodular sclerosis HL (NSHL), 15 mixed cellularity HL (MCHL), 5 lymphocyte rich HL (LRHL) and 3 lymphocyte depleted HL (LDHL). Early stages 48.1 % (n=38): IA (n=5), IIA (n=24) and IIB (n=9). Advance stage: 51.9 % (n=41): IIIA (n=14), IIIB (n=11), IVA (n=7) and IVB (n=9). In 62 patients (78.5%) 18F-FDG PET/CT were performed after second cycle: Positive in 9 patients (11.4%) and negative in 53 patients (67 %). After completed therapy 18F-FDG PET/CT was performed in 74 patients, in 8 showed positive results (10.1 %) and negative in 66 patients (83.5 %). 4 of 9 patients with positive 18F-FDG PET/CT after second cycle, became negative results when completed therapy.
Relapse HL was observed in 24 patients (30.4%), in12 (15.2%) showed early relapse (<12 months). Progression-free survival (PFS) was of 35.8 months.
Summary / Conclusion: 18F-FDG PET/CT is the most accurate tool for staging, treatment monitoring, and response evaluation in HL. In our experience to check an early 18F-FDG PET/CT could be predict a good outcome in the majority of HL patients and avoid to be over treated.

B1424

COMPARISON OF BONE MARROW INVOLVEMENT BY FDG PET/CT AND HISTOPATHOLOGY IN PATIENTS WITH LYMPHOMA

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Background: The diagnosis of either Hodgkin's disease(HD) or non-Hodgkin lymphomas (NHL) is incomplete without an accurate staging. Identification of an advanced disease changes the intensity of the theraphy as well as the duration of the treatment period. The conformity of staging is dependent on the findings of computed tomography (CT) scanning and bilateral iliac crest trephine biopsies to determine a possible infiltration of the bone marrow. Bone marrow biopsy (BMB) is a routine procedure in the algorithm of the lymphoma management and involvement of the bone marrow (BMI) is regarded as stage IV disease with poorer outcome. BMB, though precious in the initial evaluation of lymphomas, may miss focal disease, has its own complications such as infections and bleeding. The need for less invasive with less complications and also more sensitive method led hematologists towards imaging, especially nuclear imaging. ^{18}F-fluoro-2-deoxyglycose positron emission tomography (FDG-PET) is the late imaging method of choice in especially the initial staging of HH and NHL.

Parameters Hodgkin	FDG-PET positive for BMI # of patients	Histopathology positive for BMI # of patients	p values
# of patients	10	6	
LDH elevation	9	5	0.001
Presence of leucocytosis/leucopenia	8	5	0.001

Parameters Non-Hodgkin	FDG-PET positive for BMI # of patients	Histopathology positive for BMI # of patients	p values
# of patients	15	14	0.000
LDH elevation	13	11	0.000
Presence of leucocytosis/leucopenia	12	12	0.000

Aims: The aim of this study is to evaluate the value of FDG-PET in determination of BMI in comparison with histopathological findings in patients with Hodgkin (HL) and non-Hodgkin lymphoma (NHL).
Methods: 30 patients with HL, aged between 20-73 years (mean 38.87) and gender 10 female (35.5%) and 20 male (64.5%), 55 patients with NHL, aged between 28-78 years and gender 24 female (43.6%) and 31 male (56.4%) were enrolled in the study. Presence of leucocytosis or leucopenia and LDH elevation at the time of diagnosis, initial stages and findings of BMI with FDG-PET and BM histopathology were recorded.
Results: In patients with HH, FDG-PET positivity was observed to be related with pathological BMI (P=0.001). FDG-PET positivity was also related with the stage, prognostic score, LDH elevation and presence of leucocytosis/leucopenia (p values 0.000, 0.004, 0.000, 0.001 respectively). Likewise, in patients with NHL, FDG-PET positivity was observed to be related with pathological BMI (P=0.001). FDG-PET positivity was also related with the stage, prognostic score and presence of leucocytosis/leucopenia (p values 0.001, 0.000 and 0.001 respectively) while was not related with LDH elevation (P=0.009).
Summary / Conclusion: FDG-PET seems to be relevant and valuable as BM biopsy regarding BMI. As being non-invasive, it may be useful to replace with BM biopsy especially in patients who are observed to be low stage.

B1425

OUTCOME OF HIGH RISK HODGKIN'S LYMPHOMA IN THE SOUTH-EST OF ALGERIA.

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Background: Hodgkin's lymphoma (HL) is considered as one of the most curable forms of cancer, especially if it diagnosed early.
The regimen containing doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) is the common standard of care for the frontline treatment of HL. However, there is no standard of cure in advanced-stage of HL's patients; particularly in our context for several reasons: late diagnosis, frequency of bulky disease, lack of access to autologous stem cell transplantation.

Aims: To assess the poor outcomes and prognosis of adult patients (pts) with high risk advanced stage HL treated by ABVD regimen in first line.
Methods: We present a recent retrospective analysis updated on a period of 04 years (January 2009-december 2012). Adult pts with HL were staged according to the Cotswold's classification and evaluated according to the prognostic's factors of EORTC, IPS and GHSG. High-risk (HR) HL was defined in clinical stage (SC) IIB, III and IV by the presence of 04 involved lymphoid areas, and / or mediastinal mass ratio: MMR≥ 0.45 and / or ≥ 2 extra lymph node sites affected by the disease (for SC IV).
The initial treatment strategy was the ABVD (4-8 cycles) consolidated with radiation in limited stages.
Results: Until December 31, 2012, one hundred twenty six (126) pts with HL have been diagnosed. The median age of all pts was 29.2 years (range, 15-74 years), the sex ratio is 0, 89 (M: 60 / F: 67) and the mean time to diagnosis was 5.4 months (01-36) months. Early stages are 61 (48.4%), and 65 were extended (51.6%).Thirty six pts have a bulky disease (51.6%) including 18 (56%) in stage IIB. At diagnosis advanced-stage IIB (n = 34), III (n = 27) and IV (n = 38) are ninety nine (T = 99); among them 62(62%) pts are HR: IIB= 21 (33.8%), III= 16 (25.8%), and IV= 26 (42%), and 37 are in favorable advanced stages. So 49.2% of the entire series and 62% of the advanced stages IIB, III and IV are HR forms. The primary responses to ABVD protocol in 56 pts evaluated with HR HL (IIB = 20), (III = 14), (IV = 22), and 35 favorable advanced stages (IIB=12), (III=11), (IV=12) are: - Response: in 34 (60.7%) HR pts (II=12, III=09, IV=10), to 29(83%) favorable pts (II=11, III=08, IV=10). - Failure (primary failure + relapses): 22(39.3%) HR patients (II=08, III=05, IV=09), to 06(17%) favorable pts (II=01, III=03, IV=02) (P<0, 03). -Total of 13 HR pts are dead (21%) to 03(08%) favorable advanced stage's pts and a four-year overall survival rates of HR HL and favorable forms are 77% to 91% respectively.
Summary / Conclusion: This study shows several facts: - The LH is common in our region from 30 to 40 pts / year, affects young adults (29.2 years). - High risk advanced disease represents almost half of the series (49.2%), - ABVD protocol provides a response in only 60% with a high failure rate (40%), a four-year overall survival rate of 77% to 89% at five years in the literature see even 96% with the new primary intensification. -Very poor prognosis of HR HL represents clearly an area of unmet medical need, and a prospective study will be launched in comparing BEACOPP to ABVD consolidated with autologous stem cell transplantation.

Myelodysplastic syndromes and bone marrow failure syndromes incl. PNH - Biology

B1426

ANALYSIS AND VALIDATION OF THE MIRNA EXPRESSION PROFILE IN PLASMA FROM PATIENTS DIAGNOSED WITH MYELODYSPLASTIC SYNDROME

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Background: Myelodysplastic syndrome (MDS) is a group of neoplastic disorders characterized by abnormal maturation and differentiation of hematopoietic cells and frequent transformation into acute myeloid leukemia, but current prognostic scores explain only a small fraction of patient evolution variability. The pathogenesis of these complex disorders involves both the stem cells and their interactions with the microenvironment. Morphology is the basis of the MDS diagnosis, but its interpretation is subject to potential inter-observer variability. In a recent study, inter-observer disagreement was observed in 27% of the samples considered suitable for morphological evaluation. In this sense, those categories with unilineage dysplasia were the least reproducible (*Font P et al., Ann Hematol 2013*). New biomarkers might be of help in the diagnostic work-up, pathogenetic understanding as well as in prognostic stratification of MDS patients. Studies are now focused on gaining more knowledge about miRNAs. These small, non-coding RNAs sequences are involved in the regulation of biological processes. Some studies have shown that the miRNAs are stable and in detectable quantities in cell-free plasma (*Mitchell et al. PNAS, 2008*), and their expression in peripheral blood (PB) has been related with various neoplastic conditions including acute leukemia and MDS (*Schotte D et al., Leukemia 2012, Rhyasen GW and Starczynowski DT, Leukemia 2012*). Recently we have described a profile (Aragon miRNA Profile) of 14 miRNAs detected in PB and plasma of MDS patients (patent pending) that was present in higher concentration in MDS as compared to controls. Ten out of fourteen studied miRNAs had similar expression profile in PB and plasma although the increase with respect to controls was higher in plasma.
Aims: The aim of this study is to analyze Aragon miRNA profile in a larger number of plasma samples in order to validate our previous results.
Methods: We have studied a total of 243 patients diagnosed with MDS, 193 were derived from the project INBIOMED HEMA-001/2006 and 50 corresponding to patients diagnosed in the Haematology department of Miguel Servet University Hospital (2008-2011).PB and plasma samples were deposited in the Aragon Biobank. RNA was extracted from PB using Paxgene Blood miRNA kit (PreAnalytix). A miRNA signature in MDS was determined by q-PCR using Megaplex™ Primers AV2.1 and Human Pool B (Applied Biosystems, USA). A total of 754 miRNAs of the currently listed in the Sanger miRBase database were analyzed. 14 differentially expressed miRNAs were selected. To obtain cell-free plasma miRNAs, we used Total RNA Purification Kit (Norgen, Canada). A previous step of cDNA preamplification was performed with Megaplex PreAmp Primers Human Set (Applied Biosystems, USA).
Results: In a previous study, we founded 14 differentially expressed miRNAs in the PB and plasma from 40 MDS patients vs. normal controls. 71.4% of the studied miRNAs had similar expression profile in PB and plasma, but the increase with respect to controls was higher in plasma.The results obtained in the present study, performed with the same procedure in a larger MDS population, will be a test-bed for further developments. Detailed data will be provided during the meeting.
Summary / Conclusion: Aragon miRNA profile in plasma might be of help for the diagnostic work-up, pathogenetic understanding as well as prognostic stratification of MDS patients. Additional information related to specific MDS subsets, response to hypomethylating agents and disease evolution will be needed before its very role in MDS management can be established.

B1427

PROGNOSTIC SIGNIFICANCE OF GATA-1 AND WT1 LEVELS IN PEDIATRIC HEMATOLOGICAL DISORDERS

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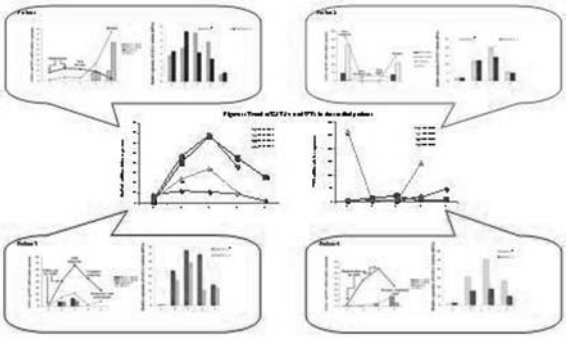
Background: Acquired mutations in hematopoietic transcription factor GATA-1 were reported in Down Syndrome (DS), Transient Myeloid Disorders and Acute Megakaryoblastic Leukemia (AMkL). These mutations occur in exon 2 and lead to a truncated shorter protein missing the N-terminal transactivation domain (GATA-1s).
Aims: This study was aimed at evaluating *in vivo* e *in vitro* results about the role of GATA-1 isoformson expression levels of specific WT1 isoforms (+/- KTS).
Methods: We monitored the GATA-1, WT1 and their isoforms expression levels in 4 pediatric patients affected by different hematological disorders (1 Acute Lymphoblastic Leukemia (ALL)-DS, 1 AMkL, 1 Acute Myeloid Leukemia (AML)-DS and 1 Myelodisplastic Syndrome (MDS)-DS). The expression levels of both GATA-1 and WT1 isoforms were evaluated to correlate changes to clinical progression of disease. For this purpose, transient transfection assays were performed in human K562 erythroleukaemic cells using constructs for the *wild-type* cDNA sequence of GATA-1 full lenght (GATA-1n) and for the sequence mutated at Tyr63 (Tyr63Stop, GATA-1mut) within eukaryotic expression vector p3XFlag-CMV.
Results: Results showed that GATA-1s was able to induce a more dramatic increase of WT1 expression than GATA-1n. Furthermore, allele-specific amplification of WT1 isoforms (+/- KTS) showed that the mutant construct was able to more dramatically increase +KTS isoform already in presence of low expression levels of GATA-1s protein, whereas the GATA-1n protein, at high expression levels, induced a significantly increase of the –KTS isoform of WT1. Total GATA-1 levels increased and/or remained high in the steady state of the monitoring without clinical worsening and in apparent patients' remission. In these conditions the prevalent GATA-1 transcription variant was GATA-1n for each patient. When GATA-1 expression levels started to decrease was to be considered as a "wake-up call" signal for a subsequent relapse. In order to this dramatic event may happen, we observed that the total GATA-1 decrease was accompanied by a drastic knock-down of GATA-1n variant and imbalance ratio in favor of low levels of GATA-1s transcript. However, although the prognostic significance of how GATA-1 isoform ratio affects the worse overall survival is still unclear because of the limited and heterogeneous sample size, we report that, following total GATA-1 decreased expression, the persistence of GATA-1s transcript, even at low levels, was associated to considerable increase of WT1 expression levels. According to our *in vitro* transfection data, relapse was found associated with a WT1 isoform ratio strongly unbalanced in favor of +KTS (Figure 1).



Summary / Conclusion: We can hypothesize that GATA-1 level and the subsequently variants displacement in favor of GATA-1s could be the trigger for the activation of WT1 expression, particularly of +KTS isoform. GATA-1 and WT1 evaluation has been performed on peripheral blood samples instead of bone marrow samples, as commonly reported. This approach is certainly less invasive and traumatic as compared to bone marrow aspirate, and can reduce monitoring intervals in the course of follow-up. Further studies need to be performed in order to clarify the genetic and epigenetic mechanisms underlying the regulation of GATA-1 isoform ratio responsible of the phase of relapse on a larger cohort of DS and non-DS patients. This would allow to contribute to clarify process of the leukemic transformation and could eventually pave the way to novel therapeutic tools.

B1428

ANALYSIS OF BONE MARROW CD34(+) SUBPOPULATIONS BY FLOW CYTOMETRY IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes (MDS) comprise a heterogeneous group of haematopoietic stem cell (HSC) clonal disorders characterized by dysplasia and ineffective haematopoiesis. But while it is assumed that MDS is a "stem cell disease", hard evidence for this claim is still lacking, and stem and progenitor alterations in MDS patients have not yet been defined.
Aims: Aim of the study was to determine the frequency of CD34+ populations (in different maturation stages) as well as CD133 (primitive cell marker) and CD96 (putative marker of myelodysplastic stem cell) expression in CD34+ subpopulations, in patients with MDS.
Methods: Bone marrow specimens of 24 patients with MDS and 6 healthy individuals (as control group) were studied using 5-color multiparameter flow cytometry. Patients were classified according to a) WHO as RCMD (n=10, 42%), RARS (n=1, 4%), RAEB 1 (n=4, 17%), RAEB II (n=7, 29%), del 5q (n=1, 4%) and CMML (n=1, 4%) and b) IPSS as low and intermediate-1 risk group (n=15, 62%) and intermediate-2 and high risk group (n=9, 38%).
CD34+ subpopulations were determined using the primitive cell marker CD90 (CD34+/CD90+ and CD34+CD90-). Our analysis included: a) investigation of the relative frequency of CD34+ subpopulations within each group and b) comparison of the prevalence of different CD34+ subpopulations between different groups.
Results: a) There was a statistically significant prevalence of CD34+CD90- compared to CD34+CD90+ subpopulation within all groups: patients (P<0,001), controls (P=0,027), low and int-1 group (P=0,001), int-2 and high risk (P=0,008), RCMD (P=0,005) and RAEB 2 (P=0,018). The addition of CD133 expression in this analysis did not alter the results. b) 1. Both subpopulations CD34+CD90+ and CD34+CD90- had a significant prevalence in both the high risk vs low risk and high risk vs control groups. 2. The expression of CD34+CD90-CD133+ subpopulation was significantly higher in high risk vs low risk and high risk vs control groups. 3. Although there was no difference in the expression of subpopulations CD34+CD90+ and CD34+CD90- between different group of patients, the co-expression of CD96 differentiated the results as the prevalent subpopulation in the high risk group was CD34+CD90-CD96+ cells and in the low risk group was CD34+CD90+CD96+ cells.
Summary / Conclusion: Even though HSC dysfunction is presumed in MDS, the exact nature of quantitative and qualitative alterations is unknown. In the present study we found that the most prevalent HSC subpopulation with high expression of CD133 and CD96, especially in patients with higher risk MDS, is the most mature CD34+CD90-. It is a challenge to investigate a number of parameters that might reveal that these cells play an important role in MDS pathogenesis.

B1429

COMPOSITION OF BONE MARROW SUBPOPULATIONS ANALYZED BY FLOW CYTOMETRY REVEALS DIFFERENCES BETWEEN PEDIATRIC APLASTIC ANEMIA AND REFRACTORY CYTOPENIA

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Background: Aplastic anemia (AA) is a disease with immunological pathogenesis. Refractory cytopenia of childhood (RCC), the most common subtype of MDS in children, is regarded as a clonal disease. Typically RCC is characterized by hypoplastic bone marrow (BM). Immunological mechanisms play a role in at least part of RCC patients. Evaluation of composition of BM populations by flow cytometry (FC) might be challenging since detailed knowledge about physiological composition in childhood is limited.
Aims: Our aim is to characterize BM by FC in AA and RCC with the focus on T cells and to define reference composition of subpopulations in physiological bone marrow.
Methods: We measured BM samples by FC in 34 AA patients (median age 10.3, 1.1-18y) and 26 RCC patients (median age 11, 4.2-18y) diagnosed in 2005-2013. We analyzed granulocytes, lymphocytes, precursors $CD34^{pos}$, $CD117^{pos}$, B cells including evaluation of immature $CD10^{pos}$ cells, T cells ($CD3^{pos}4^{pos}$, $CD3^{pos}8^{pos}$) including activation status by HLA DR expression. We also analyzed follow-up BM samples to evaluate changes following immunosuppression (IST). We established 2 reference categories: BM samples from patients examined within staging of solid tumor and a healthy donor (group A, 28 patients, median age 2.6, 0-17y) and patients at least 2 years after stem cell transplantation (group B, in total 22 patients, median age 11, 2.6-23y). For the aim of detailed characterization of T cells we introduced new polychromatic panel including markers describing T cell subsets (CD3, CD4, CD8), activation (CD57, HLA DR), cytotoxic function (Granzyme B, Perforin) and distribution from naive to memory stage (CD28, CD27, CD45RA). We measured >300 samples including 6 AA and 5 RCC paired samples before and after IST to evaluate differences in T cell composition.
Mann-Whitney test was used for statistical analyses (P<0.05 for all bellow mentioned results).
Results: Comparing both reference categories, we found higher percentage of lymphocytes and precursors $CD34^{pos}$ and $CD117^{pos}$ in patients in group A. We observed higher percentage of lymphocytes, B cells and precursors in control patients younger than 1 year. Proportion of $CD8^{pos}$ T cells out of all T cells and their activation was higher in patients older than 5 years.
Patients with AA have significantly higher number of lymphocytes compared to RCC and all reference samples. RCC patients have also higher percentage of lymphocytes compared to all reference samples. When RCC was compared to group A only the difference was not significant. Total B cells were significantly decreased in RCC compared to AA and reference group, however in both AA and RCC proportion of immature $CD10^{pos}$ B cells was significantly decreased. Following IST $CD10^{pos}$ B cells tended to increase in the majority of treated patients. Precursors $CD34^{pos}$ and $CD117^{pos}$ were decreased in both AA and RCC, but in AA patients they were significantly lower compared to RCC. T cells were increased in both AA and RCC patients, but significantly higher activation of cytotoxic T cells we observed only in AA. By T cell-oriented panel we found decrease of activated $CD8^{pos}$ T cells together with increase of naive $CD8^{pos}27^{pos}28^{pos}45RA^{pos}$ cells in AA patients with good response to IST.
Summary / Conclusion: We found reproducible differences in composition of BM populations analyzed by FC in AA and RCC patients. Decreased percentage of precursors and increased number of lymphocytes was present in both RCC and AA patients. It is important for analyses of BM composition to define control samples, which match also age characteristics.

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B1430

PERFORMANCE CHARACTERISTICS OF CONSENSUAL APPROACHES FOR SMALL AND MINOR PAROXYSMAL NOCTURNAL HEMOGLOBINURIA CLONE DETERMINATION BY FLOW CYTOMETRY

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Background: Comparison of individual approaches for paroxysmal nocturnal hemoglobinuria (PNH) clones evaluation by flow cytometry (FCM) as described in the 2010 international clinical cytometry society (ICCS) guidelines has not been reported in literature.
Aims: To rule out the influence of different consensual approaches and strategies for small and minor PNH clone evaluation by flow cytometry on final results, thus leading to consistant interlaboratory variabilty
Methods: We analyzed the performance characteristics of 4, 5 and 6 color protocols for white blood cell (WBC), one and two color protocols for red blood cell (RBC) evaluation for different target PNH clones and compared results from routine PNH patient analysis.
Results: Coefficient of variation (CV) for precision / reproducibility analysis ranged from 0.67 % / 1.49 % to 2.56 % / 3.09 % for granulocytes, from 0.93 % / 3.09 % to 7.76 % / 12.06 % for monocytes and from 0.41 % / 4.73 % to 6.53 % / 5.1 % for RBCs. Linear regression analysis revealed excellent correlation (r = 0.99 %), Wilcoxon ranks test showed no statistically significant differences (P> 0.05), Bland-Altman analysis demonstrated performance agreement with mean bias ranging from 0.02 to 2.2.
Summary / Conclusion: Our results confirmed very good performance characteristics for precision and reproducibility analysis, excellent correlation and favorable agreement between protocols, underlining the crucial role of optimally selected glycophosphatidylinositol (GPI)-specific reagents and appropriate conjugates and secondary role of the number and type of gating reagents, tubes per test and corresponding gating strategy. With respect to this, reported high interlaboratory variability is considerably related to incorrect performance and /or insufficient experience with PNH testing by flow cytometry.

B1431

DIAGNOSIS AND MONITORING OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA BY MULTIPARAMETER FLOW CYTOMETRY. OPTIMIZATION OF PROTOCOLS BASED ON CD59, CD55 AND FLAER

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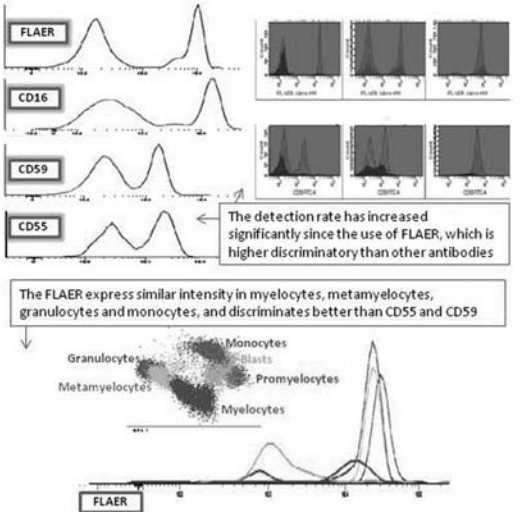
Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal disease of stem cells due to a somatic mutation in the PIG-A gene (Phosphatidil inositol glycan class A, cr. Xp22.1).
Aims: 1. Screen our reference population as recommended by Parker et al 2. Investigate the antibodies used for high sensitivity studies. 3. Quantify the size of the PNH clone and provide data of outcome and complications. 4. Monitor the clone and perform the tracing of patients.
Methods: 1. Review of results and clinical data of 457 studies (January 2009-

December 2012) in the reference area that includes different hospitals: Alicante, Andalusia and the Balearic Islands). 2. Study of the discrimination ability of the different antibodies used (FLAER Alexa488, CD24 APC H7, CD55/DAF APC, CD59/MIRL FITC and CD16 V450). 3. Study of sensitivity and specificity of our panels using dilution techniques in peripheral blood and bone marrows of healthy controls. 4. For the high sensitivity study, 100 pathological events are measured on an acquisition of 100,000 events (sensitivity 1/1000).
Results: 1. Detection of 7.7% positive cases (3.5% clinically symptomatic)
2. The detection rate has increased significantly since the use of FLAER, which is higher discriminatory than other antibodies 3. The FLAER express similar intensity in myelocytes, metamyelocytes, granulocytes and monocytes, and discriminates better than CD55 and CD59
Summary / Conclusion: · Approximately, 7-8% of patients, presenting at least one criterion of suspicion, have a detectable PNH clone. The effectiveness of the study is very different according to the clinical criteria considered; systematic screening remains controversial in patients with isolated cytopenias or thrombosis, with no evidence of hemolysis or hemoglobinuria. FLAER increases the sensitivity of the technique and avoids errors arising from the study of immature granulocyte series. FLAER allows the joint analysis of the entire population granulomonocytic in AA or MDS cases with low cellularity. The use of CD55 and CD59 allows to identify better intermediate expression clones and possibly to refine the hemolytic power and thrombotic risk in each individual case. Monitoring is essential in patients with small clones HPN/AA, especially in the regeneration phase (possible recuperation at the expense of PNH clone).



B1432
ASSESSMENT OF RESPONSE TO RECOMBINANT ERYTHROPOIETIN IN LOW GRADE MYELODYSPLASTIC SYNDROME
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Background: Ineffective erythropoiesis is characteristic of patients with low grade myelodysplastic syndrome (MDS), such as refractory cytopenia with multi-lineage dysplasia (RCMD) and refractory cytopenia with multi-lineage dysplasia and ring sideroblasts (RCMD-RS). Such patients may also have functional iron deficiency, contributing to hepcidin suppression and iron overload (Murphy PT et al, BJH, 2009:144;451).
Aims: To assess the effect of recombinant erythropoietin (R-EPO) on measures of ineffective erythropoiesis and iron metabolism in patients with low grade MDS and anaemia.
Methods: In 19 patients with low grade MDS (RAMD, n=15; RSMD, n=4) starting on R-EPO (darbepoetin alfa 150ug SC weekly) for anaemia (haemoglobin (Hb) < 10g/dl), we measured CBC, iron parameters and serum levels of EPO, hepcidin 25, tumour necrosis factor-alpha (TNF), growth differentiation factor-15 (GDF-15) and vascular endothelial growth factor (VEGF) prior to starting R-EPO and 8-12 weeks later.
Results: Pre R-EPO blood results showed a negative correlation between serum EPO and serum GDF-15 (P=0.0039), whilst serum hepcidin levels negatively correlated with serum ferritin levels (P=0.01) and positively with total iron binding capacity (TIBC) (P=0.02). Patients with a satisfactory Hb response to R-EPO (n=12) had pre serum EPO levels significantly lower than non responders (n=7) (P=0.019). Responders also had higher serum GDF-15 levels, although not reaching statistical significance. Comparing blood results pre R-EPO and after 8-12 weeks of R-EPO, no statistical difference was found in iron parameters, hepcidin 25, TNF, GDF-15 and VEGF. Analysis of blood results taken after 8-12 weeks of R-RPO therapy continued to show a negative correlation between serum EPO and serum GDF-15 (P=0.032). In addition, post R-EPO Hb correlated negatively with serum iron (P=0.014), transferrin saturation (P=0.016) and ferritin (P=0.04), whilst R-EPO responders had significantly lower serum iron (P=0.0016), transferrin saturation (P=0.0029) and ferritin (P=0.011) than non responders.
Summary / Conclusion: Our results suggest that MDS patients who respond to R-EPO have more effective erythropoiesis at baseline as evidenced by lower serum EPO levels. The negative correlation between serum EPO and serum GDF-1 is an unexpected finding, given the association between GDF-15 and ineffective erythropoiesis in other blood disorders.
R-EPO responders had significantly lower serum iron, transferrin saturation and ferritin, suggesting that R-EPO is better able to overcome functional iron deficiency in these patients with more efficient utilisation of iron to produce Hb.

B1433
ANALYSIS OF VB T CELL REPERTOIRE IN MDS PATIENTS
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Background: Myelo Dysplastic Syndromes (MDS) are clonal disorders characterised by ineffective haematopoiesis and development of Acute Myeloid Leukemia (AML). International Prognostic Scoring Systems (IPSS) define MDS prognostic groups, according with AML risk but a need for improvement in diagnostic/therapeutical procedures is largely recognised. Several data suggest the involvement of immune-selection for the emergence, expansion and dominance of dysplastic clones in a sub-group of MDS. In these patients an altered immune-tolerance control could be involved in abnormal clones selection and in AML progression. We found that Treg levels and CD54 expression on cytotoxic T cells in Bone Marrow (BM) identify a distinct subgroup of MDS patients in which it is possible to hypothesise that defective tolerance control plays a role in the selection of the dysplastic precursors likely fostering their AML progression.
Aims: This study is addressing the hypothesis that BM Treg levels and CD54 expression on CD8 T cells, by us previously associated with the occurrence of immune derangement in MDS, might provide useful tools to validate the occurrence of immune-pathogenesis in a subgroup of patients. These criteria are hypothesised to likely represent valuable predicting elements for clinical response to immune-modulating therapeutical approaches in MDS. In order to investigate on such issue we are focusing the analysis of T cell repertoire in BM and peripheral CD4 and CD8 lymphocytes of the patients, categorised according to IPSS as well as their immunological BM profile.
Methods: Eleven MDS patients, grouped according to IPSS criteria (4 Low; 4 Int-1; 2 Int-2; 1 High) have been further categorised according to their immune profile in BM. In this patients T cell repertoire has been analysed by using flow cytometric VB typing on CD4 and CD8 T lymphocytes. The analysis has been always performed in peripheral blood as well as in BM samples.
Results: Our preliminary data showed in all the Low/Int-1 Risk patients the occurrence of multiple VB expansions in both CD4 and CD8 T lymphocytes. When comparative evaluation of the VB expansions was performed between peripheral blood and BM, only one preferential BM expansion was demonstrated in CD8 effectors in all the patients analysed. This expansion was never accompanied by concomitant skewing of the CD4 population bearing the same VB specificity. None of the Int-2/high Risk patients showed a preferential BM expansion in CD8 effectors in comparison with peripheral blood where multiple VB expansions were observed in CD4 T cells.
Summary / Conclusion: Our preliminary results indicate that peripheral blood/BM comparison of VB repertoire in CD4 and CD8 T cell effectors identifies specific single expansions of CD8 repertoire in Low/Int-1, but not in the Int-2/High MDS patients. Comparative analysis of the results obtained in the patients grouped according to their BM immune-profile (Treg levels and CD54 expression on CD8 T cells) will be further discussed.

Myelodysplastic syndromes and bone marrow failure syndromes incl. PNH - Clinical

B1434

PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA CLONE IN APLASTIC ANAEMIA PATIENTS AT THE BEGINNING OF DISEASE AND IN REMISSION

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Background: The first results have been published and they show that the frequency of the Paroxysmal Nocturnal Haemoglobinuria (PNH) clone detection at Aplastic Anaemia (AA) can vary between 30 and 70%, and at that the existence of the minor PNH clone at the patients can be a favorable prognostic index induced by the Immunosuppressive Therapy (IST).
Aims: to detect the PNH clone at AA patients at different stages of desease and to reveal its' influence on the IST effectiveness.
Methods: 36 patients with severe and very severe AA (SAA/VSAA) who received combined IST with antithymocytic globulin (hATG) and cyclosporin A (CsA) have been included into the study. Mediane age - 23 years (16-54). 2 of the patients undergone the allogenic bone marrow transplantation (alloBMT).
All 36 patients were divided into 2 groups. The 1st one included de novo AA patients(n=22); the 2nd group – AA patients in complete remission (CR) after IST (n=14). The median remission duration was 3 years (2-6 y). The results of the de novo AA treatment (1st group) were evaluated at 6 and 12 months from the start of IST. We used the flow cytometry (Becton Dickinson (BD) FACS Canto II and Beckman Coulter (BC) FC 500) to evaluate the PNH clone. Peripheral blood samples were analyzed with antibodies CD45(BD), CD15(BD), CD64(BD), CD235a(BC), GPI-tying antibodies CD59 (Invitrogen), CD14(BC), CD24(BC) and FLAER (Cedarlane). Minor PNH clone was detected when the size of the clone did not exceed 1%.
Results: The PNH clone was found in 13 patients among 22 (59%) from 1st group. The minor clone was found in 3 patients, in 10 patients the clone size is exceed 1%. Median (Me) clone size on the Red Blood Cells (RBC: type II + type III) was 0,9% (0,1-1,5%), Granulocytes (GR) - 11,3% (0,1- 27,6%), Monocytes (Mon)- 23,2% (0,3-97,3%). 6 SAA/VSAA patients (46%) with the PNH clone (n=13), showed a complete remission at 6 months from the therapy beginning; 4 patients (30%), including 3 patients with the minor PNH clone, showed partial remission; in 1 patient remission was not achieved even 12 months of treatment. But regardless CR, PNH clone persisted in all patients. It worth to note, that PNH clone disappeared after allo-BMT (n=2).
Only in 3(33%) of 9 de novo AA patients with no PNH clone partial remission was obtained at 6 months. The other patients were still on the treatment.
In the 2nd group the PNH clone was detected in 10 cases from 14 (71%), only 4 of them had a minor clone. The Me of PNH clone size on RBC -2,4% (0,1 to3,6%), Gr – 74,2% (0,4-81%), Mon - 13,5% (0,1-20%)
Summary / Conclusion: The PNH clone has been detected in more than 50 % of de novo SAA/VSAA patients. The disease was characterized by pancytopenia and aplasia of the bone marrow without visible signs of intravascular hemolysis. In our study we observed the best IST response at 6 months in SAA/VSAA patients with the PNH clone (46% CR), but even in CR PNH clone persisted.

B1435

APPLICATION OF INTERPHASE FLUORESCENCE IN SITU HYBRIDIZATION IN MYELODYSPLASTIC SYNDROMES AND THE ASSOCIATION WITH CLINICAL FEATURES

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Background: Among patients with primary myelodysplastic syndromes (MDS), about 30-50 % acquired clonal chromosomal abnormalities. The chromosomal abnormalities have prognostic value, can be applied to risk factor calculation. Most of the chromosomal abnormalities are detected by conventional cytogenetic (CC) analysis. However, complex abnormalities were difficult to accurately identify by CC. Presently, multiplex fluorescence *in situ* hybridization (M-FISH), which uses fluorescent probes that bind to those parts of the chromosome with high degree of sequence complementarity to detect and localize the specific DNA sequences alteration, was applied to clarify complex karyotypes and occult defects, such as monosomy7, trisomy8, deletions of the long arm of chromosome 5 or of chromosome 7.
Aims: We performed the clinical trial in 100 cases to investigate the genetic basis in MDS patients and its relationship with clinical features and prognosis of MDS. Differences with two methods, CC and FISH, were determined separately in the abnormalities rate and estimated international prognostic scoring system (IPSS). The findings will provide a rational choice for detection of MDS chromosomal abnormalities.
Methods: All patients (60 males and 40 females aged from 12 to 82 years old) included in this study were diagnosed with MDS or MDS-Acute Myeloid Leukemia (AML), Criteria for diagnosis were made according to French–American–British (FAB) classification and were reviewed for application of World Health Organization (WHO) classification. With the application of sequence-specific DNA probes, interphase fluorescence in situ hybridization (I-FISH), is applied on bone marrow cells to detect del(5q33) (CSF1R EGR1); -5(D5S23,D5S72); 7q31(D7S486, D7S522); 7p11-q11(CSP7); 20q12(D20S108); +8(CSP8) and –Y. Chromosome studies were carried out on bone marrow cells with a Trypsin–Giemsa banding technique. The threshold was established using 10 specimens of health controls. Data were analyzed with Spearman Rank Correlation to evaluate the correlation between positive rate detected with probes and the clinical factors. Paired T test was carried to assess the difference of (IPSS) estimated by I-FISH and CC. (*P 0.05*) All of the patients involved had signed the informed consents.
Results: Chromosomal abnormalities were identified in 27% of with CC, compared to 56% with a method of FISH (*P=0.002*). Of the 56 cases with any abnormal finding, 25 (44.6%) were identified only by FISH. The proportion of IPSS categories also varied with different methods, especially intermediate-1 and -2. CC and FISH calculated the percentage of intermediate-2 as 19.5% and 50.5%, respectively. And there is link between genetic aberration and clinic pathologic characteristic. Correlation analysis indicated del(5q31) and leukopenia were negatively correlated; abnormality in chromosome 7 had relevance with the level of alanine aminotransferase (ALT); del(20q) was related to several clinical features, such as leukopenia, reduced vitamin B12 and ferritin.
Summary / Conclusion: CC plays an important role in the classification of MDS by current World Health Organization criteria. However, it may be limited when insufficient numbers of metaphase cells are obtained or cryptic or complex abnormalities, while FISH is a suitable method to complement the limitation. It is more rapid and sensitive to refine chromosome aberrations in MDS. Some of specific detection for genomic features of MDS was proved to be correlative with its clinic pathologic characteristics and the prognosis, but large-scale and long-term follow-up researches were in need to verify the correlation.

B1436

A PHASE4, OPEN-LABEL STUDY TO EVALUATE THE PHARMACOKINETICS (PK) OF SUBCUTANEOUS (SC) AZACITIDINE IN TAIWANESE SUBJECTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROMES (MDS)

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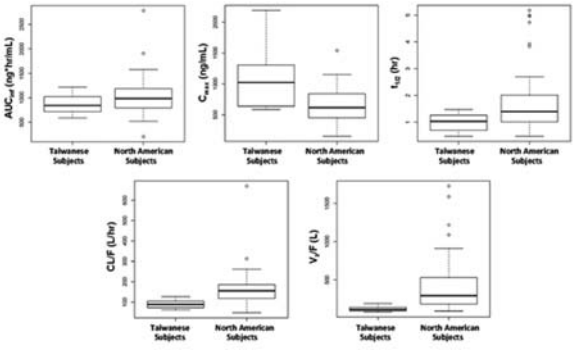
Background: PK evaluations of SC azacitidine (AZA) indicate rapid AZA absorption. Mean maximum observed plasma concentration (C_{max}) is 650 ng/mL; median time to C_{max} (T_{max}) is 0.5 hours post-dose; mean area under the plasma concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) is ~1000 ng*hr/mL; mean terminal elimination half-life ($t_{1/2}$) is 1.6 hours, mean apparent clearance (CL/F) is 175 L/hr, and mean apparent volume of distribution (Vd/F) is 410 L (Garcia-Manero, 2011). There is high intersubject variability for all PK parameters. Most AZA PK data have been collected from studies with Caucasian subjects with hematologic malignancies, although a phase I/II study showed the PK profiles of SC AZA in Japanese subjects with MDS and Caucasian subjects were comparable (Uchida, 2011). PK analysis of SC AZA has not been previously performed in Taiwanese subjects with MDS.
Aims: To characterize the steady-state PK of SC AZA during a single treatment cycle in Taiwanese subjects with higher-risk MDS; and to compare AZA PK in these subjects to AZA PK in a historical cohort of primarily Caucasian subjects from North America with MDS, AML, or CMML (Garcia-Manero, 2011).
Methods: Taiwanese subjects received SC AZA 75 mg/m^2/d x 7d. Blood samples were collected prior to dosing on Days5,6, and 7 and post-dose on Day 7 at 0.25, 0.5,1,2,3,4,6, and 8 hrs. In the historical study, North American subjects also received SC AZA 75 mg/m^2/d x 7d; blood samples were collected before dosing on Day 7 and post-dose at 0.25, 0.5,1, 1.5,2, 2.5,3, 3.5,4,6, and 8 hrs. AZA plasma concentrations were determined using a validated high-performance liquid chromatography/tandem mass spectrometric method. Using non-compartmental methods, the following PK parameters were calculated: C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$, CL/F, and Vd/F.
Results: Twelve Taiwanese subjects were studied: 58% were male (n=7); mean±SD age was 58±10 yrs; 11 subjects had IPSS Int-2 or High-risk MDS and 1 subject had IPSS Int-1 risk MDS. In the historical cohort (N=43), 81% were male (n=35), mean age was 69±12 yrs; and 30 subjects had MDS (n=13 IPSS Int-2 or High; n=16 IPSS Low or Int-1; n=1 missing IPSS), 10 had AML, and 3 had CMML. In both Taiwanese and North American subjects, high intersubject variability (large %CV) was observed for AZA exposure. Pre-dose AZA plasma concentrations were below the limit of detection indicating no AZA accumulation following multiple-dose administration. For Taiwanese and North American subjects, mean AZA plasma concentrations vs. time profiles were similar in shape with overall higher concentrations observed in North American subjects, except for C_{max}. AZA was rapidly absorbed reaching median (min, max) T_{max} within 0.25 hr (0.25, 0.50) and 0.50 hr (0.17, 1.00) post-dose, for Taiwanese and North American subjects, respectively. Subsequently, mean concentrations

declined in an apparent multiphasic manner. For $AUC_{0-\infty}$, $t_{1/2}$, CL/F, and Vd/F parameters, the ranges of values observed in the 12 Taiwanese subjects were within the ranges of values observed in North American subjects. C_{max} ranges in Taiwanese and North American subjects differed; however, overall AZA exposure (AUC) was within the same range in both groups.
Summary / Conclusion: The AZA PK profile and all PK parameters obtained in Taiwanese subjects with MDS except C_{max} were within the range of the PK profile and PK parameters obtained from North American subjects with MDS or other hematologic malignancies. Evaluation of the safety and efficacy of SC AZA in Taiwanese subjects is currently underway.



B1437
CIRCULATING FETUIN-A/A2HS-GLYCOPROTEIN LEVELS IN MYELODYSPLASTIC SYNDROME
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Background: Excess weight is now considered to be a risk factor for many types of cancer, including leukemia and lymphoma. Indeed, a strong correlation of excess body weight with insulin resistance (IR), characterized by hyperinsulinemia has been well documented. Also, there is evidence that IR is implicated in several malignancies related to obesity. Fetuin-A, also known as α2HS-glycoprotein, a hormone synthesized mainly in the liver, could cause IR via inhibiting insulin signaling, modulate adipocyte function by downregulating adiponectin expression and interact with various growth factors influencing tumor initiation and progression.
Aims: In thiscase-control study, we explored the potential role of fetuin-A in association with insulin and adiponectin, all potential mediators of the effects of obesity on IR, in relation to MDS risk taking into account body mass index (BMI), height, weight, family history of lymphohematopoietic cancer (LHC) and smoking history.
Methods: Blood samples were collected from 101 cases with incident, histologically confirmed primary MDS, and 101 hospital controls, admitted for non-neoplastic and non-infectious conditions, matched on gender, age and year/month of diagnosis (±1 month) between 2004 and 2007. Serum insulin and adiponectin were determined by radioimmunoassay. Serum fetuin-A levels were measured using an enzyme linked immunosorbent assay (Biovendor R&D) with a sensitivity of 0.35 ng/mL. The statistical analysis of the data was performed using IBM-SPSS® version 20 for Windows statistical software package.
Results: MDS patientspresented significantly higher height and weight than control subjects (P<0.001), while differences of BMI were only of borderline significance (P=0.12). Serum fetuin-A and insulin were significantly higher in patients than in control participants (P=0.04 and P=0.005 respectively).Serum fetuin-A levels were not significantly different in MDS subtypes, IPSS categories and karyotype (P>0.05). There was statistically significant evidence that elevated serum fetuin-A levels expressed by control-defined quartiles were associated with increased risk for MDS, before and after adjusting for age, gender, date of diagnosis, BMI, family history of LHC, smoking history, serum insulin and adiponectin (p value for linear trend=0.03). Stratifying by BMI revealed that mainly among overweight/obese subjects, those with elevated fetuin-A levels presented a higher risk of MDS after adjustment with MDS risk factors and hormones (for fetuin-A: OR=1.006, 95% C.I. 0.99-1.013, P=0.07 though not significant at α=0.05).
Summary / Conclusion: The interplay of elevated serum fetuin-A levels, hypoadiponectinemia and hyperinsulinemia is associated with higher risk of MDS particularly amid overweight/obese individuals, a finding with potential preventive and clinical implications. Weight loss and physical activity could decrease fetuin-A attenuating thus IR. Pharmacologic agents such as full and selective PPAR-γ agonists that decrease fetuin-A levels could be at the forefront of future therapeutic modalities for obesity-linked malignancies, including MDS. Additional research is needed to elucidate the mechanisms underlying the associations of fetuin-A and insulin with the risk of myelodysplasia and leukemogenesis.

B1438
STRIKING HEMATOLOGICAL ABNORMALITIES IN PATIENTS WITH MICROCEPHALIC OSTEODYSPLASTIC PRIMORDIAL DWARFISM TYPE II MAY INDICATE A POTENTIAL ROLE OF PERICENTRIN GENE IN HEMATOPOIESIS
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Background: Microcephalic osteodysplastic primordial dwarfism (MOPD II) is among the rare etiologies of dwarfism with similarities to Seckel syndrome. Seckel syndrome is well-known to have various associated hematological abnormalities, however the hematological findings of MOPD II patients are very limited.
Aims: To figure out the hematological characteristics of MOPD II patients.
Methods: A series of 8 patients from 7 unrelated families who were diagnosed with MOPD II syndrome by clinical evaluation were further genetically tested for pericentrin mutations. Case 3,5,7 had one visit. The patients were designated to have leukocytosis or thrombocytosis if they had persistently (excluding case3,5,7) high levels for age at multiple visits without any other cause. None of the patients had any symptom or sign of infection at work-up. The cases1,2,4,6 were screened for iron deficiency and were found to have normal iron status. Case 4 was also evaluated with bone marrow aspiration as well in order to understand the etiology of leukocytosis.
Results: All of the patients were found to have pericentrin mutations. Of the 8 patients with MOPD II, the mean age at assesment was 58.0±46.3 months (6-144 months and male:female ratio was 3:5. There was consanguinity between parents in 7 (87.5%) of the patients. Case 1 and 2 were siblings from same parents, whereas other patients were not related.
The hematological evaluation revealed leukocytosis in 6 (75%), thrombocytosis in 7 (87.5%) and anemia in 2 (25%). In 5 patients (62.5%) leukocytosis and thrombocytosis were concomitantly found, whereas in others either one was present. The mean hemoglobin level of the group was 11.8±1.4 g/dl (10-14.2); the mean WBC count and platelet counts were 22.6±10.5 $x10^9$/L (13.1-44.2) and 521.0±133.3 $x10^9$/L (285-668), respectively.
Case4, who had higher leukocyte counts was also evalauted with bone marrow aspiration, which revealed cellular bone marrow with 36% lymphocytes, 24% normoblasts, 12% metamyelocytes, 13% neutrophils, 6% monocytes, 4% eosinophils and 1% stab, with a myeloid/erythroid ratio of 1.08. No blasts were detected in the peripheral blood and the bone marrow aspirate. Immunological status of this patient was also evaluated and no cellular or humoral defects were detected. During the follow-up for 60 months, leukocytosis, thrombocytosis and anemia persisted. Case 6 developed moyamoya disease and recurrent stroke and managed with low molecular weight heparin followed with aspirin prophylaxis.
Summary / Conclusion: MOPD II was mapped to 21q22.3 and biallelic mutations in the pericentrin gene were shown to underlie the etiology. Pericentrin is an integral protein of the centrosome that serves as a multifunctional scaffold for anchoring various proteins and protein complexes.Depletion of PCNT has been shown to cause apoptosis leading to dwarfism in MOPD II, whereas overexpression has been shown to induce cell death in hematopoetic progenitor cells via mitotic defects and anuploidy, in addition to the role of overexpression of pericentrin in solid tumors and AML. The high leukocyte and thrombocyte counts in MOPD II patients may be related to the different functions of pericentrin in various cell types. In conclusion, upto our knowledge and according to the literature overview hematologic abnormalities including leukocytosis, thrombocytosis or both are very initialy being reported in a series of patients as accompanying characteristics of the disease, excluding 2 different previous case reports data giving the platelet and WBC counts of their patients within the report and were also consistent with our findings. This data may help to understand the potential function of pericentrin gene in hematopoietic cell proliferation and differentiation.

B1439
CASE SERIES OF APLASTIC ANEMIA; AN INSTITUTION BASED REVIEW
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Background: Aplastic Anemia (AA) is one of the frequently encountered Bone Marrow Failure Syndromes in Pakistan. It is idiopathic in more than 80% of cases however the major identifiable causes include drugs, ionizing radiation, chemicals and certain viruses.
Aims: We have performed a prospective data collection of patients suffering

from AA with detailed analysis of their baseline characteristics and same characteristics were compared with the control population of same physical characteristics without AA.
Methods: We designed an extensive database which incorporated all detailed baseline characteristics of patients suffering from AA and certain epidemiological factor exposure prior to the development of AA. These parameters included their socioeconomic details, nature of work, living conditions, exposure to certain known industrial toxic materials. The data was analyzed on SPSS version 17 and frequencies were determined and compared with 150 control population without AA.
Results: From December 2010 till December 2012, we collected detailed data of 153 confirmed cases of Aplastic Anemia. Out of them 105 (68.6%) were male and 48 (31.4%) were females. We also collected 150 control cases with 58 males (50.4%) and 57 females (49.7%). The mean age at the time of presentation was 21 years (range 3-69 years). The urban population constituted 93(60.7%) while rural was 59 (39.3%). Overall 31.3% of patients belonged to Karachi. Mean hemoglobin was 7.73, mean while cell count was 2.23 and mean platelet counts were $14x10^{10}$ at the time of first visit in clinic. Very Severe AA in 22.3%, Severe AA in 43.5% and Non-Severe AA was identified in 13.5%. Chronic use of NSAID could be established in 23.5% of patients with AA while 5.2% in control group. Benzene exposure in 7.8% versus 0.9% of control group, organophosphates in 20.3% while 11.3% in control population. There was no significant difference with excessive use of fluoride, lead containing chemicals, hair dye or microwave oven use. HLA matched Allogenic stem cell transplant was performed in 36(23%) with complete response seen in 21(72%) cases in past two year. 71 (48.4%) continued with supportive care therapy alone and 29 (21.2%) continued Cyclosporin alone or with ATG.
Summary / Conclusion: There probable identifiable risk factors include use of NSAIDS, Benzene and exposure with organophosphorous compounds. The affect need to be further established by serum levels of theses injurious toxins and its in-vitro effect on pleuripotent stem cells and it might contribute in disease prophylaxis in future. The overall mortality can be reduced if HLA identical donor identified (Allogenic transplant outcome 72%) and also with good supportive care if other treatment options utilized.

B1440
POSITIVE OUTCOMES OF THROMBOSIS IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA WITH ECULIZUMAB THERAPY
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Background: Thrombotic events (TE) are the most feared complication in paroxysmal nocturnal hemoglobinuria (PNH), accounting from 40 to 67% of the deaths in the pre-eculizumab era. The likelihood of TE in the PNH patient population is up to 62 times higher than in the general population. In PNH, TE often happens despite correct anticoagulation. Eculizumab, a MoAb designed to block complement's C5, has proven to be safe and effective in the prevention of TE in PNH.
Aims: The aims this study were to analyse the number of TE and their complications before and after initiating eculizumab on PNH patients, and its effects on both the number and consequences of TE and the QoL of the patients.
Methods: The study reviews the medical history of a Spanish cohort of 22 patients with medical history of TE caused by PNH and their clinical course before and after receiving eculizumab.
Results: Fourteen men and 8 women, ages between 30 and 72 (mean 52.7, SD 12.1), were included in the study. Eight patients (36.3%) did not have a previous diagnosis of PNH before suffering their 1st TE; average time from diagnosis to 1st TE was 5.9 yrs (SD 5.4, range 0.2 – 15.9). In the other 14 cases (63,6 %) the diagnosis of PNH preceded the 1st TE by 2.2 yrs on average (SD 3.3, range 0.1 – 6). The mean granulocyte clone size at diagnosis (n=13) was 73.1% (SD 21.1, range 35-100). The average number of TE per patient before starting eculizumab was 2.1 (SD 1.3, min=1, max=7). In total, the 22 patients presented 42 TE before eculizumab, despite the fact that all of them were under anticoagulation prophylaxis. The most frequently affected territory was abdominal (18 TE, 42.9%), out of which 9 were Budd-Chiari syndromes, followed by brain (11 TE, 26.2%), lower extremities (9 TE, 21.4%), and atrial, dermal, retinal and pulmonary (1 case each, 2.1%). The TE was venous in 35 occasions (83.3%), and arterial in 7 (16.7%). The mean granulocyte clone size at the time of the 1st TE (n=13) was 78.9% (SD 21.2, range 30-100). Fifteen patients (68.2%) presented a total of 30 serious complications due to TE. Ten patients (45.5%) presented 18 abdominal complications, including hepatomegaly (7), splenomegaly (4), increased liver enzymes (2), jejunectomy (2), cirrhosis (1), ascites (1) and abdominal pain (1). Eight patients (36.4%) presented 9 neurological complications, including hemiplegia (5), hemiparesia (3) and lose of conscience and dysarthria (1). One patient presented papiledema, one varicose veins and one dehiscence of suture in a previous surgery. Two patients died without receiving eculizumab, a 72 year old man after hemiplegic consequences of a sepsis by E. Coli, and a 48 year old woman after a failed allogeneic BMT. Both patients had been under treatment with eculizumab for more than 2 years each with good QoL. The mean follow-up (until February 26, 2013) of the remaining 19 was 3.1 years (SD 1.9, range 0.6 – 8.1). One patient suffered a possible pancreatic micro-thrombosis 15 days after the first infusion of eculizumab, of which the patient recovered well in the next few days. None of the others have had any TE event, and all are well and with good QoL. Eighteen of them are still anticoagulated.
Summary / Conclusion: TE are a severe and recurring complication of PNH. The most frequent territories are abdomen and brain, leading to severe, sometimes life-long sequelae. TE can appear with clones of any size, are difficult to predict and are not adequately prevented with standard anticoagulation. Eculizumab is a safe treatment for PNH that effectively prevents TE and their complications.

B1441
DETECTION OF PNH-CLONE IN PATIENTS WITH DEPRESSION OF HEMOPOIESIS
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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, life-treatening clonal blood disorder. Its immunologic diagnostics attracts more and more attention during the recent years as there appeared standardized flow cytometric protocols using a panel of monoclonal antibodies, and such drug as Eculizumab has been developed. It is known that beside patients with confirmed clinical diagnosis of PNH, cells with PNH-phenotype (PNH-clone) are often revealed in patients with aplastic anemia (AA) and myelodysplastic syndrome (MDS). Nevertheless, the frequency of detection of PNH-clone and its dynamics vary broadly, according to different data.
Aims: to study the frequency of detection of PNH-clone and its size in different groups of patients with blood diseases.
Methods: PNH-cells were revealed using multi-color flow cytometry (FC 500, Beckman Coulter), detecting PNH-clone in granulocytes (FLAER/CD24/CD15/CD45), monocytes (FLAER/CD14/CD64/CD45) and red blood cells (CD235a/CD59), revealing II and III type PNH-RBCs. The corresponding cells lacking expression of listed surface antigens were considered PNH-clone.
Results: During the period from June 2011 to March 2013 in laboratory of immunohematology of Russian Research Institute of hematology and transfusiology we have examined 148 patients in order to reveal PNH-clone. Of them the diagnoses on admission to laboratory were distributed as follows: AA – 69 patients, MDS – 41, suspicion for PNH – 16 patients, other (hypercoagulation, thrombophilia, cytopenia of unknown origin, autoimmune hemolytic anemia) – 22 patients. In total PNH-clone was established in 62 patients (41.8% of cases), among them with PNH diagnosis – 43.8% (7 of 16 examined patients), AA – 63.8% (44 of 69), MDS – 21.9% (9 of 41), other diagnoses – 9.1% (2 of 22). The size of detected PNH-clone varied from 0.01% to 100%. It was less than 1% in 12 (27.3%) patients with AA, 6 (66.7%) – with MDS, 2 (100%) – with other diagnoses. PNH-clone from 1.1 to 10% was observed in 13 (29.5%) patients with AA and in no cases in patients of other groups. PNH-clone sized 10.1-99.9% was detected in 7 (100%) PNH patients, 19 (43.2%) AA patients, 3 (33.3%) MDS patients. It should be noted that in MDS patients its size did not exceed 20%. In examined patients the symptoms of latent intravascular hemolysis (moderately increased LDH, transient hemosiderinuria) usually developed when pathologic clone exceeded 25%. Clinically manifested hemolysis with skin colour changes, darkening of urine and laboratory signs of hemolysis were observed in patients with large PNH-clones, exceeding 80%. The dynamics of PNH-clone was evaluated in 21 patients, studies were performed every 6 months. Gradual increase of PNH-clone was observed in 8 (38.1%) of cases.
Summary / Conclusion: Thus, PNH-clone occurs more frequently in AA than in MDS patients, and its size in MDS is mostly less than 1% and in other cases it does not exceed 20%.

B1442

LENALIDOMIDE (LEN) IN MYELODYSPLASTIC (MDS) DEL(5Q) PATIENTS: A SINGLE INSTITUTION POPULATION-BASED EXPERIENCE

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Background: LEN induces red blood cell transfusion independence (TI) and complete cytogenetic response (CCR) in MDS with del (5q). Published results of large multicentre MDS-003 and MDS-004 studies are well known, but only limited information on population-based experience is available.
Aims: To evaluate safety, efficacy and long-term outcome in a strictly consecutive population-based series of MDS pts with anemia and del(5q), treated with LEN at a single Institution.
Methods: All consecutive MDS pts classified according to WHO criteria and IPSS, and treated with LEN at our Institution between July 2007 and February 2013 were analysed. Institutional guidelines include observation until transfusion need, then epoetin (EPO) treatment and LEN for EPO non-responders. Initial LEN doses was 10 mg po days 1-21 on 28-day cycles, without thrombosis primary prophylaxis. Responses were evaluated according to IWG criteria after 4 months. LEN was continued until loss of TI, toxicity, leukemic evolution or death. Bone marrow blasts count and cytogenetic analysis were performed in all responsive cases treated for at least 6 cycles.
Results: Twenty patients were recorded (7,5 % of 264 newly diagnosed MDS). Median age was 75,5 years (range 53-87) female were 65%. WHO categories included unclassificable (2), MDS del(5q) (12), RCMD (2) and RAEB I, RAEB II, RARS and RARS-T (one each). Eighteen cases (90 %) had IPSS Low/Int-1 and bone marrow blast count ≤ 5%. By conventional cytogenetics 5(q-) was isolated in 18 (90 %) and associated with one additional abnormality in 2 [+8 and del(17p)]. Median time from MDS diagnosis was 13,5 months (range 1-87), all patients were transfusion-dependent. Fourteen cases were pre-treated and non-responders to epoetin. Median number of cycles was 6 (range 1-43). Median follow-up was 33 months (1-152) after MDS diagnosis. Among the 18 cases evaluable for erythroid response (2 too early), TI was reached in 15 cases (83 %), haematologic improvement in1, failure in 1; one case refused treatment after 2 cycles. Cytogentic response was evaluated in 9 pts. There were 6 CCR (66 %) and 3 partial response. Haematological toxicity (neutropenia and/or thrombocytopenia WHO grade 3-4) was observed in 55 % of cases. Non haematological toxicity included: thrombosis (3), diarrhea (2), headache, abdominal pain, rash, transaminases increase, fatigue (1 each). It caused LEN discontinuation in 2 cases in TI (cutaneous rash and deep venous thrombosis with pulmonary embolism). The incidence of deep venous thrombosis/pulmonary embolism was higher than expected occurring in 3/20 cases, (15 %), two of whom had haematologic comorbidity as risk factor (asymptomatic multiple myeloma and indolent lymphoma). Two pts with isolated del(5q) evolved to acute myeloid leukemia (AML) during treatment, respectively after 1 and 7 months (the latter while in CCR). The 5-year actuarial risk of progression to AML was 15,6 % (SE ± 13,4). The 5-year overall survival probability was 67,7 % (SE ± 13,8). Causes of death were: congestive hearth failure in one, gallbladder carcinoma in one, infection in one, hemorrhage in one and AML in 2. At present 9 patients are still on treatment after a median of 23,5 months (range 4-68+).
Summary / Conclusion: In a population-based series of MDS del(5q) pts median age was higher than in clinical trials. EPO was ineffective in transfusion dependent pts and is currently avoided at our Centre. LEN confirmed to be effective and manageable. TI was obtained at high frequency and was durable even after LEN interruption. Thrombosis was frequent suggesting that prophylaxis should be considered, at least in pts with other risk factors. LEN did not increase the risk of leukemic evolution. Survival was satisfactory and causes of death weren't treatment related.

B1443

MORPHOLOGICAL FEATURES OF HEMATOPOIESIS IN MDS PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION WITH REDUCED INTENSITY CONDITIONING

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Background: The use of conditioning regimens with decreased intensity extend the availability of allo-HSCT for elderly MDS patients and MDS patients with comorbidities. However, data on the morphological characteristics of the recovering hematopoietic tissue in MDS patients at different times post-transplant are still uncertain.
Aims: To study morphological features of hematopoiesis in bone marrow aspirates and trephine biopsies of MDS patients taken at different time points after allo-HSCT with reduced intensity conditioning.
Methods: We have analyzed 98 bone marrow aspirates and 94 trephine biopsies from 2 high risk, 3 intermediate-2, 5 intermediate-1 MDS patients before and after1,2,3,6, 12 and 24 months allo-SCT.
Results: Abnormal karyotypes before transplantation were found in 5 patients. Engraftment was achieved in 9 of 10 patients. One patient died of graft failure6,5 month post transplant. Another patient died from a severe infection that developed on the background of the extensive form of chronic GVHD 13 months after allo-HSCT. Rapid recovery of blood parameters after transplantation was noted for the rest of the patients. Threphine biopsies after allo-HSCT indicated hypoplasia of bone marrow with erythroid predominance for majority of patients. However the numbers of megakaryocytes were reduced. Bone marrow cellularity in patients with severe GVHD was not lower compared to those without GVHD. However delayed engraftment in 2 patients with acute GVHD was observed. Focal fibrosis of the stroma, revealed by pre transplant biopsies in 4 patients, persist in 2 of them in the follow-up period. Magaloblasts were found in 6 of 10 patients during early post transplant period. Significant reduction of erithropoiesis was observed later. Granulocytopoiesis was not affected in most of the patients. Dysplastic changes in megakaryocytes persist during all periods of observation in most patients, but were less pronounced compared to the baseline.
Summary / Conclusion: Recovery of hematopoietic tissue after allo-HSCT in MDS patients is similar to that in other hematological malignancies. Hypoplasia of hematopoietic tissue was still detected in the majority of patients after transplantation. However disease-specific signs of dysplasia were significantly reduced.

B1444

AZACYTIDINE THERAPY FOR MDS AND AML PATIENTS: RETROSPECTIVE MULTICENTRE REGIONAL EXPERIENCE IN PATIENTS NOT ENROLLED INTO CLINICAL TRIALS.

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Background: In higher-risk patients with myelodysplastic syndromes (MDS), the DNA methyltransferase inhibitors produce responses in 20% to 30% of patients and azacitidine (AZA) has demonstrated a survival advantage when compared with conventional therapies. AZA is increasingly used in low-risk patients failing to respond to recombinant human erythropoietin (r-EPO) and in acute myeloid leukaemia patients (AML) with low marrow blast count or considered not fit for intensive chemotherapy.
Aims: We report a retrospective review on AZA treatment of MDS and AML patients in the common clinical practice of an Italian region (Liguria).
Methods: Of 67 patients who started azacitidine therapy only 46 patients received at least 4 courses of therapy, and were therefore considered evaluable for response and included in the study. Median age was 74 years (56-84), male/female ratio was 25/21. Nine patients (19%) had untreated AML with marrow blasts ranging from 25 to 41%. MDS patients had RA or RARS (n.7, 19%), AREB-1 (n. 13, 35%), AREB 2 (n. 15, 40%), other forms (n.2, 5%). In MDS patients the IPSS score was low / int-1 in 18 pts, int-2 / high in 13 pts, not assessed in 7 pts. Twenty-six patients had a transfusion-dependent anaemia and the median number of packed erythrocyte units transfused weekly was 1 (range 1-2). All low and int-1 risk MDS patients had transfusion dependent anaemia and were unresponsive to r-EPO.
Results: After a median of 8 courses (range 4-44) 26 patients (63%) achieved a haematological response (CR in 26%, PR in 24%, HI in6,5%) whereas 20 (43,5%) were unresponsive. According to diagnosis and IPSS score responders were 6 (66,6%) among AML patients (CR1, PR4, HI 1), 10 (55,5%) in low /int-1 risk MDS patients and 7 (54%) among int-2 / high risk MDS patients (CR4, PR 3). Response was achieved after a median of 5 (range 3-6), 3 (range 2-12) and 5 (range 1-12) AZA courses in AML, low/int-1 risk and int-2 / high risk MDS patients, respectively. Grade 1-2 myelotoxicity was commonly observed but no life threatening infections were reported. Eight-teen patients concomitantly received r-EPO therapy. Response lasted a median of 16 months (range 4-40) and median survival was 6 months in AML patients (4-9) and 23 months (6-48) in MDS patients.
Summary / Conclusion: These preliminary data confirm efficacy and feasibility of AZA therapy in the common clinical practice, for both AML and all risk MDS patients.

B1445

CLINICAL FEATURES AND ANALYSIS OF PROGNOSTIC RISK FACTORS OF 124 PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background: Myelodysplastic syndrome is a malignant clonal disease of hematopoietic stem cells, with the risk of eventually progress to acute leukemia. Large number of foreign clinical data analyzed and summarized the clinical features of MDS. But whether these features of domestic patients equally have, needing clinical data further confirming.
Aims: To gain more the understanding of myelodysplastic syndrome as they

occur in south China, a retrospective clinical analysis was conducted in patients diagnosed as MDS from January 2007 to October 2011 at our hospital Department of hematology.
Methods: Our country reviewers independently examined the bone marrow and peripheral blood smears of all the patients and classify the disease according to the WHO(2008) classification. There were a total of 117 eligible patients.
Results: The median age of the patients was 57.5 years (range17-94). The male:female ratio was 1.8:1. Twenty five percent of the patients were younger than 40 years□57.3% were younger than 60 years. The frequency of the WHO subtypes was RCUD 12.1 % , in which RA was 8.1% , RN 1.6 % , 2.4% in RT , RARS 4%, RCMD 38.7%, RAEB -1 20.2% , RAEB -2 23.4% , the MDS - u 1.6% , 5q - not statistics. Anemia was the most common symptom presenting in 70.3% of the patients. In the 31 patients in whom the cytogenetics in the bone marrow were analysed, 35.3% revealed abnormalities. Of these, trisomy 8 was the most common aberration, and monosomy 7 was the worst prognosis. Transfusions were the main therapeutic modality in 49.2% of the patients. The median survival of patients is 17.9 months. Fourteen point six percent of the patients had progressed to acute myelogenous leukemia (AML) with a median time to disease-progression of 5.6 months. Single factor derived from the KM survival analysis obtained age, gender, LDH , WBC , peripheral primitive cell count , bone marrow blast cell count , Plts , the Plt quality statistically significant .The Cox regression analysis revealed peripheral primitive cell count□the number of bone marrow blasts□LDH * age , and the PLT quality as the parameter significantly associated with survival and disease progression. The establishment of a new prognostic scoring system for domestic is the R- WPSS (domestic) , patients were divided into four groups , namely: the low risk group, risk group, risk group, very high-risk group; median survival times were as follows: 46□36 □12□3 months .
Summary / Conclusion: The median age of our MDS is 57.5 years, and domestic and Asian countries with a median age similar , but smaller than the United States and Europe more than 10 years. The reasons is may biological and environmental factors. Consistent with the proportion of gender of domestic data. The most common for hospitalising with the disease is lung infection (14.5%), chronic diseases are more common in hypertension and diabetes, respectively, 10.4% , 4%. Anemia is the most common symptoms (70.2%) , signs of liver and spleen and lymph nodes are rare. Trisomy 8 was the most common aberration, and monosomy 7 was the worst prognosis. The Cox regression analysis revealed peripheral primitive cell count□the number of bone marrow blasts□LDH * age , and the PLT quality is independent risk factor. The establishment of a new prognostic scoring system for domestic is the R- WPSS (domestic) , patients were divided into four groups , namely: the low risk group, median group, high group, very high-risk group; median survival times were as follows: 46□36□12□3 months.

B1446
OVERVIEW OF RUSSIAN PNH REGISTRY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal hematopoietic stem cell disease which can lead to life-threatening complications including thrombotic events (TE), chronic kidney disease (CKD) and pulmonary hypertension. Recently, the international PNH Registry has been established in Russia to assess disease burden and examine the natural history of PNH.
Aims: We aim to update the spectrum and natural history of the disease in Russia according to International PNH Interest Group Classification (Parker C et al, Blood 2005;106:3699-3709).
Methods: The international PNH Registry is a non-interventional, prospective, multicenter, observational study. Study populations: Russian part of international PNH Registry, patients with a confirmed diagnosis of PNH according to international guidelines. Demographics, clinical characteristic and medical history were stratified by PNH clone size and history of bone marrow disorders (BMD).
Results: As of February 2013, the international PNH Registry has enrolled 189 patients from Russia (Table).
Summary / Conclusion: Patients in the international PNH Registry enrolled from Russia represent a broad age range, clone size and degree of hemolysis, two-thirds having a history of BMD. History of TE, CKD and signs of chronic hemolysis were present in patients regardless of PNH clone size. The likelihood of TE was significantly higher in patients with PNH clones ≥20%. Data are essential for the design of treatment trials and for planning services for PNH patients in Russia.

Table. Patient characteristics and symptoms.

Demographics	All patients	Classic PNH	BMD-PNH	BMD-Subclinical PNH
Total patients, n	189	39	97	53
Age at enrollment (years), median	30	35	29	27
Gender female, n (%)	112 (59.3)	28 (71.8)	60 (61.9)	24 (45.3)
Time to diagnosis (months), median	17.3	51.2	10.1	27.9
Clinical characteristics				
Granulocytes clones (%) at enrollment, median	7.4	93.0	16.6	0.2
LDH ratio x ULN at enrollment, mean ± SD, median	2.6 ± 3.2 1.1	6.3 ± 3.5 6.3	2.6 ± 3.1 1.3	0.8 ± 0.2 0.8
History of TE, n (%)	9 (4.8)	4 (10.5)	4 (4.2)	1 (1.9)
CKD at enrollment, n (%)	42 (22.5)	14 (36.8)	16 (16.7)	12 (22.6)
Pulmonary hypertension at enrollment, n (%)	5 (2.7)	3 (7.9)	2 (2.1)	0 (0)
Symptoms stratified by PNH granulocyte clone size				
			<20%	≥20%
Total patients, n			104	80
History of TE at enrollment, n (%)			1 (1.0%)	8 (10%)
CKD at enrollment, n (%)			20 (19.4)	22 (27.5)
Pulmonary hypertension at enrollment, n (%)			0 (0)	5 (6.3)
Lactate Dehydrogenase (LDH) ratio x UNL, mean ± SD (median)			1.1 ± 1.2 (0.8)	4.8 ± 3.8 (4.0)

B1447
ACQUIRED APLASTIC ANEMIA : DIAGNOSTIC AND THERAPEUTIC ASPECTS

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Background: Aplastic Anemia (AA) is a quantitative marrow failure resulting in pancytopenia. The prognosis depends on the risk of infection and bleeding.Thebone marrow allograftis the best curative treatment. In Tunisia, this technique has been available since 1998 at the National Centre for bone marrow transplantation in Tunis.
Aims: The purpose of our study is to investigate the diagnostic, therapeutic and evolutive characteristics of patients presenting with AA followed in hematology department of Sfax.
Methods: This is a retrospective study concerned with all cases of acquired AA, diagnosed in hematology department of Sfax, during a period of 13 years (between January 1999 and December 2011). The diagnostic confirmation of AA is based on the bone marrow biopsy for all patients. The etiological assessment involved: karyotype on blood and on marrow, liver function tests, viral serology and Ham Dacia test and withflow cytometry. For the severity of the disease we adopted the criteria of Camitta. The proposed treatment was bone marrow allograft (indicated for all patients younger than 45 years with an identical HLA donor), immunosuppressive therapy (cyclosporine+/- antithymocyte globulin: ATG) and / or androgenic and symptomatic treatment.
Results: We collected 92 patients; the mean age was 26 years, ranging from 2 to 87 years. The sex ratio was 1.42%. Sixty-five percent of patients had severe form and 35% moderate form. AA was idiopathic in 78%, post-hepatitic in 16%, toxic in 4% and associated with paroxysmal nocturnal hemoglobinuria in 2%. The allograft was indicated for 55 patients. Thirty-three patients (60%) had a compatible HLA donor. Among them, 82% had a bone marrow allograft. The graft was rejected, due to unfavorable economic conditions in 4 patients and a genetic disease of the donor in one case. In total: 27 among 92 patients were grafted (29%). Thirty seven patients (34%) were treated by immunosuppressive therapy with an objective response up to 37%. Androgen and symptomatic treatment were used respectively in 20and 8 patients (8% and 18% respectively). The 5-year survival was 87% for allograft patients and 36% for patients treated with immunosuppressive therapy.
Summary / Conclusion: The diagnostic and the epidemiological aspects of AA in our series are similar to those seen in the literature (rare disease, young population, predominance of severe and idiopathic forms). Compatible HLA donor was found in 2/3 of cases which is higher than the western series rate, due to the important number of families in our country. Thebone marrow allograftis the potentially curative treatment in our series as well as in the literature. But, objective response was found in 1/3 of our cases after immunosuppressive therapy because of the difficulty of using the ATG in some patients.

B1448

COMBINED OVEREXPRESSION OF WT1 AND BAALC GENES MAY PREDICT AML EVOLUTION IN MDS PATIENTS

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Background: Current international Guidelines of Myelodysplastic Syndromes (MDS) indicate that management's strategy of MDS patients should be based on prognostic scoring systems such as the International Prognostic Score System (IPSS). Most used MDS scoring systems do not include biological parameters that are potentially able to influence the clinical course.
Aims: Our goal was to verify if biological markers utilized in acute myeloid leukemia may have any predictive role in high and low risk MDS patients.
Methods: From September 2007 to December 2012 we analyzed 54 MDS patients [40 low-intermediate-1 (LR) and 14 intermediate 2-high IPSS score (HR)] observed in our Hematology department. FLT3 gene ITD, NPM1 gene mutations, WT1 and BAALC expression were evaluated. Abnormal karyotype was observed in 8 cases (3 high and 5 intermediate risk karyotypes). Nineteen patients were treated with azacitidine plus erythropoietin, 2 with chemotherapy , 18 with hematopoietic growth factors only. Three patients underwent allogeneic bone marrow transplantation. The remaining patients received supportive care alone.
Results: A molecular analysis was performed at diagnosis on bone marrow samples in all the patients. No patients showed either FLT3-ITD or NPM mutations. WT1 overexpression was observed in 9 patients (2 LR, 7 HR), BAALC overexpression was present in 15 patients (3 LR, 12 HR). Neither WT1 nor BAALC overexpression were related to cytogenetic abnormalities. We observed a simultaneous overexpression of WT1 and BAALC genes in 8 patients. All these patients had been classified, at diagnosis, as high risk MDS with a IPSS score ≥ 1.5. In all these cases an evolution to acute myeloid leukemia was reported at a median follow up period of 6 months (range 2-9). Among 31 low risk patients only 2 patients evolved to AML. Both these patients had an aspecific diagnostic molecular profile. Three more cases showed an isolated overexpression of WT1 and one of these experienced an evolution. Two are still leukemia free after a follow-up period of 1 year and 5 years respectively. An isolated BAALC overexpression was observed in one patient who had a disease evolution.
Summary / Conclusion: Thank to this analysis we can conclude that the combined more than the isolate overexpression of WT1 and BAALC may be associated to a MDS evolution. The prognostic value of this molecular pattern may overcome that of IPSS score. We suggest that molecular evaluation at diagnosis of MDS should include WT1 and BAALC evaluation whereas there is no reason to perform FLT3 and NPM analysis in this setting.

B1449

RABBIT ANTITHYMOCYTE GLOBULIN IN CHILDHOOD ACQUIRED SEVERE APLASTIC ANEMIA

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Background: Acquired severe aplastic anemia (SAA) is a life threatening bone marrow failure characterized by pancytopenia and hypocellular bone marrow. Matched sibling donor is not available for majority of the patients and many children receive immunosuppressive therapy (IST). Although horse anti-thymocyte globulin (ATG) is usually included as a first line treatment, it is not available in Turkey and all the patients received rabbit ATG.
Aims: In this study, we wanted to evaluate the efficacy of rabbit ATG in children with acquired SAA.
Methods: We retrospectively reviewed the medical records of children with SAA who were treated with rabbit ATG, cyclosporine, G-CSF and short term prednisone between 2006 and 2012.
Results: Fifteen children with very SAA (9 boys and 6 girls) aged between 1.5 and 17 years received rabbit ATG as a first line treatment in our institution during six-year period. Only one patient showed partial response and the others failed to achieve any response at3,6,12 months of the first course of IST. Eight patients received second course of ATG. While rabbit ATG was again used at the same dosage in 3 patients, 5 patients received horse ATG. None of these 3 patients showed complete or partial response to the second course of rabbit ATG. Two patients showed partial response to the treatment of horse ATG while the remaining 3 showed no response. Three patients received the third course of rabbit ATG, however they did not show any response. Invasive fungal infection which observed in 60% of the patients was the major reason of death.
Summary / Conclusion: Six-year experience with rabbit ATG has led us to suggest that this option is not proper as the first line treatment in children with very SAA. Fungal infections are very frequent and complete response is very low.

B1450

PROGNOSTIC MARKERS FOR MYELODYSPLASTIC SYNDROME

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Background: Along with certain progress achieved in treatment of myelodysplastic syndrome (MDS) during recent years, issue of accurate prognostic stratification of this disease remains an important task.
Aims: This study was intended to determine clinical parameters, easily accessible, which could be used for evaluation of myelodysplastic syndrome disease course and prognosis of the risk of its transformation into acute leukemia (AL).
Methods: A group of 108 patients with MDS (64 with refractory anemia (RA), 6 – with refractory anemia with ringed sideroblasts (RARS), 28 – with refractory anemia with excess of blasts (RAEB) and 10 – with chronic myelomonocytic leukemia (CMML)) were monitored for their disease course and rate of transformation to acute leukemia. Clinical, morphological, cytogenetic and statistical methods were used within the study.
Results: Among the investigated patients transformation into AL was detected in 28 of the cases. The highest rate of transformation was revealed in CMML and RAEB (40% and 32% respectively) and the lowest – in RA (19%). Retrospective analysis of peripheral blood counts, bone marrow aspirate and cytogenetics in patients with transformation showed that anemia, neutropenia and thrombocytopenia should be considered as prognostically unfavorable. However, the adverse influence on MDS course appeared to depend not only on severity of cytopenia but also on its type (uni-, bi- or trilinear). Accordingly, patients with severe pancytopenia had more aggressive disease course and shorter median time to transformation (8,5±3,65 months), as compared to subjects with bi- or unilinear cytopenia transforming after 13,5±1,45 months and 14,0±2,41 months respectively. Patients whose bone marrow blast counts exceeded 10% (RAEB) developed disease transformation in less than 12 months whereas no dependence between bone marrow blast counts and time to transformation was revealed in RA patients. Another factor to influence disease course prognosis was bone marrow cellularity. The majority of patients who had transformed into AL showed increased (54% of investigated group) and normal (25%) bone marrow cellularity. On the contrary patients with hypoplastic marrow had more favorable disease course and responded well to immunosuppressive treatment with cyclosporine A. This fact could be partially explained by the level of cytokines produced by microenvironment cells and playing important role in disease pathogenesis. In about a half of the patients with disease transformation cytogenetic abnormalities were detected in bone marrow cells already at the first diagnosis of MDS. Leukemia developed faster in these patients as compared to those with normal karyotype. Results of cytogenetics performed at different time points suggested that MDS transformation was preceded by evolvement of new cytogenetic clones, namely additional chromosome8, chromosome 5 deletion and polyploidy, which in turn can also serve as prognostic markers of MDS evolution. Among the investigated patients the disease was more likely to transform in women whereas the very diagnosis of MDS was more frequent in men. Age of more than 60 years can also be considered as prognostically adverse since the rate of MDS transformation in these patients exceeded 65%. Higher frequency of AL development was detected also for transfusion dependent MDS patients.
Summary / Conclusion: Collected data support suggestion that MDS transformation is a multi-step process depending on a number of factors. The obtained results suggest that prognosis of MDS transformation into AL can be influenced not only by laboratory parameters outlined within international prognostic scoring system (IPSS), but also by bone marrow cellularity, age, gender and transfusion dependency status of the patients.

B1451

MONITORING OF THE CYTOGENETIC ALTERATIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA/MYELODYSPLASTIC SYNDROME TREATED WITH AZACITIDINE

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Background: After the AZA-001 study (Fenoux et.al, ASH/2007) azacitidine has become a standard of treatment for patients with MDS and AML (20-30% blasts in bone marrow) included risk cytogenetic, with higher rates of response and survival. Hellström-Lindberg et.al (ASCO/2008) stated that there is no relationship between pretreatment and worsening of cytogenetics in patients who evolved to AML.
Aims: Determine the effect of treatment with azacitidine (75 mg/m2/day x 5 days) on the cytogenetic alterations and the response according to the IWG (Cheson 2006) in patients with MDS and AML who have received at least 4 cycles of treatment.
Methods: we have reviewed the patients who had received at least 4 cycles of azacitidine in first line, between May 2009 and December 2012. They were

14 patients (9 men / 5 women) with a median age 76.5 years (range 40-84). 7patients with secondary-AML,4 RCMD (3 IPSS low and 1 IPSS intermediate-1), 2 RAEB (1 RAEB and 1 RAEB2, both with IPSS intermediate-2) and 1 patient with CMML-2 (WHO/2008). In the diagnostics, 50% of patients had > 20% of blasts in the bone marrow study. 9 (64.2%) had normal karyotype, 2 (14.2%) complex karyotype, 1 patient with monosomy7, 1 patient with (5) + Trisomy 8 and 1 patient with normal karyotype but with molecular positive AML-ETO study. Bone marrow aspirate with cytological, cytogenetic and molecular studies were made after 4-6 cycles and the disease progression.

Results: Median of 8.5 (range 5-19) cycles of azacitidine administrated, after 4-6 cycles, 85.8% of our patients hadat least stable disease(4 SD; 4 SD with hematologic improviment; 2 CR, 1 CR with molecular persistence and 1 PR). No patient with less than 20% blasts had progressed after the 4-6 cycle and 42.9% of those who had more than 20% blasts to the Diagnostics (AML, WHO 2008) presented cytologic improvement (< 20% blasts) in the reassessment (P<0.035). 57.1% of patients achieved transfusion independence. This wasn´t related to the quality of the response, the number of blasts in the diagnosis (P=0.999) nor to the 4 - 6th month (P=0.999) and neither is related to transfusion independence with survival (P=0,416). To assess cytogenetic in revaluation changes: 1 patient (7.1%) presented a new disturbance in the revaluation after 4-6 cycles, disappearing in the progression of the disease and 2 patients (14.2%) presented new cytogenetic alterations at the time of the progression. All of them without relevance in the progression of the disease. Median overall progression-free survival was 19.1 (95% CI 10,5 – 25,5) months in patients < 20% of blasts to the diagnostic vs 7.1(95% CI2,7 – 9,3) months in patient > 20% blasts in the bone marrow to the diagnostics (P=0.005), undetermined impact on the small subset of patient AML to have < 20% blasts after reassement. Only 1 patient remains in treatment with azacitidine and the treatment has been suspended in 8 (57.1%) patients because of the progression (1 submitted to the allo-BMT program), 3 patients due to the lack of response to the treatment and 2 patientes by éxitus of non-related cause to the disease.

Summary / Conclusion: Our study confirms the low incidence of the appearance of new alterations cytogenetic described by Hellström-Lindberg (ASCO/2008) following use of azacitidine.

Azacitidine 75 mg/m2 x 5 days has a high rate of response (85.6%), with a high rate of progression-free survival, including patients with a high cytogenetic risk and secondary AML similar data from the AZA-001 study.

It has been detected a high rate of transfusion independence (57.1%) independent of the quality of the response to treatment and without impact on progression-free survival.

B1452

SMALL IMPACT OF NEW CYTOGENETIC SCORE SYSTEM FOR MYELODISPLASTIC SYNDROMES ON CLINICAL PRACTICE

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Background: A new comprehensive cytogenetic scoring system in myelodysplastic syndromes (MDS) was recently proposed with the major goal of defining the prognostic impact of rare, single cytogenetic abnormalities not included at the current Prognostic Scoring System (IPSS) model.Cytogenetic abnormalities are important in determining prognosis in MDS. 50% of this patients display clonal chromosome abnormalities. The availability of new therapeutic options for low and high risk MDS targeted against entities characterized by specific chromosome abnormalities underlines the important role of cytogenetics for the clinical.

Aims: Analyse differences between former and new cytogenetic classifications in 49 consecutive MDS patients in a single centre from January 2011 to September 2012.

Methods: Bone marrow samples were obtained from 49 consecutive MDS patients. 38 were new diagnoses (78%) and 11 (22%) were patients already under follow up. Sex: Male: 28, female: 21. Median age: 72 years. WHO diagnoses: CMML: 9 (18%), MDS with del5q: 7 (14%), RCUD: 8 (16%), RCMD: 13% (27%), RAEB 1: 4 (8%), RAEB 2: 6 (12%), t-MN: 2 (4%). Cytogenetic study was performed to all marrow aspirates. Results were excluded if less than 20 metaphases were analyzed. FISH analysis for chromosomes5,7,8, 20 were available in 35 patients.

Cytogenetic results were available in 29 (59%) and not available in 20 (41%): normal: 20/29 (69%), abnormal 9/29 (31%). Abnormalities: del5q: 4 (44%), trisomy 8: 3 (33%), trisomy 21:1 (11%), del7q: 1 (11%), complex karyotype: 0.

FISH anylisis: normal: 22/35 (63%), abnormal: 13/35 (37%). Abnormalities: del5q: 7/13 (54%), del7q: 3/13 (23%), trisomy 8: 3/13 (23%).

Results: No differences were found between former cytogenetic risk classification and the new one at any category: Good risk (83% vs 82%), Intermediate risk (10% vs 14%), Poor risk (7% vs 3%). No very good nor very poor patients were detected. Four patients with no previously cytogenetic result available obtained prognostic abnormalities after FISH analysis (20%).

Summary / Conclusion: FISH analysis is able to find prognostic genetic abnormalities in up to 20% of previously not evaluable samples with karyotyping. Overall impact of the new cytogenetic classification is small in daily clinical practice. Molecular testing for relevant mutations in MDS will be probably a more useful tool.

B1453

COMBINED USE OF AZACITIDINE (AZA) & RECOMBINANT HUMAN ERYTHROPOIETIN (RHUEPO) INDUCES RAPID HAEMATOLOGICAL RESPONSE WITHOUT AFFECTING RESPONSE RATE IN HIGHER RISK MDS: RESULTS OF A RETROSPECTIVE STUDY

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Background: AZA, a DNA hypomethylating agent, provides 50-60% responses in higher-risk MDS after administration of 6 courses of treatment. Recent laboratory data suggests that demethylation with AZA upregulates EPO-receptor mRNA (Wallach, 2009). AZA might also affect several genes involved in cell cycle, metabolism and signal transduction which are down-regulated in bone marrow erythroid cells in MDS patients non-responsive to rHuEPO.

Aims: To evaluate preliminary data for the combined use of AZA and rHuEPO in MDS patients.

Methods: We explored retrospectively the efficacy of the AZA-rHuEPO combination in a small cohort of 10 (M/F: 5/5) patients (pts) with a median age of 75(67-83) years. Diagnosis (WHO classification) was: RAEB-2:6, CMML:2, RAEB-1: 1 and acute myeloid leukemia: 1; IPSS was: int-2 in 8/10 and high in 2/10 pts. Median time from diagnosis was 6(1-31) months. 9/10 pts were transfusion dependent, 8/10 were refractory to previous rHuEPO administration, while 2/10 pts were not previously treated with rHuEPO but their serum EPO levels at diagnosis were >200 U/L. Patients were given AZA at FDA/EMEA-approved schedule (75 mg/m^2/d x7d /4-weekly) initially for 6 courses and continued if response was obtained. rHuEPO (40,000IU/week) was given until achievement of steady Hb level >10.5 g/dL or until AZA discontinuation.

Results: Median follow-up was 21.5 (range 2-45) months. Patients received a median of 12 cycles (range 2-33) of AZA; half the patients received 10 or more courses. The median time on rHuEPO was 20.5 weeks (3.71-34) after AZA start. Best response (IWG 2006 criteria) was CR in 1/10 pts (RAEB-2), marrow CR in 1/10 pts (AML), and stable disease with hematological improvement (HI) in 4/10 pts (RAEB-1:1, RAEB-2:2, CMML: 1) leading to an overall response rate of 60%. Transfusion independence was achieved after a median of 32.5 (range 3-62) days and it lasted for a median time of 10.15 months (range 4-40.8). Three patients (33%) were transfusion independent for more than 2 years. Median progression-free survival (PFS) was 15.3 months for those who responded to AZA therapy versus 4.2 months for the non-responders, P=0.003. Median overall survival (OS) was 29.5 months for responders versus 15.4 months for non-responders, P=0.23. The estimated 2-year OS was 62.5% for responders.

Summary / Conclusion: Our data suggests that the combination of AZA and rHuEPO can produce rapidly a haematological response even in patients previously resistant to rHuEPO. In our cohort, response to AZA was not affected by addition of rHuEPO. This highlights the potential for rHuEPO to be used as supportive treatment in patients treated with AZA in need of transfusions.

B1454

AZACITIDINE: AN ALTERNATIVE TREATMENT REGIMEN FOR ELDERLY HIGH-RISK MYELODYSPLASTIC PATIENTS

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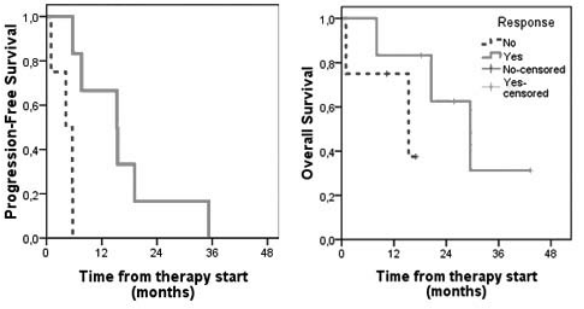
Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of disorders that manifest bone marrow failure with risk of life-threatening infections and bleeding. MDS occurs with a frequency of 15-50 new cases per 100,000 per year. Around a third of the patients progress to acute myeloid leukaemia (AML). However, the majority of mortality and morbidity relates to infections and bleeding complications. In the majority of patients, the standard care is supported in the form of blood and platelet transfusions, along with growth factors such as G-CSF and antibiotic therapy. Methyltransferase inhibitors (MTI), such as Azacitidine and related analogues are useful in treating MDS. Potentially reversing slicing of genes, such as CDKN2B by parameter methylation has been shown to occur in MDS and increases risk of disease progression. The MTIs have demethylating properties proven both *in vivo* and vitro and hence are useful in treating patients with MDS. Patients with AML have also been treated with Azacitidine intravenously with varying regimens.

The majority of the toxicity related to Azacitidine was myelosuppression, fatigue, and myalgia, along with gastrointestinal symptoms of nausea and vomiting. Injection site erthyrema is also common. Hence tolerance and compliance are a concern.

Aims: Altering the treatment regime of Azacitidine to improve compliance and reduce adverse events without compromising response rate for elderly patients (65+).

Methods: Sixteen (16) patients were enrolled over an 18 month period, in our study of modified subcutaneous Azacitidine therapy to treat high-risk MDS/AML. Age ranged between 67 and 86 with a median of 77. Follow-up, after the therapy initiation, ranged between 6 months and 36 months. All patients have completed at least a trial of four months of therapy. Azacitidine was given at 75 mgs/m^2, three times per week for two consecutive weeks per month (Monday,

Wednesday, and Friday each week). Myelosuppression, as well as overall tolerance and side effect profile was assessed.
Results: In this cohort, 93.75% (15) showed haematological improvement in at least one cytopenia. Decreased transfusion requirement was seen in 75% (12). No haematological improvement was seen in 6.25% (1). Mean survival at 18 months was 56.25%. There has been no grade II, or III complications. Results are summarised in Figure 1 (to be presented).
Summary / Conclusion: In our experience, Azacitidine therapy given subcutaneously three times per week for 2 weeks each month is extremely well tolerated and has good efficacy in terms of disease control with an overall response rate of 75%, and mean survival at 17 months of 56.25%. This is comparable to results achieved in trials with different regimens[1-2]. Therapy was initiated as an outpatient and did not require regular hospitalisations. Symptomatically, they have been able to cope quite well with no Grade II or III complications and decreased transfusion requirement.



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B1455
COMPARATIVE EFFICACY OF HOMOHARRINGTONINE PLUS CYTARABINE AND DECITABINE IN PATIENTS WITH MDS/AML
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Background: Myelodysplastic syndrome (MDS) is a malignant hematological disease that comprises a heterogeneous group of clonal hematopoietic stem and progenitor cell disorders, with peripheral cytopenias, bone marrow hypercellularity, high-risk of evolving into acute myeloid leukemia (AML). MDS/AML is a special refractory and palindromic AML characterized by poor therapeutic effects and low complete response rate, as well as high treatment-related complications and mortality. Patients with MDS/AML are often elders and represent more intolerance to routine or intensive chemotherapies. Homoharringtonine, an alkaloid found as the major active component in Chinese plants cephatotaxus fortuneif. , has been widely used in AML since the 1970s in China. Decitabine, a hypomethylating agent, is active and has been approved for the treatment of myelodysplastic syndrome (MDS) in recent years.
Aims: In order to compare the efficacy, toxicity and long-term prognosis of HA (Homoharringtonine plus cytarabine) with Decitabine in MDS/AML.
Methods: A total of 26 MDS/AML patients consisting of 14 males and 12 females were included in this study. They were randomly assigned to receive either HA (HHT 4mg m^{-2} d^{-1} ,d1-3; Ara-C 100 m^{-2} d^{-1}, d1-7) or decitabine (20 mg m^{-2} d^{-1}, d1-5). The effects were evaluated by hazard ratios (HR) for overall survival (OS), progression-free survival (PFS) and freedom from first progression. Relative risks were used to analyse complete response rate, total response rate, treatment-related mortality and adverse events. A Log-rank test was used in survival analysis, and a Chi-square test was performed for other outcomes.
Results: The complete remission (CR) rate in HA arm according to MDS/AML criteria was 33% and 36% in decitabine arm (*P*>0.05). HA group had no lower total response rate than Decitabine group(53% *versus* 64%, *P*>0.05). The freedom from first progression in chemotherapy with HA and decitabine was 20% and 18% (*P*>0.05), respectively. PFS was not statistically longer for two comparators with HR was 0.41 (95% confidence interval (CI) 0.09722 to 1.740).There was no statistical difference in OS between the HA group and decitabine group with HR was 0.799 (95% CI 0.2992 to 2.133); median survival: 300 days vs 291 days (*P*>0.05, 95% confidence interval (CI) 0.6165 to 1.445). The treatment-related mortality was 13% with HA regimen versus 18% with decitabine at 3 weeks (*P*>0.05) and 40% with HA regiment versus 18% with decitabine at 3 months (*P*>0.05). The incidence of haematological toxicities and liver function lesion of WHO grade III or IV were not higher in the HA group than that in the decitabine group (*P*>0.05). The total secondary infection rates in all sections of chemotherapies were 58% and 19% (*P* =0.005) in the two groups, respectively. Secondary infection rate was significantly lower in the decitabine group than that in the HA group.
Summary / Conclusion: This study showed that Homoharringtonine combined with cytarabine regiment in treating MDS/AML had a similar therapeutic effect and long-term benefit with decitabine, both regiments were associated with relatively safe and effective outcomes in MDS/AML patients. However, HA regiment showed a higher risk of secondary infection than decitabine. Longer follow-up and further studies will prospectively evaluate the results of HA regiment versus decitabine in this setting.

B1456
LIPOSOMAL IRON IS BETTER THAN IRON SULFATE IN LOW-RISK MYELODYSPLASTIC SYNDROMES (LR-MDS) WITH MILD ANEMIA. MONOCERNTRIC STUDY.
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Background: LR-MDS frequently shows a chronic inflammatory status, ferritin high values and impaired capacity of iron absorption and utilization. Liposome has a described anti-inflammatory effect and transports its content directly in blood, beyond gastric and enteric wall.
Aims: Aim of this study is to verify if liposomal iron support in refractory anemia (RA) and refractory cytopenia with multilineage dysplasia (RCMD) with mild anemia is safe and effective in increasing hemoglobin level.
Methods: In group A 7 patients (5RCMD and 2RA), with normal cytogenetics, M/F:4/3, median age 65 years (R64-75), Hb 10.7 g/dl (R10-11.5), saturation of iron binding capacity > 20%, with a median ferritin level of 480 ng/ml (R380-550), ESR 28 mm/1st hour (R20-32), CRP 6 mg/l (R4-7), normal B12 and folate, received liposomal iron 30 mg/day orally for 3 months.
In group B 7 patients (3RCMD and 4RA), with normal cytogenetics, M/F:5/2, median age 63 years (R62-70), Hb 11 g/dl (R10.8-12), saturation of iron binding capacity > 20%, with a median ferritin level of 430 ng/ml (R370-580), median ESR 30 mm/1st hour (R18-38), median CRP 7 mg/l (R5-7), normal B12 and folate, received support with iron sulfate 105 mg orally/day.
Results: Group A showed a median hemoglobin increase of 1.5 g/dl (R0-2), a ferritin decrease to a median of 160 ng/ml (R 100-250), a ESR decrease to a median value of 15 mm/1st hour (R 8-20) and a median CRP 3 mg/l (R2-5).
In group B no significative increase of hemoglobin or decrease of ferritin, ESR and CRP were recorded. 2 patients showed hepygastralgia, 2 stipsis, 2 diarrohea.
Summary / Conclusion: Liposomial iron is safe, effective, well tolerated, effective in increase hemoglobin level and reduce inflammatory markers in low-risk MDS.

B1457
EXPERIENCE WITH 5-AZACYTIDINE IN EAST KENT: WHEN TO QUIT?
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Background: 5-Azacytidine is a DNA hypomethylating agent which has recently been licensed for the treatment of high-risk myelodysplastic syndrome. It was subsequently approved by NICE in the UK with a significant financial impact on drug budgets, as its administration is continuous in responding patients until intolerance or loss of response. The recommendation from the manufacturers is for at least six cycles to be administered before stopping for lack of efficacy.
Aims: This analysis was performed to identify non-responders as early as possible to quit unproductive 5-Azacytidine therapy in order to reduce unnecessary expense and visits.
Methods: 26 patients with high-risk MDS were treated with 5-Azacytidine between Jan 2009 and July 2012 in EKHT and respectively analysed. To enter the analysis patients needed to have completed 6 cycles of 5-Azacytidine or suffered disease progression prior to this. The response was assessed according to the IWG criteria for MDS.
Results: Eight out of twenty six patients achieved a major response lasting at least 6 months. Six of these patients achieved a major response prior to commencing cycle 3 and the other two patients, whilst not achieving a major response, had improvement in platelet count. No patient who had failed to show any response prior to the 3rd cycle went on to achieve a major response.
Summary / Conclusion: We conclude that response at the end of the 2nd cycle predicts the long term outcome with 5-Azacytidine therapy in high-risk MDS. As a result of this audit local practice is now as follows: Start 5-Azacytidine at 75mg/m^2 d5+2+2 and assess after two cycles If no response – stop If

responding continue for a further 4 cycles and reassess after cycle 6 If borderline response continue for a further 2 cycles and reassess If patient reaches cycle6, and is still responding, convert to 105mg/m^2 d1-5 (max 200mg)

B1458

LENALIDOMIDE TREATMENT IN 20 PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS). MULTICENTER REGISTRY

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Background: Lenalidomide is a drug approved by the FDA for the treatment of low risk MDS with associated 5q deletion and transfusion dependence. In Europe, its approval is being studied by the EMEA and its prescription is usually made respecting the approved indication in the United States. Use of Lenalidomide presents controversial issues in both the timing of its onset, and maintenance if complete remission is achieved.
Aims: To evaluate the use of Lenalidomide in MDS in the clinical practice of several hospitals in our geographic area, in order to analyze the results.
Methods: Between July 2007 and January 2013, we performed a multicentre and retrospective registry, which was carried out in seven hospitals from northern Spain. Data was collected from patients diagnosed with MDS, treated with Lenalidomide. Information for the karyotype at diagnosis and subsequent evaluations was included. Similarly we obtained demographic data, risk stratification, transfusion-dependence and response to treatment.
Demographic data, WHO classification and risk prognosis scores are listed in Table I. Karyotype / cytogenetics: 15/20 patients (75%) had deletion 5q, of which 3 had other abnormalities (2 had the V617F JAK-2 gene mutation and one patient was a carrier of the translocation t (2; 11)(p21: q23)).

Table I.	
Patients	20 (13 female/7 male)
Age	Mean 69.15 (range 50-78)
WHO classification	RARS 4/20 (20%) RCMD 4/20 (20%) RAEB-1 2/20 (10%) RAEB-2 1/20 (5%) MDS 5q deletion 8/20 (40%) Unclassifiable MDS 1/20 (5%)
IPSS	Low-risk 14/20 (70%) Int-1 5/20 (25%) Int-2 1/20 (5%)
WPSS	Very-low risk 8/20 (40%) Low risk 7/20 (35%) Intermediate 3/20 (15%) High risk 1/20 (5%) Very-high risk 1/20 (5%)

Results: Response to treatment: 65% of the patients (13/20) achieved complete hematological response in a median time of 136 days (31-396), equivalent to 5 treatment cycles of 21 days of Lenalidomide 10mg/day. Of these only one patient had no 5q deletion. In the group of patients showing no hematological response (7/20, 35%), we observed that one of them was the patient presenting a 5q deletion and translocation t (2; 11) (p21: q23), while the rest of the group lacked the 5q deletion. Cytogenetic remission (CyR) was evaluated in 10 patients (50% of cases), noticing that 5 of them (4 with deletion 5q, and 1 non-5q) showed complete CyR (defined as normal karyotype and/or 0% of 5q - by FISH). The average time to reach complete CyR was 304.8 days (range 123-518). In 2 patients, we found partial CyR and 3 patients there was no response, of which 2 showed increased percentage of 5q- clone (90 to 92%, and 32 to 74%). We analyzed the relationship between the percentage of mitosis 5q- and the time required to reach the complete CyR (Pearson correlation). As a result we observed that the higher percetage of the 5q- clone was, the longer it took the patient to reach complete CyR (p: 0.44). Discontinuation of treatment: Lenalidomide was suspended in 11/20 patients (55%): 3 patients due to no answer, 2 patients due to disease progression, 2 patients due to adverse events, and 4 patients due to the physician's decision since they had achieved complete CyR. The 4 latter patients had received Lenalidomide on average 10 cycles (4-24) for 21 days. They presented a disease relapse at a median time of 23 months (10-42). In the bone marrow cytogenetic study, one of them showed clonal evolution towards a complex karyotype, with worse prognosis than prior to treatment. These four patients were resumed on Lenalidomide therapy at the same dose (10 mg/day x 21 days), 2 of them reached CyR again with complete hematological remission at 6 and 9 months respectively, and 2 progressed to acute leukemia.
Summary / Conclusion: Data from this registry intended to be "a snapshot" of clinical practice today and reflect: a) Lenalidomide is sometimes prescribed off-label FDA approval (35% of patients had no transfusion-dependence at baseline). b) Percentages of hematological and cytogenetic response are generally similar to those described in the literature. c) Treatment is interrupted in most of the patients who achieve complete hematological and cytogenetic remission, and relapse is common. d) Progression towards acute myeloid leukemia is observed in 10% of cases. It is essential to carry out controlled clinical trials to assess if the start of Lenalidomide prior to transfusion dependence increases the number of cytogenetic responses and survival and whether the cessation of treatment in patients who achieved cytogenetic remission does not mean a worse prognosis karyotype at relapse.

B1459

RESPONSE TO ECULIZUMAB COMBINED WITH CYCLOSPORINE AFTER FAILURE OF ECULIZUMAB MONOTHERAPY IN 2 PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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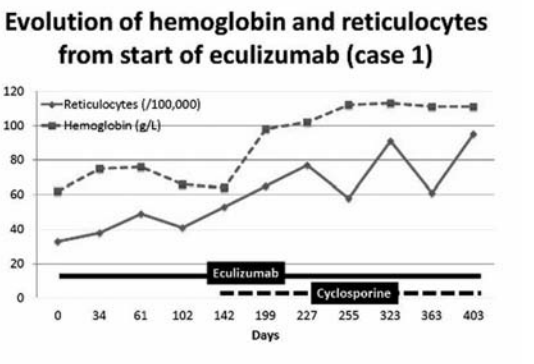
Background: Eculizumab is a monoclonal antibody that inhibits terminal complement activation. In patients with paroxysmal nocturnal hemoglobinuria (PNH), eculizumab stabilizes hemoglobin concentration in the absence of transfusion in half of the patients.
Aims: We hypothesized that in patients who failed on eculizumab, the addition of an immunosuppressive drug could improve the biological and clinical patient's status.
Methods: Here we report on 2 patients who remained transfusion dependent on eculizumab. When eculizumab was given combined to cyclosporine, the first patient became transfusion-independent, while in second patient, the number of packed red cells transfused was cut by half.



Results: Patient1, a 51-year-old man, developed aplastic anemia in October 2008. A small PNH clone (0.6% of granulocytes) without hemolysis was present at diagnosis. The patient eventually developed red cell and platelet transfusion dependence and was treated with rabbit anti-thymocyte globulin (ATG), cyclosporine, and steroids in January 2009. He failed to respond except for an improvement of the neutrophil count. A second course of ATG-cyclosporine was started in May 2009. The patient became transfusion-free and cyclosporine was withheld in February 2010. In May 2011, transfusions were again needed with, in addition, obvious signs of hemolysis and an increasing proportion of granulocytes with PNH phenotype, up to 15%. Eculizumab infusions were started in November 2011 but did not decrease the red cell transfusion rate (10 packs transfused between November 2011 and April 2012). In April 2012, cyclosporine was resumed, in addition to eculizumab. No red cell transfusion was needed since April 2012 (see figure). In January 2013, hemoglobin concentration was 122 g/L with $79x10^9$ platelets/L and $2.75x10^9$ neutrophils/L.

Patient2, was a male aged 74 years in September 2008 when a diagnosis of aplasia was made with 30% of PNH-type neutrophils but without hemolysis. ATG, cyclosporine, and steroids were started in October 2008. Platelet count and hemoglobin concentration improved and transfusion could be omitted until December 2009, when abdominal painful crisis and hemolysis were noted. Cyclosporine was stopped in December 2009 and, as hemolysis was prominent, eculizumab was started in May 2010 but failed to reduce the transfusion rate (2.2 packed red cells monthly). Eculizumab was stopped and cyclosporine was started in November 2010 without any response. Eculizumab was added to cyclosporine in January 2011, which was followed by an improvement of abdominal pain, and by a decrease of the transfusion rate down to 1.5 packs monthly. In January 2013, hemoglobin concentration was 89g/L with $66x10^9$ platelets/L and $2.89x10^9$ neutrophils/L.
Summary / Conclusion: These two cases suggest that in some patients with aplastic anemia evolving to PNH, a poor response to eculizumab can be improved to a large extent by the addition of cyclosporine. The mechanism of the beneficial effect observed when both drugs are combined remains speculative but could conceivably rely on the additive effect of eculizumab on hemolysis and of cyclosporine on aplasia, as suggested by the reticulocyte count rise in case 1 (figure) after cyclosporine was started.

B1460
EFFICACY OF ERYTHROPOIESIS-STIMULATING AGENTS IN ANEMIC PATIENTS WITH MYELODYSPLASTIC SYNDROME
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Background: Anemia in Myelodysplastic Syndrome (MDS) patients is the most frequent symptom and can be observed above 85% patients. Anemia decrease survival rate and overall quality of life. Red blood cell (RBC) transfusions are routinely used to treat anemia. However numerous RBC transfusions may cause iron overload and hemosiderosis. While erythropoiesis-stimulating agents (ESA) treatment has been shown significantly to increase hemoglobin concentration, reduce transfusion dependency, improve overall survival and quality of life.
Aims: To study efficacy ESA in anemic patients with MDS and find out the correlation between positive response to ESA and initial serum erythropoietin (SE).
Methods: The investigational group of patients (n=42) were diagnosed myelodysplastic/ myeloproliferative neoplasms (by WHO classification) and concluded patients only with refractory anemia: RA (n=18), RCMD (n=2), RARS (n=6), del(5q) (n=4), RAEB-1 (n=5) and RAEB-2 (n=4), chronic myelomonocytic leukemia (CMML) (n=3). All patients were determined anemia (Hb concentration 3.5-10.0 g/dL). The median age was 70.0 years (range 52-88). RBC transfusions were administrated to the patients whose Hb concentration was below 8.0 g/dL, to increasing up to 8.5-9.9 g/dL. Transfusion dependency showed 22 (52.4%) patients, they were received 4.0±1.2 (2-7) RBC units every 8 weeks. ESA were administrated to patients subcutaneously: epoietin alpha (n=17) and epoietin beta (n=14) were injected 150 IU/kg three times a week, darbepoietin alpha (n=11) – 500 µg every three weeks. The target Hb concentration was 11 g/dL. Erythroid response (ER) rate was estimated by IWG 2006 criteria as major and minor ER. One group of MDS patients (with RAEB-1, RAEB-2 and CMML) were administrated ESA and chemotherapy (Decitabine, Cytarabine, Hydroxyurea) but another group (with RA, RCMD, RARS, del(5q) received ESA only.
Results: The overall ER rate was 15 of 42 (35.7%) in ESA patients. Major ER showed 11 patients (26.2%): 6 patients increased Hb concentration >2 g/dL (no-RBC transfusion) and 5 patients stopped RBC transfusions. Minor ER was observed in 4 patients (9.5%): 2 ones reduced RBC transfusions more than 50% and 2 patients increased Hb at 1.0 and 1.6 g/dL. On the whole in different groups of patients positive ER (major + minor) vs no-ER was ascertained following: RA 5/13, RCMD 1/1, RARS 3/3, del(5q) 0/4, RAEB-1 3/2 and RAEB-2 2/2, CMML 1/2. ESA treatment was 4-8 months. During this time Hb level increased from 7.9±1.1 g/dL to 10.4±1.1 g/dL ($P<0.05$) in the major ER group patients, from 8.5±1.0 g/dL to 9.7±1.2 g/dL in the minor ER patients and from 7.3±1.8 g/dL to 8.7±1.2 g/dL in the no-ER patients. Even though Hb concentration increased in the no-ER group of patients but the reason of this situation was connected with regular RBC transfusions. In a small group MDS patients we determined negative correlation between positive response and initial level of SE ($r=-0,57$; $P<0,05$; n=17). 6 (60%) patients showed positive ER with SE <200 IU/L (26-180 IU/L), and none positive ER were observed in patients with SE >500 IU/L (540-970 IU/L).
Summary / Conclusion: The study was shown that ESA is effective at increasing Hb concentration, reducing RBC transfusion dependency in MDS patients with anemia. Initial level of SE (<200 IU/L) may be used as prognosis of positive response to ESA.

B1461
DELETION 5Q MYELODYSPLASTIC SYNDROMES (MDS) TREATMENT AND DIAGNOSTIC PATTERNS IN MEXICO: RESULTS FROM A PHYSICIAN SURVEY
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Background: Deletion of the long arm of chromosome 5 (5q) is the most common chromosomal abnormality seen in MDS; however, there is little information about treatment and diagnostic patterns for this disease, especially in Latin America.
Aims: This study aimed to understand the real-life approach to treatment and diagnosis in Mexico in 2012.
Methods: Overall clinical practice for management of del(5q) MDS was investigated through a physician face-to-face survey. Eligible participants were hematologists and/or hemato-oncologists with at least 5 years of clinical experience treating patients with MDS. 10 physicians (90% hematologists; 10% hemato-oncologists) practicing in one of 5 major public hospitals in Mexico City (IMSS, ISSSTE or MoH) and/or two private hospitals answered the questionnaire.
Results: Significant differences were found in treatment patterns when comparing public and private healthcare physician responses. The patient flow in the public practice starts at Family Medicine Units, which care for basic health issues, as well as in second level hospitals and centers that tend to basic specialties before being referred to a hematologist in third-level high-specialty hospitals. Moreover, these patients arrive in a more advanced stage of the disease than those in private practice. The only way to diagnose del(5q) is through karyotyping; however, there are several important challenges, including a) lack of expertise in performing karyotyping as well as diagnosis; b) karyotyping for del(5q) is not covered by the major social security system, IMSS, which covers 43% of the population; c) most performed tests are paid out-of-pocket and, considering that about half of the population is considered to have income below the poverty line, karyotyping is expensive; and, d) there is a lack of good quality laboratory testing facilities for performance of karyotyping for del(5q) and the majority are located in 3 major cities (Monterrey, Guadalajara and Mexico City), making access difficult for ~80% of the population. Likewise, since most treatment centers are also located in major cities, patients must travel from other parts of the country to get diagnosed and treated. Physicians report that most MDS patients receive treatment for anemia prior to referral; moreover, they acknowledge lenalidomide as the best treatment option for MDS del(5q). However, due to its high cost and restricted availability, they use whatever is readily available at the institution. Thalidomide is used in 1st line treatment, cyclosporine and prednisone as 2nd line and hypomethylating drugs, such as azacitadine or bone marrow transplant as 3rd line. Additionally, due to misdiagnosis and/or co-morbidities, patients are treated with erythropoietin (EPO) (for anemia), granulocytic colony stimulating factors (for leucopenia), platelet agonists or transfusions (for thrombocytopenia), and iron chelators (for iron excess due to transfusions).
Summary / Conclusion: In order to have appropriate del(5q) MDS diagnosis and treatment, following accepted treatment guidelines in Mexico, there is a clear opportunity to increase continuous medical education on the disease and diagnosis as well as training to spur more highly qualified geneticists for MDS in the country. Moreover, there is a need to have more reference centers and genetic testing laboratories and greater availability of del(5q) karyotype testing in the public sector. In addition, improving access to novel drugs, such as lenalidomide, will expand the treatment options indicated for del(5q) MDS in Mexico.

B1462
A RETROSPECTIVE REVIEW OF THE TREATMENT OF APLASTIC ANEMIA
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Background: Introduction: Aplastic anemia is a syndrome of bone marow failure (pancytopenia and marow hypoplasia). However, current therapies (bone marow transplantation, immunosuppresive therapy with cyclosporine and antilimfocyte or antithymocite globuline) are curative.
Aims: Aims: The primary objective was to review the management of treatment for severe aplastic anemia at patients in order to elucidate differences in treatments and review the mortality and morbidity which relate to the variation of treatment.
Methods: Methods: We treated 36 patients in the Clinic of Hematology of Craiova for aplastic anemia (both severe and moderate) over a period of 16 years. A total of 36 patients of aplastic anemia were treated in our institution from 1997 to february 2013. Thus, a total a of 36 patients were included in the analysis.
Results: Of the total of reviewed patients, 21 were male and 15 were female; The mean age at diagnosis was of 46±2 ,4 years. Nine patients (25%) of patients had severe aplastic anemia, 25 patients (69,5%) had severe anemia

and two (5,05%) patients had moderate aplastic anemia. Four patients (9%) developed aplastic anemia in the setting of hepatitis (B2 patients and C2 patients) and 4 patients (9%) were diagnosed with toxic aplastic anemia; 89,9% of patients were treated with cyclosporine and ATG or ALG and had a complete response.The average time to C.R. was of 23±11 months. Average duration of treatment was of 12±10 months. Three patients died: one from complications unrelated to aplastic anemia and two related to a second neoplasm (unrelated to treatment). The average time to transfusion independence for all patients was of 8±8 months. The time to transfusion independence was associated with immunossupresive failure (P=0,001). Patients who did not respond to treatment had an average time to transfusion independence of 15±12 months as compared to those who were complete responders and who had an average time to transfusion independence 2±2 months. Additionlly, there was an association between immunosuppresive failure and cyclosporin levels (P=0,002). There was a non-significant association between immunosupressive failure and bone marrow cellularity (P=0,02). Other 4 patients had evidence of a cytogenetic abnormality (iso-17 q, del 16 q, 5q), but never progressed to LAM, MDS or myeloproliferative neoplasms.

Summary / Conclusion: With immunosupressives regimens, aplastic anemia is currative in the majority of cases. For patients with confirmed clonal disease, it is possible that immunosupressive failure and the ultimate development of MDS, PNH or LAM are related.

B1463

LONG TERM AZACYTIDINE THERAPY IN RESPONDING MDS IS FEASIBLE AND PROLONGS THEAREPEUTIC BENEFIT: ANALYSIS IN 18 ELDERLY PATIENTS

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Background: The only potentially curative treatment for Myelodisplastic Syndromes (MDS) is allogeneic stem cell transplantation (allo-SCT). Nevertheless, MDS is more frequent in elderly patients (pts). The majority of these pts are not candidate for transplant. Azacytidine (AZA) compared with conventional supportive care may improve quality of life and long-term outcome of higher-risk (HR) MDS (IPSS Int-2 or high) and is therefore the reference frontline therapy of pts ineligible for allo-SCT. No indications have been established regarding how long AZA treatment should be continued. Pts responding to AZA should continue the treatment until relapse. Outcome after AZA failure is poor. Rapid loss of response to AZA has been observed in case of inter-cycle interval prolongation or withdrawal of treatment.
Aims: To assess long term tolerability to AZA in HR MDS/AML pts who responded to treatment and underwent more than 6 cycles of therapy.
Methods: We retrospectively evaluated the tolerability profile of AZA in a series of 18 consecutive pts treated at our Institute from 11/2010 to 2/2013. We used the approved schedule of AZA 75 mg/m(2)/d subcutaneously for days 1-7 of every 28-day cycle. To assess treatment related toxicities weekly blood examinations and clinical monitoring were performed. After the first 6 cycles a bone marrow evaluation was also planned in order to assess response.
Results: Between 11/2010 and 2/2013 we administered AZA to 18 pts (**table 1**: pts' characteristics). 9/18 pts (5 HR MDS, 4 AML with <30% of blasts) received more than 6 cycles, median number of cycles being 13 (range 8-18); after VI cycles 6/9 pts were in Partial Response (PR), while 3/9 pts in Stable Disease (SD). All 9 pts continued with further cycles. After a follow up (fu) of 366 days (median, range 231-637), 7/9 pts are alive, still responding to AZA, 2/9 died of disease progression. In this series of pts, we documented a good profile of tolerability to AZA, maintained during all cycles. As it has been previously reported, the most frequent adverse event was gastrointestinal (GI) constipation (no grade III-IV severity). We also observed neutropenia e thrombocytopenia of grade III-IV (5/9 pts). In order to maintain the correct cycle timing, we decided as our policy to administer AZA despite the detection of neutrophil values <1000/mmc (but >500/mmc). All neutropenic pts received anti-bacterial, anti-fungal and anti-viral prophylaxis. During fu the incidence of severe infectious events leading to hospitalization was low: only two cases of febrile neutropenia were observed and both cases were successfully treated with large spectrum anti-microbial therapy.
Summary / Conclusion: In HR MDS pts, AZA improves survival vs conventional care regimens. AZA treatment is associated with delayed disease progression, reduced transfusion requirements and enhanced quality of life and it is the standard of care for pts with HR MDS ineligible for allo-SCT. The likelihood of having a sustained response to AZA is increased by maintaining a correct cycle-timing and maximizing treatment duration. Our preliminary data suggest that AZA is well tolerated even after several cycles: we observed a low percentage of severe adverse events, especially infectious ones despite the occurrence of severe neutropenia. Our data support AZA administration until disease progression. The favorable toxicity profile of the drug even in presence of prolonged neutropenia allows the maintenance of a correct interval between consecutive cycles, thus maximizing the therapeutic potentialities of this hypomethylating agent.

B1464

EVALUATION OF FLOW CYTOMETRIC SCORE FOR THE DIAGNOSIS OF LOW-GRADE MYELODYSPLASTIC SYNDROMES

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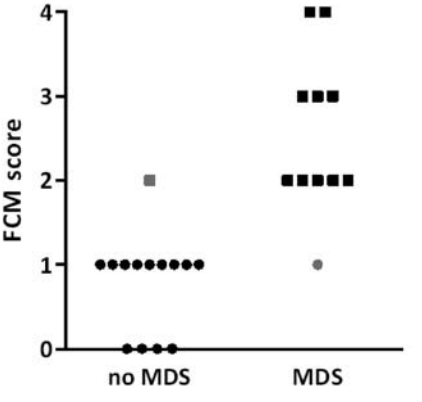
Background: The diagnosis of myelodysplastic syndromes (MDS) is not always straightforward when patients lack specific diagnostic markers, such as clonal cytogenetic abnormalities and/or ringsideroblasts. Recently, a flow cytometric score (FCM score) has been developed and validated to be able to discriminate low-grade MDS from non-clonal cytopenias (1).
Aims: We aimed to test the applicability of the FCM score in our hospital population.
Methods: Immunophenotypic data generated in the diagnostic work-up of bone marrow samples from patients suspected of having MDS in 2012 were retrospectively analyzed. The flow cytometric algorithm as proposed by Della Porte et al (1) was used to determine an FCM score. The FCM score was composed of four cardinal parameters: CD34+ myeloblast-related and B-progenitor-related cluster size, myeloblast CD45 expression and granulocyte side scatter value. An FCM score of 2 or more was considered suggestive for the diagnosis of MDS.
Results: A correct diagnosis of MDS was formulated in 10/11 cases (sensitivity 91%), while 1 false-positive result was noted among 14 control patients (specificity 93%) (Figure 1). Eighty percent (4/5) patients without specific markers of dysplasia (ring sideroblasts and/or clonal cytogenetic abnormalities) were correctly classified. The positive likelihood ratio of the flow cytometric score was 12.7.



Summary / Conclusion: The evaluation of the proposed FCM score in low-grade MDS showed a high sensitivy and specificity, and a clinical important positive likelihood ratio. This study confirms the applicability of the described FCM score in our hospital population and may help to establish the diagnosis of myelodysplastic syndrome, especially when morphology and cytogenetics are indeterminate. However, the relatively low number of patients included in the analysis warrants further experience with the use of the FCM score in the diagnostic work-up of patients suspected of having MDS.

Reference

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B1465

A NEW CASE OF REFRACTORY ANEMIA WITH RING SIDEROBLASTS AND THROMBOCYTOSIS SF3B1 AND JAK2 V617F POSITIVE, RESPONDING TO LENALIDOMIDE MONOTHERAPY.

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Background: Refractory anemia with ring sideroblasts has recently been associated with SF3B1 mutations in more than 60-80% of cases according to the published series. The revised WHO classification of hematopoietic tumors introduced the new category myelodysplastic/myeloproliferative neoplasms (MDS/MPD). Refractory anemia with ring sideroblasts associated with marked

thrombocytosis (RARS-TE) is the best characterised hematologic entity in this category of MDS/MPD. Besides the SF3B1mutation more than 50% of cases carry also the JAK2 V617F or MPL mutations. Gerwin Huls et al (Blood 2010; 116: 180-182) reported two such cases responding to lenalidomide single agent treatment. Both patients became transfusion independent and one attained complete molecular remission.
Aims: We report another case of refractory anemia with ring sideroblasts and thrombocytosis positive for SF3B1 and JAK2 V617F mutations, who was successfully treated by lenalidomide. We present clinical, haematological data and molecular biology markers at diagnosis and after four months treatment.
Methods: 65 years old male patient from Spain. At diagnosis Hb: 98 g/L, MCV 108 fl, WBC 6 G/L with 66% neutrophils, Plt: 400 G/L. Four months later Hb 92 g/L, WBC4,9 G/L, reticulocytes 68 G/L, Plt 465 G/L. Bone marrow aspiration with iron staining was performed. Caryotype (G-banding) was determined in culture of bone marrow unstimulated and GM-CSF and G-CSF stimulated cells. RNA and DNA was extracted from peripheral blood and the mutations of the gene SF3B1 and JAK2 V617F were looked by PCR and sequencing and pyrosequencing respectively. JAK2 mutation analysis was repeated 4 months after initiating lenalidomide treatment.
Results: Bone marrow was hypercellular (70-75%) with marked dyserythropoiesis and dysmegakaryopoiesis. Blasts cells were less than 2%. 75% of erythroblasts were ring sideroblasts upon iron staining. Caryotype was normal, 46XY. Serum iron 39,6 µmol/L with 82% of transferrin saturation, ferritin 536 µg/L. JAK2 V617F mutation was present (30% by pyrosequencing) and we also found the K700E mutation of the gene SF3B1 (PCR and direct sequencing) in heterozygous state. On September 2012 we started treatment with lenalidomide 5mg per day, 21 days out of 28 per cycle. Beginning cycle2, lenalidomide was increased to 10mg per day 3 weeks out of 4. An hemogramme done after 4 cycles, gave the following results: Hb 121 g/L, MCV 102 fl, reticulocytes 86 G/L, WBC4,7 G/L, Plt 228 G/L. JAK2 V617F mutation analysis at that point was repeated and showed no mutation (by pyrosequencing). Lenalidomide is tollerated very well with no side effects particularly with no clinical sign of polyneuropathy.
Summary / Conclusion: This patient satisfies all WHO criteria for the diagnosis of RARS-TE. He presented a specific mutation for sideroblastic anemia and was JAK2 V617F positive. Treatement with lenalinomide was motivated because of deterioration of anemia. The response to the treatment is evident with a gain of more than 29 g/L of Hb, correction of platelet number and the disapperance of JAK2 V617F mutated clone. Our case confirms the two previous cases presented by Gerwin Huls et al, particularly regarding anemia improvement, correction of thrombocytopenia and complete molecular response concerning JAK2. Furthermore our results agree with the rapid effect of lenalidomide. In our opinion these three cases consist a strong argument to perform a multicenter International prospective clinical study on the effect of lenalidomide in cases with RARS-TE.

B1466
OUTCOME OF 5-AZACITIDINE AND DECITABINE TREATMENT IN ELDERLY PATIENTS WITH MYELODYSPLASTIC SYNDROME
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Background: Myelodysplastic syndrome (MDS) are heterogenous group of hematologic disorders broadly characterized by cytopenias associated with a dysmorphic and usually cellular bone marrow, and by consequent ineffective blood cell production. For patients with MDS an epigenetic therapy with hypomethylating agents is considered standard of care.
Aims: We evaluated the outcome of 5-Azacitidine and Decitabine treatment in elderly patients with myelodysplastic syndrome in our veterans hospital.
Methods: For analysis, patients were included if they were ≥ 60 years, had morphological evidence of MDS per bone marrow examination at the time of diagnosis and were treated with either hypomethylating agent, 5-azacitidine and decitabine. The patients received 5-azacitidine 75mg/m2, d1-7 or decitabine 20mg/m2, d1-5 at four weeks interval. The primary end point was overall response rate.
Results: A total of 27 patients were analyzed. Median age was 68 years (range 61-81 years). 10(37%) patients had received 5-azacitidine and 17(63%) patients had received decitabine. The International Prognostic Scoring System risk category was Intermediate-2/High in 44%. A median of 5 courses (range 1-16) were delivered and 56%(15/27) patients were treated 4 cycles over. The overall response rate was 59%. Patients who showed response (CR+PR+HI) had significantly longer overall survival than those who did not (30 months vs 5months, P=0.01). With a median follow-up duration of 12 months, median overall survival was 14.7 months. The difference in overall survival was evident in the Intermediate-2/High risk group but not in the Intermediate-1 risk group. However, the difference between 5-azacitidine and decitabine group was not stastically significant.
Summary / Conclusion: In our study, both 5-azacitidine and decitabine were feasible and effective in elderly patients with myelodysplastic syndrome. The overall survival was significantly longer in patients showing response. There were needed additional large-scale prospective studies.

B1467
MELATHONIN PLUS DANAZOLE, PREDNISONE AND ERYTHROPOIETIN ALPHA IS EFFECTIVE IN TREATMENT OF MYELODYSPLASTIC SYNDROMES WITH ANEMIA AND THROMBOCYTOPENIA.
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Background: Melathonin was reported effective in some cases of ITP because of its thrombopoietic effect.
Aims: Aim of this study is to verify if danazole, prednisone, melathonin and erythropoietin alpha is effective and safe in patients with refractory cytopenia with multilineage dysplasia (RCMD) with anemia and thrombocytopenia.
Methods: This study is a multicentric study.
20 patients with RCMD with IPSS intermediate or low showed anemia and thrombocytopenia. Cytogenetics was normal in 15 patients and not evaluable in 5 patients. In group A 10 patients received orally danazole 200 mg/day, prednisone 25 mg/day, melathonin 60 mg/day, B12 400 mg/day, calcium levofolinate 7.5 mg/day, liposomal iron 30 mg/day, erythropoietin alpha 40000 IU subcutaneous weekly (5 originator and 5 biosimilar) for at least 3 months. In group B 10 patients received the same treatment except melathonin. In group B 7 patients received originator erythropoietin alpha and 3 biosimilar. In group A M/F was 6/4, median age was 68 years (R62-80), median follow-up was 4 months (R2-6), median Hb 9 g/dl (R8.5-10), median PLT count 40000/mcl (R30000-50000). In group B M/F was 5/5, median age was 66 years (R60-84), median follow-up was 3 months (R2-5), median Hb 8.7 g/dl (R8-9.5), median PLT count 27000/mcl (R20000-45000).
Results: In group A median platelet count after treatment was 55000/mcl (R40000-60000), median Hb 10 g/dl (R9-11). In group B median platelet count after treatment was 38000/mcl (R25000-50000), median Hb 10.2 g/dl (R9-10.5). In group A the 5 patients receiving biosimilar erythropoietin alpha showed a median platelet count of 55000/mcl vs a median platelet count of 40000/mcl in patients receiving originator molecule. No side effects were noted in the two group.
Summary / Conclusion: Melathonin, danazole, prednisone and erythropoietin alpha is safe and effective in RCMD with anemia and thrombocytopenia.

B1468
CLINICAL ANALYSIS OF 24 PATIENTS WITH SEVERE APLASTIC ANEMIA AND VERY SEVERE APLASTIC ANEMIA TREATED WITH ANTITHYMOCYTE GLOBULIN(ATG) COMBINED WITH CYCLOSPORINE
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Background: Severe aplastic anemia(SAA) and very severe aplastic anemia(VSAA) are serious hematopoietic disease,most of the patients would die within 6-12 months if they couldn't receive effective treatment. If the patient is younger than 40 years old and has a histocompatible sibling donor,the first choice is hematopoietic stem cell transplantation (HSCT);but If the patient is older than 40 years old and has no histocompatible sibling donor, Immunusuppressive therapy (IST) is a key treatment strategy ,the standard IST is antithymocyte globuline (ATG) plus cyclosporine A (CsA). Because the majority of patients have no histocompatible sibling donor. so IST is a key treatment strategy to the patients.
Aims: Here we summarized the recent therapeutic effect and adverse reaction of treatment with ATG plus CsA in 24 patients with SAA and VSAA as follows.
Methods: There are 13 patients with SAA and 11 patients with VSAA including 10 male and 14 female ranged from 6 to 63 years old median age was 18 years. All the patients were treated with rabbit ATG from France at a dose of 3-5mg/kg/day for 5 days, and received the CsA a week later at a initial dose of 3-5mg/kg/day, then adjusted the dose of CsA according to the blood concentration and reduced the dose after one year gradually.we also treated the patients with the hematopoietic stimulating factors(eg.G-CSF,L-11,EPO) after the ATG treatment. We strengthened the anti-infective therapy within 2 months after ATG treatment , And we emphasized the erythrocyte suspension and platelet infusion.
Results: We evaluate the effects according to the Camitta criteria 4 months after the ATG treatment, 6 patients achieved CR,12 PR,the other 6 patients had no response,the overall response rate is 75%, during the treatment,22 patients suffered from Varying degree of inflammation, including 10 sepsis 4 of them whose history was longer than 6 months died early,We haven't found MDS or PNH evolution for 2 years.
Summary / Conclusion: ATG plus CsA is an effective treatment for SAA and VSAA,we can overcome the severe infections and allergic reactions.

B1469

EFFICACY AND TOLERABLITY OF AZACITIDINE TREATMENT IN PATIENTS WITH MDS, AML AND CMML

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Background: Azacitidine (AZA) is a hypomethylating agent approved for the treatment of adults patients (pts) who are not eligible for haematopoietic stem cell transplantation with: Int-2 and high-risk myelodysplastic syndromes (MDS) according to the International Prognostic Scoring System (IPSS); chronic myelomonocytic leukemia (CMML) with 10-29% marrow blasts without myeloproliferative disorder, and acute myeloid leukaemia (AML) with 20-30% blasts and multi-lineage dysplasia, according to World Health Organization (WHO) classification (1). AZA efficacy has been firmly established in higher risk MDS patients; however, in CMML its efficacy remains uncertain (< 10 patients have been treated in the AZA trials) (2). Regarding AZA efficacy in AML, an AZA-001 trial sub-analysis showed that AZA significantly prolonged overall survival (OS) vs. conventional care regimens (CCRs) in 113 patients with WHO-classified AML and 20 – 30% BM blasts (3)
Aims: We describe the clinical experience with azacitidine in patients with MDS, AML and CMML with safety data included.
Methods: Multicenter, observational, post market, retrospective study in the AZA indicated population (patients with MDS, AML and CMML) treated with AZA (≥ 3 months) before September 2011.
Results: Twenty one patients were included (intent to treat population). AZA treatment was administrated in 5-2-2 and 7 days schedules in 15 (66.7%) and 6 patients (28.6%), respectively (11.7 cycles; mean); with concomitant G-CSF in 18 pts. Six (28.6%) patient continued with the AZA treatment; 11 pts (52.4%) achieved transfusional independence (TI) (mean: 33.9 weeks), and 3 maintained it at the end of the study. Thirteen (61.9%), 10 (47.6%), 7 (33.3%), 7 (33.3%) and 2 (9.52%) patients, achieved erytroid, plateled, granulocytic and morphological medullar response, respectively at a mean of 5.08, 5.80, 3.29, 5.29 and 3 cycles. The best response to AZA treatment was complete response (CR) in 4 patients (19%), partial response (PR) in 3 (14.3%) and stable disease (SD) in 10 (47.6%). Overall response rate (ORR) (RC+RP+EE)= 17 patients (81%). The median overall survival (OS) was 612 days (20.4 months) (95% CI 233 – 800). At the end of study 7 patients (33.3%) remained alive with none exitus related with AZA treatment. Adverse event (AE) was reported in 13 patients, among them 11 required dose reduction/withdrawal treatment. The AE included: Grade2-4 neutropenia (n=12), Grade 3-4 thrombocytopenia (n=2), Grade 3 cytopenia (1), Grade 2 injection site reaction (n= 1), Grade 1 polycythemia (n=1) and Grade 3 digestive toxicity (n=1).

ITT population n=21					
	n	%		mean	range
Men	15	71.4			
Previous treatment before AZA			Age (years)	72.5	55-83
	n	%	Time from dg to tt (months)	9.85	0-44
GCSF	10	47.6			
Transfusion	7	33.3			
Steroids	7	33.3	ECOG PS	1.38	0-4
BM Aspirate					
	mean		SD		
Blast	0.13		0.14		
Ringed sideroblasts	0.15		0.18		
Cellularity			Dysplasia		
	n	%		n	%
Normal	11	52.4	Multilineage	17	81
High	1	4.76	Eritroyd	4	19
Augmented	2	9.52			
Conserved	1	4.76			
Hypercellular	6	28.6			
MDS classification					
WHO			FAB		
	n	%		n	%
RAEB-1	2	9.52	RA	2	9.52
RAEB-2	7	33.3	RAEB	8	38.1
RARS	1	4.76	RAEB-t	1	4.76
RCMD	6	28.6	RARS	6	28.6
RCUD	1	4.76	AML	2	9.52
AML	2	9.52	CMML	2	9.52
CMML	2	9.52			
Risk classification					
IPSS			WPSS		
	n	%		n	%
High	3	14.3	High	10	47.6
Low	5	23.8	Low	5	23.8
Int-1	6	28.6	Int	3	14.3
Int-2	7	33.3	Very high	3	14.3
Hemograma					
	n	SD		n	SD
Leucocytes (/mm3)	10693	18553	Hemoglobin (g/dL)	8.28	1.95
Ferritin (ng/mL)	713	675	Platelet (/mm3)	97443	84054
Granulocytes (/mm3)	4566	9113	LDH (U/L)	335	282
Creatinine (mg/dL)	0.78	0.53	Blast (%)	0.04	0.08

Summary / Conclusion: The current results of our study showed that azacitidine treatment of patients with MDS, CMML and AML is effective with an ORR of 81%. The therapy was well tolerated with cytopenia as more commonly reported haematological adverse event and digestive toxicity and injection site reaction as nonhaematological events. In conclusion azacitidina is effective and safety in the treatment of these patients.

B1470

INTESTINAL ANGIODYSPLASIAS AND MYELOSYPPRESSION: 3 CASE REPORTS- THE ROLE OF LONG ACTING OCTREOTIDE

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Background: Vascular malformations of the gastrointestinal tract are one not so common cause for gastrointestinal bleeding and iron deficiency anemia. The co – existence of myelodysplastic syndrome in cases like these has been registered, leading the patients to be treated independently for the 2 co – existing diseases.
Aims: Our goal was to investigate the possibility of correlation between the gastrointestinal and the hematological condition of such patients. We took under consideration the new data of international literature, where the role of certain cytokines – that are produced from the intestinal vascular malformations – on the suppression of the bone marrow, is suggested.
Methods: 3 patients, all 3 above the age of 65, were treated with blood transfusions and iron supplementation for a period of at least two years for iron deficiency anemia due to gastrointestinal bleeding, which was provoked by angiodysplasias of the small intestine (as were diagnosed after a thorough endoscopic approach and the use of wireless video capsule). At the same time, these 3 patients were treated with erythropoietin, since they were thought to suffer from myelodysplastic syndrome. With the beginning of the second year of treatment, therapy on all 3 patients seemed to fail and the results of the bone marrow biopsy were reconsidered. We found out that at all 3 patients, myelodysplastic syndrome was not a definite diagnosis because the histology report concluded that the findings were not compatible with any certain type of myelodysplastic syndrome but merely with myelosuppression. Having in mind the suggested in such cases thalidomide and octreotide therapeutic approach, we started treating the patients with long-acting octreotide, in a dosage of 20 mg, given intragluteally at 4 – week intervals for at least one year.
Results: The hemoglobin levels, blood transfusions, iron supplementation, hospitalizations and the need for erythropoietin on all 3 patients were monitored during the period of their octreotide treatment. The results were satisfactory, since all 3 of them maintained an Hb level above 9mg/dl without being submitted to their previous treatments.
Summary / Conclusion: The suppressive role of intestinal vascular malformations via cytokines on the bone marrow productive procedure is a topic with great interest and prospective. The identity and role of intermediate cytokines is discussed and the benefit deriving from the usage of long acting octreotide,as well as thalidomide, is yet to be proved.

B1471

CLINICAL TRIAL OF PROPHYLACTIC ANTIFUNGAL AND ANTIVIRAL THERAPY IN SAA/VSAA PATIENTS UNDERGOING IMMUNOSUPPRESSIVE THERAPY

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Background: Persistent neutropenia associatedInvasive fungal infections predominantly with aspergillus, and bacterial infections are a major cause of death in patients with SAA.Patients of SAA/VSAA undergoing immunosuppressive therapyare also susceptible to viral infections. In multivariate analysis, younger age, absolute neutrophil count > 200 cells/μL before IST, absence of IFIs, and use of voriconazole were independently predictive of survival .
Aims: The aim of this study was to investigate the effects of prophylactic antifungal and antiviral therapy in SAA/VSAApatients undergoing IST .
Methods: 26 patients with SAA/VSAAundergoing IST were evaluated. The patients were divided into two groups. We reviewed the records of 14 patients (group1, non- prophylactic group) with SAA/VSAA admitted to our Hematology department from 2005 to 2012 who received immunosuppressive therapy (IST) without prophylacticanti-infective therapy. 12 patients (group2, prophylactic group) undergoing IST during April 2012- February 2013 with prophylactic antifungals , antiviralstherapy, Valaciclovia 0.3 po bid plus Micafungin 50mg intravenously qd.
Results: In 12 patients of prophylactic group , 8 were response to IST, 4(33.3%) were found neutropenic febrile (1 patient wasserum sickness, 1 patient with bacterial infection, 2 patients with bacterial and fungal infections and serum sickness). In 14 patients of non- prophylactic group , 10 were response to IST , but had longer hospitalized time than prophylactic group, 13(92.8%) patients

occurred neutropenic febrile (each patient developed 1 or more febrile episodes) (2 patients were associated with serum sickness, 3 patients with bacterial infection, 7 patients with clinically documented bacterial and fungal infections,1 patient with bacterial and viral infections).
Summary / Conclusion: Our study showed thatprophylactic antifungals , antivirals treatment is very imperative. Prophylactic antifungal and antiviral therapy is effective in reduce neutropenic febrileand hospitalized timefor SAA/VSAA patients undergoing immunosuppressive therapy.

B1472
SUCCESSFULL AZACITIDINE BASED TREATMENT OF THERAPY RELATED MYELODYSPLASTIC SYNDROME WITH NORMAL KARYOTYPE OCCURRED AFTER ACUTE MYELOID LEUKEMIA
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Background: Therapy-related myelodysplasia (t-MDS) occurs as a complication of cytotoxic treatment given for cancer. Typically at presentation chromosomes abnormalities and/or complex karyotype are present. Standard chemotherapy is often not effective with very low complete remission rates so prognosis remains poor.
Aims: Azacitidine is an hypomethylating drug that showed to be effective in MDS. We describe here 3 patients with t-MDS secondary to chemotherapy for de novo acute myeloid leukemia (AML), successfully treated with Azacitidine.
Methods: Between April 2011 and November 2012 we observed in our centre 2 women and one man previously successfully treated for AML (M2,M2 NPM+, and M5) who were then diagnosed with MDS, defined by blast count <20% and typical cytological morphology in bone marrow aspirate. Patient 1 was a 48 years-old lady diagnosed in March 2011 with RAEB-1, not transfusion dependent and with 2 cytopenias. Patient 2 was a 74 years-old lady diagnosed in February 2012 with RAEB-1 but with a mild anemia; patient 3 was a 64 years-old man presented in February 2012 with RCMD was transfusion dependent for Red blood Cells and Platelets. All patients were Intermediate-1 and Intermediate according to IPSS and WPSS scores respectively. Karyotype was evaluable in all patients and was normal. t-MDS occurred after 44 months of complete remission (CR) for patient1, after 22 months from 2nd CR for patient 2 and after a 8 months CR for patient 3. Azacitidine was started at a median of 1 month from t-MDS diagnosis at the dose of 75 mg/sqm daily for 7 days every 4 weeks. A median of 9 courses were administered (range 7-15). Response was assessed according to the International Working Group criteria.
Results: Patient 1 received 15 courses of azacitidine and after obtaining complete remission underwent allogenic stem cell transplantation. Patient 2 received 9 courses of azacitidine achieving complete remission and she is still on treatment. Patient 3 achieved stable disease with persisting severe thrombocytopenia and anemia after 7 courses of azacitidine but died because of hemorraghic stroke. Therapy was generally well tolerated except for patient 3 who experienced several grade 2- 3 infective episodes . Median overall survival of t-MDS patients was 12 months (range 6-22)
Summary / Conclusion: Currently there is no standard treatment for patients wit t-MDS. In our small experience, azacitidine given upfront showed to be effective improving overall survival with an accetable toxicity profile. Further studies are needed to confirm these results.

B1473
RAPID HAEMATOLOGICAL RESPONSE WITH 5-AZACYTIDINE IN HIGH RISK MYELODYSPLASTIC SYNDROME
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Background: High risk myelodysplastic syndrome (IPSS intermediate 2 and above) and CMMLII have poor prognosis. Median survival with best supportive care is less than one year. 5-azacytadine has significantly changed the median survival in these patients. Median cycles to complete haematological response has been 4 cycles.
Aims: We report two patients who achieved complete haematological response just after one cycle of azacytidine. It is not commonly seen that patients acheive such rapid response.
Methods: 67 years old male patients presented with Hb-7gm/dl, WBC-54X10^9 /L and platelets-17x10^9 /L. Peripheral monocyte count was 5.5x10^9 /L. Bone marrow showed Chronic myeloonocytic leukaemia (CMMLII) with 11% promocytes and blasts. Karyotype was normal with FISH for chromosome 5 and 7 being negative. He was requiring 2 units RBC support every 2 weeks, and one pool of platelets every week for past 2 months before starting 5 Azacytidine. He had splenomegaly of 10cm below the costal margin. He was started on 5-azcytidine with the standard dose of 75mg/m^2 for 7 days .2 weeks after the last day of 5 –Azacytidine, his haemoglobin rose to 9gm/dl and platelets improved to 33x10^9/Lwithout any transfusion support. White cell count started to reduce and neutrophils normalized. Just before starting the second cycle he achieved complete haematological remission and transfusion independence. He continued to receive further 3 cycles before bone marrow which revealed 4 % blasts and continuing tri-lineage dysplasia He has completed 9 cycles of Azacytidine and continues to be in complete haematological remission. Second patient is a 79 years old male who presented in 2011 with isolated thrombocytopenia of 40 X109 /L and normal haemoglobin and white cell count. Bone marrow revealed MDS RCMD with 3 % blasts. He had complex cytogenetics with deletion5,-22, +22, deletion 20, +marker. He chose to remain on watch and wait initially. 5 months later he was admitted through the accident and emergency department with septicaemia and swollen joints. Blood test: Hb-7.9 gm/dl, WBC-1.3xX10^9 /L, Neutrophils-0.4x10^9 /L, Platelets-20x10^9 /L. Repeat bone marrow after sepsis revealed RAEB II with 17% blasts with same complex cytogenetics abnormality as presentation. He was started on Azacytidine of 75mg/m2 for 7 days. Immediately after completing the drug he was again admitted with second episode of sever sepsis and sever pancytopenia. As he recovered from sepsis his counts recovered and he achieved complete normal blood counts with Hb-14.5gm/dl, WBC 6.8x10^9 /L, neutrophils 2.5x10^9 /L and platelets 340x10^9 /L. Patient refused to have any more chemotherapy and further tests thinking he has been cured. His last blood test in January 2013 which was3 months after last dose of Azacytidine still shows complete haematological remission
Results: We have reported that both patients acheived complete haematolgical response just after one cycle of azacytidine. In one patient we could document bome marrow dysplastic remmission also but the otherpatient unfortunately declined further investigation and treatment.
Summary / Conclusion: Azacytidine treatment results in significantly higher response rates, improved quality of life, reduced risk of leukaemic transformation, and improved survival compared with supportive care. Complete haematological repose just after one cycle is not common. It has not been reported till date in the literature. Early platelet response has been a marker of long term survival and long progression free survival. It would be interesting to see if very rapid response in haemoglobin and white cell, just after one cycle could also translates in to long progression free survival

B1474
A RARE DISEASE IN CHILDHOOD: PNH AND ITS TREATMENT WITH ECULIZUMAB
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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired syndrome in which glycosyl phosphatidylinositol–anchored proteins (GPI-AP) are deficient on the surfaces of blood cells. PNH can present at any age, most commonly between 20 and 50 years. It is extremely rare in childhood and adolescence. Only 70 well-documented pediatric cases of PNH could be retrieved from the literature and most of these have arisen in a previous setting of bone marrow failure. There are few pediatric case reports presented with reccurrent hemolytic episodes. Treatment is mainly supportive in the form of folic acid, iron supplements, and blood transfusions of filtered red cells when ever necessary. Hematopoietic stem cell transplantation is the only curative option for patients with PNH. A novel alternative treatment option for adult patients with PNH includes the humanized monoclonal antibody eculizumab.
Aims: Eculizumab is not licensed for use in patients <18 years of age. The experience regarding to the use of eculizumab therapy under 18 years is lacking. So, we find it significant to share our experience regarding to the use of eculizumab for a 10 year old patient that was diagnosed as PNH at 4.5 years of age and have been managed successful by eculizumab for more than one year in Kanuni Sultan Süleyman Education and Research Hospital.
Methods: The diagnosis of the patient was made at 2007 with immunphenotyping and then verified with FLAER method.She had been given blood transfusions, hyperhydration therapy, low-dose oral prednisolone continuously at the beginning and then during hemolytic attacks.During follow-up, attacks had become more frequent and recur at 2-3 weeks intervals.With the informed consent of the family, eculizumab therapy was commenced. The dose of eculizumab was set according to the weight of the child as 600 mg/week for 4 week, then 900 mg/every other week parenterally.
Results: After introduction of eculizumab both the frequency and the severity of the attacks had declined significantly. She has been under eculizumab therapy for more then one year without any considerable advers effect.
Summary / Conclusion: Depending on our experience; we can say that, like in adults, eculizumab may have a substantial role in controlling hemolytic episodes and preventing thrombotic complications and improving quality of life in children

Myeloma and other monoclonal gammopathies - Biology

B1475

THE NOVEL PAN-HDAC INHIBITOR, CKD-581 ENHANCES THE ANTI-MYELOMA EFFECTS OF BORTEZOMIB

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Background: Despite recent advances in myeloma treatment with the advent of new targeted agents such as bortezomib, thalidomide and lenalidomide, multiple myeloma (MM) still remains incurable. To improve clinical outcome of MM patients, many studies have been trying to develop novel targeted drugs.
Aims: We wanted to examine the efficacy of CKD-581, a novel HDAC inhibitor, either alone or combined with bortezomib in MM.
Methods: Antimyeloma effect of CKD-581 was tested in 6 commercial MM cell lines, either alone or combined with bortezomib. The same experiment was done with the presence of bone marrow stromal cells. We also examined the effect of CKD-581, either alone or combined with bortezomib in an animal model and freshly isolated MM cells from MM patients' bone marrow. For an animal study, we used NRG(RAG2−/−gc−/−) mice that were injected with MM cells via tail vein.
Results: CKD-581 had a potent anti-myeloma effect in 6 MM cell lines. At the molecular level, CKD-581 caused accumulation of acetylation of histones and activated cleavage of PARP and caspase 3 in MM cells. In combination with bortezomib, CKD-581 synergistically increased bortezomib-mediated apoptosis in MM cells. In addition, this synergistic effect was maintained despite co-culture with bone marrow stromal cells (BMSCs). Mice treated with CKD-581 alone and CKD-581 combined with bortezomib survived longer than non-treated controls, with the longest survival in combination arm. MM cells were not detected in bone marrow of treated mice. In *ex vivo* study, CD138+ cells decreased significantly when cells were treated with CDK-581 alone and CKD-581 combined with bortezomib.
Summary / Conclusion: Our data indicated that CKD-581 had apoptotic effects in MM and it showed synergism with bortezomib.

B1476

IMMUNOPHENOTYPIC ANALYSIS OF BONE MARROW B LYMPHOCYTE PRECURSORS (HEMATOGONES) IN PLASMA CELL DISORDERS: A NEW MARKER OF DISEASE?

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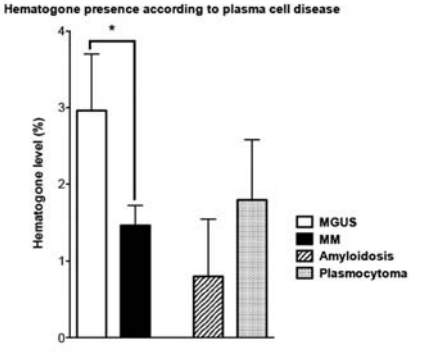
Background: Hematogones (HGs) are normal B-cell precursors present in bone marrow, including pro-B, pre-B and immature B cells, and characterized by phenotypic co-expression of CD19 and CD10, and the acquisition of CD20. HGs level in bone marrow aspirates is inversely correlated with age, but can be increased after chemotherapy or stem cell transplantation. This increase may reflect B cell reconstitution. Recent studies suggested that HGs level in bone marrow was statistically correlated with favorable outcome in acute myeloid leukemia and allogeneic hematopoietic stem cell transplantation. However, no data exist on the presence of these B-cell precursors in patients with plasma cell disorders.



Aims: Our goal was to describe the HGs level in plasma cell disorders, and to determine any correlation with plasma cell infiltration or disease activity.
Methods: We analyzed by cytology and flow cytometry cells obtained from bone marrow aspiration in 73 patients at the time of diagnosis for plasma cell diseases: monoclonal gammopathy of undetermined significance (MGUS, n=16), multiple myeloma (MM, n=48), AL amyloidosis (n=6) and isolated plasmocytoma (n=3). HGs were isolated as CD10+/CD19+ CD45low and CD20+ cells by flow cytometry with a 4-colour combination. The data were visualized using a plot of side scatter scale versus CD45 staining. The sensitivity cut-off was 0.01% of the total number of nucleated cells. Plasma cells were analyzed using a CD38/CD138 plot and for CD19, CD20, CD56, CD117, CD33 and CD28 expression.
Results: The mean age of the 73 patients was 64.8 years old. Sixty-two patients (84%) had detectable HGs in the bone marrow. The median and mean percentages of total HGs were 2.04% and 1.81% (0-10%) respectively. The mean level of HGs was similar in both men and women (1.80%). The percentage of HGs was not correlated with age, and high values have been observed in very old patients (4.6% in a 89 years old patient for example). The percentage of HGs was inversely correlated with plasma cell infiltration, as assessed by both flow cytometry and cytology (P=0.0089 and P=0.0126, respectively). Moreover, the percentage of bone marrow HGs was significantly lower in multiple myeloma patients (smoldering and/or active myeloma) than in monoclonal gammopathy of undetermined significance (P=0.04, figure 1). There was no correlation between HGs level and the expression of CD56, CD117, CD28, CD33 or beta2-microglobinemia at diagnosis.
Summary / Conclusion: At our knowledge, this is the first study to show HGs presence in plasma cell disorders. Our results demonstrate that B-cell precursors could be identified in the majority of patient with monoclonal protein. Moreover, we found that the percentage of HGs was inversely correlated with plasma cell infiltration and disease activity. More data and a longer follow-up of our patients are needed to determine the prognostic impact, in MGUS and multiple myeloma progression and survival.

B1477

POTENTIAL ROLE OF PPAR-G AND HMOX1 IN MULTIPLE MYELOMA (MM) CELL DEATH

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Background: Peroxisome proliferator-activated receptor γ (PPAR-γ) is a multifunctional transcription factor that regulates immune and inflammatory responses. It has been previously shown that normal and malignant B cells, including multiple myeloma, express PPAR-γ but its physiological role remains unknown. Moreover, while certain PPAR-γ ligands can induce apoptosis of multiple myeloma cells and PPAR- γ over-expression decreases MM cell growth, silencing PPAR-γ expression by RNAi increases survival of B lymphoma cells (Garcia Bates Clin Can Res 2009). Heme Oxygenase 1 (HO1) is a microsomal enzyme that catalyzes the degradation of heme into carbon monoxide, biliverdin and ferrous iron. It plays a pivotal role in inflammation, oxidation and apoptosis. It is able to regulate PPAR-γ by enhance its expression as well as different PPAR-γ agonists upregulates HO1 via a PPAR-g dependent-pathway. Moreover, it has been recently described that Bortezomib is able to increase HO1 expression (Barrera, Cell Cycle 2012).
Aims: The aim of this study was to evaluate the correlation between Bortezomib, HO1 and PPAR-γ in multiple myeloma cells.
Methods: Three human multiple myeloma cell lines (ARH77, U266, SKMM1) were incubated with rosiglitazone (1μM, 72h), a synthetic agonist of PPAR-γ currentlyused to treat type 2 diabetes, and with Bortezomib (10nM, 24 h). Cell viability was evaluated by flow cytometer (Becton-Dickinson, San Jose, CA, USA) with annexin V and 7-Aminoactinomycin D (*7-AAD*) assay. Real-time PCR (7900 Fast Real Time PCR Applied Biosystems) was used to analyze PPAR-γ and HO1. HO1 enzymatic activity was evaluated by a colorimetric assay.
Results: Rosiglitazone was able to induce apoptosis in human multiple myeloma cells (apoptotic cells 48%±9,3 vs 35%±7 of control) and a relevant increase of PPAR-γ (30 folds) and HO1 (12 folds) expression. Bortezomib, as expected, was able to decrease cell viability of about 50% and, more notably, was able to increase HO1 protein and mRNA expression (40 fold) and PPAR-γ expression (16 folds). We have also treated myeloma cells with Bortezomib plus or not an inducer (Hemin 10uM) or an inhibitor (Tin, Sn-Mesoprotoporphyrin, 10uM) of HO1 enzyme activity. The treatment with Hemin and Tin alone did not change cell viability whereas combination of Bortezomib with Hemin induced higher mortality levels than Bortezomib alone and, in contrast, Bortezomib plus Tin induced a reduced cell mortality.
Summary / Conclusion: Our results indicate that the antidiabetic drug rosiglitazone, a PPAR-γ activator, and proteasome inhibitor Bortezomib are able to induce apoptosis, HO1 and PPAR-γ expression in multiple myeloma cells. In addition, HO1 expression is induced by Hemin that enhances the pro-apoptotic activity of Bortezomib, suggesting a potential role of peroxisome proliferator-activated receptor γ and HO1 in multiple myeloma-drug induced cell death

B1478

INFLAMMATORY MONOCYTES ARE INCREASED IN MM AND CORRELATED TO EXTENSION OF BONE DISEASE

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Background: The immune function in MM is impaired consequently to an immunologically hostile microenvironment and cellular defects, differently from MGUS. Inflammatory CD14+/CD16+ monocytes (IM) have a wide range of chemokine pathways for recruitment into tumour microenvironment, in which they differentiate into macrophages or alternatively in dendritic cells and as shown exclusively in mouse model they might enhance oxidative stress and endothelial dysfunction. In mouse model increased levels of sIL-2R induce expansion of IM. Activin-A is potently up-regulated in monocytes as well as stromal fibroblasts by cognate interaction with activated T cells in the bone marrow milieu and plays a functional role in the suppression of inflammation.
Aims: Evaluation of IM in patients with advanced bone disease.
Methods: In a cohort of 40 newly diagnosed MM , 30 MGUS and 20 Healthy subjects, we evaluated in peripheral blood the absolute count of monocytes and their phagocytic activity and IM by flow cytometry. Circulating levels of Activin A, sCD44 and sIL-2-R were detected by a commercially available ELISA kit in sera of 20 newly diagnosed MM, 20 MGUS and compared to 10 healthy subjects matched for age and sex.
Results: Even though absolute count of monocytes was similar between healthy subjects vs MGUS vs MM, they have a reduced phagocytosis (90.6±3.3 vs 80.6±4.7vs 71.2±2.2). IM in MM were higher than in MGUS or healthy subjects (respectively, mean 51.39±4.47/mmc vs 48.32±7.82/mmc vs mean 37.75±3.45/mmc, P=0.027). Absolute count of MI was positively correlated with presence and extension of osteolytic MM-related bone disease (evaluated at MRI) and cytogenetics risk (according to Mayo criteria).
MM patients with bone disease exhibited an increased amount of circulating Activin A (260.8±29.24 ng/mL) compared to MGUS (189.5±32.43 ng/mL) and healthy subjects (mean 62.18±8.83 ng/mL) (P<0.0001, one way ANOVA). Among MM patients, Activin A was significantly increased in presence of osteolytic lesions (280.4±26.82 vs 173.5±24.15 , P=0.014, unpaired T test). In MM sCD44 and sIL-2R were increased when compared to healthy subjects (respectively mean 6.4±0.4 vs 3.4±0.5 ng/mL vs , P=0.02 and 2.1±0.2 vs 1.2±0.01 ng/mL vs , P=0.02).
Summary / Conclusion: Taken together, our findings suggest a role for MI MM contributing to osteolysis and aggressive disease.

B1480

INCREASED SERUM LEVELS OF MIP-1ALPHA CORRELATE WITH BONE DISEASE AND ANGIOGENIC CYTOKINES IN MULTIPLE MYELOMA PATIENTS

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Background: Many cytokines possess multiple roles in the pathogenesis of multiple myeloma. Macrophage inflammatory protein-1alpha (MIP-1alpha) is an osteoclast activating factor with a major role in myeloma bone disease.
Aims: The aim of the study was to examine the participation of MIP-1alpha in the angiogenic process of the disease, by correlating its levels with major angiogenic factors, such as basic-fibroblast growth factor (bFGF), hepatocyte growth factor (HGF) and interleukin-18 (IL-18).
Methods: Fifty-six newly diagnosed myeloma patients (30 male and 26 female; median age 66 years, range 40-84 years) were enrolled in the study. The types of monoclonal proteins were: IgG for 34 patients, IgA for 16 patients and light chain disease for 6 patients. According to international staging system, 16 were in stage I, 19 in stage II and 21 in stage III of the disease. Bone involvement was graded according to standard X-ray evaluation, into four scores: grade 0, with no lesions (16 patients), grade1, with involvement of one bone or with diffuse osteoporosis (10 patients), grade2, with more than one but less than four bone lesions (10 patients) and grade3, with more than four lesions or presence of bone fracture (20 patients). Twenty-five, age and sex matched, healthy volunteers were used as controls.
Serum samples were collected from all subjects, stored at -70^{o}C, and assayed at the end of the study. Serum levels of the MIP-1alpha, IL-18, bFGF and HGF were measured by ELISA, according to manufactures' instructions.
Results: All analyzed parameters were found higher in myeloma patients (P<0.001 for all cases). They were all also increasing levels with the advance of the disease (P<0.001 for all cases). Similarly with disease staging, all of them were higher while bone involvement was progressing (P<0.04 for MIP-1alpha, P<0.001 for other cases). In the group of myeloma patients, serum levels of MIP-1alpha correlated positively with IL-18 (r=0.596), b-FGF (r=0.509) and HGF (r=0.424). Furthermore, IL-18 correlated positively with b-FGF and HGF (r=0.676 and r=0.511 respectively), while b-FGF correlated positively with HGF (r=0.508) (P<0.001 for all cases).
Summary / Conclusion: MIP-1alpha seems to be a predominant factor responsible for the enhancement of bone resorption and increased angiogenesis. The positive correlation between MIP-1alpha and the angiogenic chemoattractants supports the involvement of these factors in the biology of myeloma cell growth. Moreover, they could be used as possible therapeutic targets as well as markers of disease activity.

B1481

EPILEPSY DOSES OF VALPROATE COMBINED WITH THE ANTI-HELMINTHIC, NICLOSAMIDE, SYNERGISTICALLY KILL MYELOMA CELLS: A POTENT NEW ANTI-MYELOMA DRUG COMBINATION.

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Background: Multiple myeloma (MM) is a plasma cell neoplasm which is characterized by a clinical course of remission and relapse. Progressions in its treatment have largely focused on developing novel agents; however, drug redeployment offers an inexpensive and efficacious strategy for identifying new therapeutic options with minimal adverse effects. Using a drug-redeployment screening strategy, we identified and have shown that the anti-helminthic niclosamide is an effective anti-myeloma agent that targets the mitochondrial respiratory chain inducing production of mitochondrial superoxide (mitosox) and triggering apoptosis and autophagy. Rescreening of the library with low dose niclosamide identified valproate, an anti-epileptic and Histone Deacetylase Inhibitor (HDI), as potentiating cell killing. Here we show that valproate used at anti-epilepsy doses has little or no activity alone, but in combination with niclosamide, demonstrates potent synergistic anti-myeloma activity. Our data shows that this synergy arises through enhanced production of oxidative stress, reduction of antioxidative responses and modulation of acetylation state of non-histone proteins.
Aims: The aim of this project is to identify redeployed drug combinations with anti-myeloma activity that will offer therapeutic hope for relapsed/refractory myeloma patients.
Methods: Cell viability, cell death and reactive oxygen species (ROS) production was measured using flow cytometry. N-acetylcysteine (NAC) was used to modulate ROS levels. Molecular, cellular and biochemical studies were performed to measure Superoxide dismutase (SOD) activity, changes in gene expression and protein levels. Acetylation state and levels of proteins were assessed using proteomics approaches.
Results: Cross-titrations of niclosamide and valproate demonstrated synergistic killing of MM cell lines associated with both apoptosis and autophagy. We have shown previously that niclosamide induces mitochondrial superoxide (mitosox) in MM cell lines. Here we show that valproate induced production of non-mitosox ROS, alone and in combination with niclosamide treatment whilst also potentiating production of mitosox in the combination treatment. The role of oxidative stress was verified with the antioxidant N-acetylcysteine partially rescuing MM cell lines from cell death induced by the drug combination. The increase in mitosox production correlated with decreased mitochondrial SOD (SOD2) activity, whereas activity of the cytosolic SODs (SOD1, SOD3) was unaffected. Although we did observe perturbations in sirtuin gene expression and in mitochondrial genes, these together with the changes observed in SOD2 mRNA or protein levels did not explain the enhanced oxidative stress levels in the combination treatment. Since many non-histone proteins have been shown to be regulated by acetylation, we have performed an acetylome analysis using tandem mass-tagging (TMT) and mass spectrometry to dissect the role of low dose valproate on protein acetylation in myeloma cells. Preliminary data demonstrates changes in SOD2 acetylation, a modification that regulates SOD2 activity.
Summary / Conclusion: We have successfully identified a redeployed drug combination with anti-myeloma activity using small scale screening of a drug library. The combination of valproate and niclosamide (VaN) demonstrated potent synergistic anti-myeloma activity. Importantly, the concentrations of both agents used are clinically achievable, safe and affordable which will expedite progression of VaN therapy to phase I/II clinical trials.

B1482

TRANSLOCATION T(14;16): FREQUENCY AND SIGNIFICANCE IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Together with translocations t(4;14) and t(14;20) and deletion of the TP53 gene, translocation t(14;16) is a high-risk cytogenetic aberration in patients with multiple myeloma (MM). Translocation t(14;16) with subsequent

increased expression of the *MAF* proto-oncogene and deregulation of the *WWOX/FOR* gene are associated with an aggressive course of the disease, decreased sensitivity or resistance to chemotherapy and early progression/relapse of the disease. Due to its very low incidence in MM patients (2-10%), the significance of t(14;16) has not been fully elucidated.
Aims: The goals were to determine the frequency of translocation t(14;16) and to analyze cytogenetic, molecular cytogenetic and clinical data in a group of patients with MM or monoclonal gammopathy of undetermined significance (MGUS) diagnosed and treated at the Department of Internal Medicine III and cytogenetically investigated at the Department of Hemato-Oncology, University Hospital Olomouc in 2000-2013.
Methods: In all MM or MGUS patients, bone marrow was tested by conventional cytogenetic methods and FISH with locus-specific, centromeric, whole chromosome and BAC probes; one patients was investigated using mFISH and arrayCGH.
Results: Translocation t(14;16) was found in 10 (2.9%) out of a total of 339 patients investigated for *IgH* gene rearrangement. The group of 10 patients comprised 5 males and 5 females with a median age of 67 years. The only monoclonal immunoglobulin isotype detected in the patients' blood serum was the IgG isotype; in two patients, a Bence Jones protein was detected. The ratio of kappa to lambda free light chains was 5:5. Using the Durie-Salmon staging system, clinical stage I was found in 2 patients and stages II and III in 3 and 5 patients, respectively. Significant renal insufficiency was observed in 3 patients. Cytogenetic and molecular cytogenetic assays revealed other additional changes in all 10 patients with s t(14;16): deletion of the RB1 gene (7 patients), trisomy or tetrasomy of chromosome 15 (7 patients) and structural and numerical aberrations of chromosome 8 (7 patients). To determine the frequently altered regions of chromosomes 8p and 8q, BAC-specific probes were used as follows: 8p23.1 (RP11-589N15), 8p21.3 (RP11-177H13), 8q24.21 (*MYC* FISH DNA probe), 8q24.3 (RP11-639O3), RP11-639O3 (8q24.3) and a chromosome 8 centromere probe. The most frequently detected chromosome 8 aberration was deletion of the short arm region (8p21.3).
Summary / Conclusion: Cytogenetic and molecular cytogenetic analysis of 339 patients with MM or MGUS confirmed a low incidence of t(14;16) (2.9%). In no patient, this was the only aberrations; it was a part of complex changes in 9 out of 10 patients. Recurrent aberrations in the complex karyotype of patients with t(14;16) were aberrations of chromosomes 13, 15 and 8. Out of 10 patients, only 2 are alive, with the OS rates of 5 and 20 months, confirming the adverse prognostic significance of this aberration in this small group of patients.

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B1483

THE RELATIONSHIP OF MYELOMA CELLS, STROMAL CELLS AND MONOCYTES UNDER BONE MARROW MICROENVIRONMENT

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Background: Multiple myeloma (MM) is a disease characterized by clonal B-cell tumors of slowly proliferating plasma cells within the bone marrow. The current data on MM disease progression indicate thatbone marrow microenvironment plays crucial role in pathogenesis of MM. Myeloma cells contacts with bone marrow stromal cells (BMSCs), which secrete factors/cytokines, promoting tumor cell growth and survival. Paracrine secretion of cytokines such as interleukin-6 (IL-6), insulin-like growth factor-1, and inflammatory protein-1a in BMSCs promotes multiple myeloma cell proliferation and protects against drug-induced cytotoxicity. These cytokines provide stimulatory signals for multiple myeloma growth and survival.
Myeloma is closely associated with lytic bone disease, the most debilitating manifestation of the malignancy, found in 40-75% of patients. Disease-related skeletal complications in MM patients result in significant morbidity due to pain, pathologic fractures and spinal cord compression. The BM microenvironment creates a supportive niche for myeloma cell growth. Osteoclasts and BMSCs, along with extracellular matrix and cytokines stimulate tumor cell proliferation and confer chemoresistance. Therefore, dynamic reciprocal interactions among myeloma cells, osteoclasts, osteoblasts, and BMSCsimpact both the establishment and progression of MM.
In current study, monocyte can directly promote osteogenic differentiation of BMSCs through cell contact interactions and production of osteogenic factors. This mechanism is mediated by the activation of STAT3 signaling pathway in the mesechymal stem cells that leads to the upregulation of Osteoblasts-associated genes such as Runx2 and alkaline phosphatase (ALP), and the down-regulation of inhibitors such as DKK1 to drive the differentiation of mesechymal stem cells into osteoblasts.
Aims: The aim of this study is to expose a role of monocytes that supports myeloma cells in BM microenvironment.
Methods: The growth inhibitory effect of MM cell lines, monocytes and BMSCs was assessed by measuring 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide (MTT) dye absorbance.
To evaluate growth stimulation and signaling in MM cells, MM cells were cultured in BMSC coated 96-well plates in the presence or absence of monocyte. DNA synthesis was measured by [3H]-thymidine uptake, with [3H]-thymidine added during the last 8 h of 48 h cultures. All experiments were performed in quadruplicate.
Results: we observed increased proliferation of MM cell lines in the presence of either BM stromal cells or monocytes compared to cell line-only control. Furthermore, the co-culture of BM stromal cells plus monocytes induced the greatest degree of proliferation of myeloma cells. In addition to increased proliferation, BMSCs and monocytes decreased the rate of apoptosis of myeloma cells.
Summary / Conclusion: Our results therefore suggest that highlights the role of monocyte as an important component of the BM microenvironment.

B1484

BORTEZOMIB UPREGULATES OSTERIX AND OSTEOCALCIN IN OSTEOBLASTS AND REDUCED MYELOMA-INDUCED APOPTOSIS

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Background: Multiple myeloma is the disease with the highest incidence of bone involvement among the malignant diseases. Complex interactions between myeloma cells and bone marrow stroma cellspromote osteoclastic bone resorption and suppress osteoblast activity, there is uncoupled or severely imbalanced bone remodeling with increased bone resorption and decreased or absent bone formation. Bisphosphonates are specific inhibitors of osteoclast activity. They reduce bone breakdown but do not restore bone formation.Clinical data indicate that Bortezomib has potential to regulate bone turnover and increase serum alkaline phosphatase and osteocalcin concentrations.The underlying mechanisms of Bortezomib's action on osteoblasts proliferation and apoptosis remain to be clarified. We therefore investigated roles of Bortezomib on cultured osteoblast in the present study.
Aims: To explore Bortezomib's effects on osteoblasts in the presence of myeloma cells and culture supernatants.
Methods: To mimic the microenviroment of osteoblasts in myeloma bone disease, the mouse calvaria-derived MC-3T3E1 osteoblast cells were cocultured with RPMI8226 myeloma cells and the supernatants. Cell proliferation and apoptosis were evaluated by MTT assay and flow cytometry respectively. Formation of mineralized nodules was calculated by alizarin red staining. RT-PCR and Wstern blot were employed to access expressions of Runx2/cbfa1, osteocalcin (OCN) and osterix (OSX).
Results: MTT results showed Bortezomib dose-dependently inhibited MC-3T3E1 cells growth with an IC50 of 38.1 nM at 48 h. Bortezomib lower than 5 nM was tolerable and non-toxic to both MC-3T3E1 and RPMI8226 cells. MC-3T3E1 cells cocultured with RPMI8226 cells or supernatants underwent extensive apoptosis while 5 nM Bortezomib significantly attenuated apoptosis in conditioned culture. OSX and OCN at mRNA and protein levels markedly increased as response to 5 nM Bortezomib. However, no significant changes were observed in Runx2/cbfa1 expressions and mineralized nodules formation.
Summary / Conclusion: Bortezomib at low concentrations prevented apoptosis and protected osteoblasts in myeloma bone disease. These effects might be mediated by the activation of OSX and OCN through a Runx2/cbfa1-independent mechanism.

B1485

ANALYSIS OF B-CELLS AND PLASMA CELLS IN MONOCLONAL GAMMOPATHIES

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Background: Monoclonal gammopathy of undetermined significance (MGUS) is non-malignant disease, which can progress into malignant form-multiple myeloma (MM). MM is characterized by the presence of clonal plasma cells (PC) arising from malignant transformed B-cells. It is still not clear which stage of B-cell differentiation is responsible for the development of MM and for eventual relapse after treatment, so nowadays there is an effort to identify the source of myeloma-initiating cells.
Aims: Analysis of the phenotypic profile and enumeration of B and PC subpopulations in monoclonal gammopathy patients.
Methods: Total of 38 newly diagnosed MM patients, 18 MGUS patients and 18 controls without MM were analysed. There were identified $CD19^+$ cells in peripheral blood (PB) or bone marrow (BM) and surface expression of CD38, CD45, IgD, CD24, CD20, CD138, CD19, CD27 and IgM was studied by 8-color flow cytometry. The enumeration of transitional, naive, preGC and memory B-cells (with/without isotype switched), switched $CD27^-$ B-cells and plasmablasts was done in PB. Moreover, immature B-cells and normal/abnormal PCs were detected in BM. Comparison of MM samples with controls and MGUS samples was done.
Results: There was found significantly lower % of the total $CD19^+$ cells in MM than in control samples for both PB [3.9% (0.4-8.9) vs. 6.8% (3.8-12.2), $P<0.005$] and BM [4.2% (0.6-50.9) vs. 11.3% (3.5-20.9), $P<0.05$]. Moreover in

BM the decrease of CD19+ cells in MM was significant also compared with MGUS [7.9% (3.9-21.1), P<0.005]. In PB, transitional B-cells were reduced in MM when compared with controls [0.9% (0.0-12.2) vs. 2.5% (0.8-5.8), P<0,05], while reduction of naive B cells in MM was significant compared with both controls [36.4% (2.4-70.9) vs. 56.0% (45.2-70.6), P<0.01] and MGUS [56.7% (7.7-77.2), P<0.05]. On the contrary, switched memory B-cells were increased in MM compared with controls [20.5% (4.9-67.2) vs. 12.5% (6.5-17.1), P<0.01] and MGUS [11.3% (4.2-41.3), P<0.01]. In comarison with controls, higher number of CD27- switched B-cells was found in MM [10.0% (2.6-38.0) vs. 5.2% (2.4-9.0), P<0.01], as well as preGC B-cells were increased in MM compared with controls [0.7% (0.0-2.4) vs. 0.3% (0.0-0.8), P<0.01]. No difference was found when compared plasmablasts in all three groups. In BM there were found similar results as reduction of naive B-cells in MM compared with controls [37.0% (0.6-67.9) vs. 48.0% (27.3-86.3), P=0.05] and MGUS [50.1% (13.8-74.9), P<0.05], as well as increase of switched memory B-cells in MM compared to both controls [14.0% (1.4-41.3) vs. 5.7% (1.7-11.5), P<0.05] and MGUS [6.0% (0.5-21.3), P<0.05] and increase of CD27- switched B-cells in MM compared with controls [8.1% (1.3-96.4) vs. 5.9% (1.4-13.7), P<0.01]. In contrast to PB, plasmablasts in BM were increased in MM compared with MGUS [2.6% (0.1-18.0) vs. 1.2% (0.4-31.6), P<0.05]. As expected, higher % of PC was found in MM mostly with abnormal phenotype CD19- compared with both MGUS and controls (P<0.005).

Summary / Conclusion: Polychromatic flow cytometry is capable to identify minimum of 10 B-cell and PC subpopulations. Reduction of CD19+ cells in MM should be mediated by an increased number of abnormal PCs. The enhancement of isotype-switched memory CD27+ and isotype-switched CD27- B-cell subpopulations in MM patients suggests that some of these could be a potential source of myeloma-initiating cells. Analysis on DNA level will be done.

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B1486

SPHINGOSINE KINASE 2 IS OVEREXPRESSED IN PATIENTS AFFECTED BY MULTIPLE MYELOMA

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Background: Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid generated upon phosphorylation of Sphingosine by two kinases, Sphingosine Kinase 1(SHPK1) and 2 (SHPK2). S1P was first characterized for its role in controlling apoptosis-survival balance, however in the last years many data showed that this molecule has a peculiar role in embryology and maturation of vascular compartment, trafficking and function of cell of the immune system, inflammation, cell transformation. SHPK1 is found in the cytosol while SHPK2 is found mainly in the nucleus. SHPK2 seems to be able to affect gene transcription. It is now accepted that these two kinases may have different role in cell functions. Recent findings suggest that these two kinases may play an important role in the growth and behaviour of cancer cells. There are no data concerning the expression of SHPK1 and SHPK2 in MM.

Aims: The aims of this work was to assess the expression of SHPK1 and SHPK2 in bone marrow of patients affected by Multiple Myeloma (MM).

Methods: We analyzed the bone marrow of fourteen individuals who underwent bone marrow biopsy for diagnostic purposes. Six individuals had MM at the first diagnosis, one individual had MM and was at that moment in remission after chemotherapy, two individuals were found to be normal, five individuals had other hematological malignancies (ALL, AML, CML, CLL). cDNA was prepared acording to standard protocols and quantitative PCR was performed using validated specific primer for SHPK1 and SHPK2 using a 7500 Thermal Cycler. The results were analyzed using a dedicated software.

Results: We found that SHPK2 was overexpressed in marrow of MM patients (P<0.001) when compared to normal marrow and to other hematological malignancies (P<0.05). Intriguingly, the patients with MM in remission showed levels of SHPK2 comparable to normal marrow. Same levels of SHPK1 were measured in normal individuals and in patients regardless of the disease.

Summary / Conclusion: These preliminary data indicate that SHPK2 is highly expressed in bone marrow of MM patients. SHPK1 was found to be expressed always at similar levels in normal individuals, individuals with MM or other hematological malignancies. Therefore, it is likely that the role of SHPK2 in MM is not, or not only, linked to phosphorylation of sphingosine. It is possible to speculate that here SPHK2 has a function due to its capacity to control gene transcription. More data are needed to confirm these results and to understand the meaning of the expression of this kinase in MM. However, these data may lead to new therapeutical approaches in MM through specific targeting of SPHK2.

B1487

ESTABLISHMENT OF A BORTEZOMIB-RESISTANT CELL LINE KM3/BTZ

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Background: Multiple myeloma is an incurable plasma cell malignant disease.As the first generation proteasome inhibitor,bortezomib already displays significant single-agent anti-tumor activity in the treatment of MM.However,drug-resistance has been a major challenge.So,we will establish the cell line resistant in bortezomib,and investigate its biological characteristics and mechanisms of drug-resistant in MM preliminarily.

Aims: To establish a bortezomib-resistant cell line KM3/BTZ of human multiple myeloma (MM) and investigate its biological characteristics and mechanisms of drug-resistant in MM preliminarily.

Methods: The KM3 cell line was exposured to bortezomib at increasing doses to obtain a stable bortezomib-resistant KM3/BTZ in vitro. The growth curve was drawn and the doubling time was counted. MTT assay was used to evaluate the sensitivity of drug resistance. The expression of MDR-1 mRNA was determined by RT-PCR in KM3 and KM3/BTZ cell lines.

Results: The KM3/BTZ cell line resistant to bortezomib was established successfully with the resistance index of 19.7 (KM3/BTZ to KM3). Compared with the parent cells, KM3/BTZ exhibited a significant longer doubling time (p□0.05) .The expression of MDR-1 mRNA was not observed in either resistant or parent cells.

Summary / Conclusion: We have successfully established bortezomib resistance cell line KM3/BTZ and its drug-resistant character is stable. The KM3/BTZ cell line might serve as an ideal model to explore the drug-resistance mechanisms and to reverse the drug resistance.

B1488

IMPACT OF P53 PERCENTAGE IN SURVIVAL OF PATIENTS DIAGNOSED OF MULTIPLE MYELOMA

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Background: The natural history of multiple myeloma (MM) is highly variable despite the homogenous morphologic appereance of malignant plasma cells. Clinical outcome for MM appears to be defined by genetic events. Genetically high risk disease is defined as any of the following, t(4;14), t(14;16), t(14;20) deletion 17p by FISH, or deletion chromosome 13 or hypodiploidy by conventional metaphase cyogenetics. Chromosome 17p13 deletion seems to be an independent prognostic factor and also a progression marker.

Aims: To retrospectively investigate the prognostic impact of p53 on the outcome of newly diagnosed MM.

Methods: We analyzed the impact of p53 mutation in a series of patients who were diagnosed of MM since the year 2010. A series of 12 patients was analyzed: 6 male and 6 female. Median age at diagnosis was 63.16. We performed a comprehensive stratification of the disease, using the Durie Salmon staging system and the ISS. Fluorescence in situ hybridization (FISH) analysis identified genetic abnormalities such as +14 in 1 patient, t(4;14) in2, rearranged IgH in3, while the rest of our patients (6) only showed chromosome 17p13 deletion. 5 out of the 12 patients were included in the VISTA study, the other 7 patients were treated using a combination of Bortezomib-Dexamethasone. We show a table with our patients prognostic factors in Figure 1.

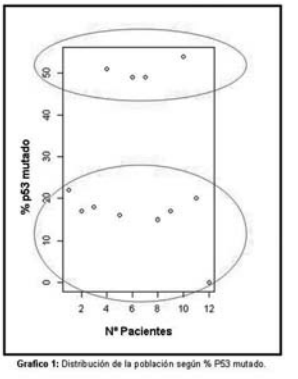

Grafico 1: Distribución de la población según % P53 mutado.

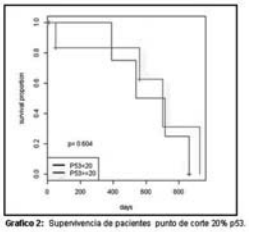

Grafico 2: Supervivencia de pacientes punto de corte 20% p53.

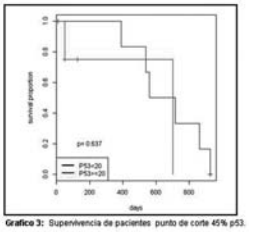

Grafico 3: Supervivencia de pacientes punto de corte 45% p53.

Results: Two patients died in the first two years because of MM progression, one of them within the first month presenting central nervous system (CNS) involvement. 1 patient died after 4 m. because of refractory disease and 3 patients died the first month after diagnosed; 2 of septic shock and the other because of rapid progression. 4 patients are receiving maintenance chemotherapy, 1 has relapsed, and 2 patients are not taking any medication, being closely followed on their disease evolution. The presence of mutated p53 in the medical literature in MM has been related with CNS involvement and bad response to conventional treatment, including autologous hematopoietic stem cell transplantation. It is well known that mutated p53 worsens the prognosis in patients diagnosed with MM, though there is still a controversy about wether the percentage of p53 mutated cells is important in these patients progress. Since we were looking for a cut-off point to predict a worse prognosis, we divided the sample (graph 1) into two different groups, establishing a cut-off point of 20 % and 45 % to evaluate the survival. Even though our sample has a small number of patients and we could not find any statistically significant difference using both cut-off point of 20 % (graph 2) and 45 % (graph 3); out of the 5 deceased patients, two presented a mutated p53 under 20 %; 3 of them over 45%. One out of the 3 patients that presented a p53 over 45 %, showed another cytogenetic alteration that is related to bad prognosis t(4;14); leaving the rest only to present p53 mutation as the isolated prognostic marker. Though our results have not been statistically significant, in our group of patients, those who showed a p53 mutation over 45 % turned out to have decreased OS because of a rapid disease progression. We could consider the high percentage, over 45, of mutated p53 as an independent bad prognostic factor.

Summary / Conclusion: Even though we present here a very small sample, the poor prognosis of the chromosome 17p13 deletion is obvious, with the following survival graphics.Since there are no studies about the importance of percentage of mutated p53 cells in MM and its relation with other prognostic factors, we suggest that studies with bigger samples are needed.

B1489

CYTOTOXIC EFFECTS OF APIGENIN ON MULTIPLE MYELOMA CELLS

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Background: Apigenin, a common plant flavonoid, has been reported to suppress the proliferation of prostate, breast cancer and various types of leukemic cells. It also inhibits cell cycle progression and induces apoptosis. Apigenin triggers its anti-carcinogenic effects by inducing modulation of several kinase activities, inhibition of NF-KB and proteosomal activation and proteosomal degradation of Her2/neu proteins. Multiple myeloma is the second most common hematologic cancer. However, an approach that can cure multiple myeloma completely via current approach is not achieved yet.

Aims: The purpose of this study is to determine the cytotoxic and apoptotic effects of Apigenin on U266 Multiple Myeloma cells.

Methods: Time-dependent antiproliferative effect of Apigenin was determined by MTT cell proliferation test. Apoptotic effects of Apigenin was determined by changes in activity of caspase-3, loss of mitochondrial membrane potential and localization of phosphatidylserine on plasma membrane (Anexin-V by flow cytometry).

Results: The results demonstrated that Apigenin has time and dose-dependent anti-proliferative effect on U266 Multiple Myeloma cells. IC50 values obtained for Apigenin at 48- and 72 hours on U266 Multiple Myeloma cells were 36.6- and 31.4 μM, respectively. It was shown that caspase-3 enzyme activity was increased 164- and 186% in response to 30-, and 40 μM Apigenin, respectively. There were 21.5-, 36.7-, and 63% of the cells in apoptosis in response to 20-, 30-, and 40 μM Apigenin, respectively. As compared to control group, there were 69- and 162% increases in loss of mitochondrial membrane potential of U266 cells exposed to 20-, and 30 μM Apigenin.

Summary / Conclusion: In conclusion, all these results showed for the first time that Apigenin has anti-proliferative and apoptotic effects on multiple myeloma cells in a dose-dependent manner.

B1490

TREND ANALYSIS OF P53 AND AMPLIFICATION OF CYCLIN D1 GENE IN MULTIPLE MYELOMA AFTER BORTEZOMIB AND DOUBLE AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: In patients with multiple myeloma (MM) risk stratification by chromosomal abnormalities may enable a more rational selection of therapeuthic approaches. Patients presenting with del (17p) are known to have a short event-free survival (EFS) and overall survival (OS); patients with t(11;14) and amplification of cyclin D1 have relatively favorable prognosis; instead patients with up-regulation of cyclin D1 independently of a t(11;14) have indefinite prognosis. Some data suggest that Bortezomib-based treatment is able to improve outcome in patients with MM, including patients with high-risk chromosomal aberrations, while double autologous transplantation can not overcome the poor prognosis of high-risk cytogenetics.

Aims: We evaluated the correlation between bone marrow FISH analysis changing (from the diagnosis to the consolidation therapy) and response to therapy after induction therapy, first and second autotransplantation.

Methods: A female patient 58 years old was diagnosed with Multiple Myeloma IgG/K, Durie&Salomon stadium III, ISS 2. She underwent to 7 cycles of VTD (Bortezomib, Thalidomide, Dexametasone) and then she received double autologous stem cell transplantation. Fish analysis was performed on bone marrow sample of patient at diagnosis, after 7 cycles of Bortezomib-Thalidomide-dexametasone, after I and II autologous transplantation. We performed bone marrow biopsy and laboratory test at the same time of FISH analysis. Patient underwent also to MR whole body and CT scan of bone to assess the response to therapy.

Results: Our patient achieved a poor partial response after 7 VTD cycles; after the first auto-transplantation she achieved a good partial response which was confirmed after the second one. FISH analysis at diagnosis showed the presence of p53 in 4.6% of analysed nucleus and the amplification of D1 cyclin in 20.7% of nucleus; after induction therapy (7 VTD) p53 was in 15.5% of nucleus and amplification of D1 cyclin in 3.3%. After the first autotransplantation p53 was in 11.4% of nucleus and amplification of D1 cyclin in 2.5%; after the second transplantation percentage of p53 and D1 cyclin were respectively 4.4% and 0. FISH analysis at diagnosis showed the presence of p53 in 4.6% of analysed nucleus and the amplification of D1 cyclin in 20.7% of nucleus; after induction therapy (7 VTD) p53 was in 15.5% of nucleus and amplification of D1 cyclin in 3.3%. After the first autotransplantation p53 was in 11.4% of nucleus and amplification of D1 cyclin in 2.5%; after the second transplantation percentage of p53 and D1 cyclin were respectively 4.4% and 0.

Summary / Conclusion: Our data show a better response to therapy at the same time of reduction of p53 after double autologous stem cell transplantation and not after bortezomib-based induction therapy. We suggest that double autotranplantation after high-dose chemotherapy in high-risk multiple myeloma can still have a role for treatment of high-risk multiple myeloma in the present. The prognostic role of amplification of D1 cyclin indipendently of a t(11;14) in not so clear nowday. Our patient is now receiving consolidation therapy with Bortezomib-Thalidomide-Dexametasone and she will undergo to a new FISH analysis at the end of the second course of consolidation.

B1491

PROGNOSTIC VALUE OF 13Q14 DELETION AND IGH REARRANGEMENT BY INTERPHASE FISH IN EGYPTIAN MULTIPLE MYELOMA PATIENTS

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Background: Multiple myeloma (MM) is a clonal bone marrow disease characterized by the neoplastic transformation of differentiated B cells with the accumulation of malignant plasma cells in the bone marrow compartment. In Egypt, its extrapolated prevalence is 17,630/76,117,421 with annual rate around4,085/76,117,421. Chromosomal aberrations in MM are typically complex and represent a hallmark of the disease, the most frequent structural aberrations are 13q14 deletion detected in 33-50% of the cases and 14q32 IgH rearrangement detected in 60-70% of the cases with t(11;14) (q13;q32) being the most frequently detected 14q32 translocation seen in 15% of these patients.

Aims: To detect both 13q14 deletion and 14q32 IgH rearrangement by I-FISH on 100 newly diagnosed Egyptian myeloma patients and their relation to patients outcome and prognosis .

Methods: Patients were subjected to complete history, clinical examination and laboratory. Also we tested the expression of 13q14 deletion by LSI D13S319 probe assay and 14q32 IgH rearrangement by IGH dual color FISH break apart assay on bone marrow samples collected from the patients at diagnosis (before starting therapy). Staging and follow up of patients were carried out to detect the outcome of the disease.

Results: we found that 65% of the examined myeloma patients showed genetic aberrations (13q14 deletion, 14q32 IgH rearrangement or both) by I-FISH. Of which, 40% were positive only for 13q14 deletion, 20% were positive only for 14q32 IgH rearrangement and only 5% was positive for both aberrations.
13q14 deletion was associated with more patients being resistant to chemotherapy or dying indicating its poor impact on patients' prognosis, whereas 14q32 IgH rearrangement was associated with more patients in remission indicating its good impact on patients' prognosis. Comparing myeloma patients who went into remission to those who died or were resistant to chemotherapy revealed highly statistical significance (p value ≤ 0.001) as regards to their serum Calcium, Albumin, B2 microglobulin and bone marrow clonal plasma cell levels with p values (0.0009,0.0001,0.0001 and 0.0001 respectively). Moreover, there was a statistical significance (p value ≤ 0.05) found in these patients as regards to their total protein and serum M-component with p values (0.0288 and 0.0182 respectively).

Summary / Conclusion: 13q14 deletion detected by LSI D13S319 FISH probe in myeloma patients was seen in 40% of the studied cases and was associated with patients being resistant to chemotherapy or dying. Whereas, 14q32 IgH rearrangement detected by IGH dual color break apart FISH assay in these patients was seen in 20% of the studied cases and was associated with patients in remission.

Myeloma and other monoclonal gammopathies - Clinical

B1492

ROLE OF 18 F-FDG PET/CT IN THE ASSESSMENT OF PATIENTS WITH SMOLDERING MULTIPLE MYELOMA

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Background: Smoldering multiple myeloma (SMM) is a proliferative plasma-cell disorder without organ damages (defined as presence of hypercalcemia and/or renal insufficiency and/or anemia, and/or lytic bone lesions, CRAB), and without need to treat. Whole body X-ray (WBXR) and magnetic resonance imaging (MRI) are the cornerstone imaging procedures in the initial staging.
Aims: The aim of the study was to determine the role of ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET) / computed tomography (CT) for the assessment of bone disease in patients with SMM without lytic lesions in standard imaging.
Methods: The study population consisted of 46 patients (M/F 23/23) with diagnosis of SMM referred at our institution from February 2008 to December 2012; the median age was 67.5 years (range 39-88). Diagnosis of SMM was established according to the criteria of the International Myeloma Working Group. In particular, SMM was characterized by the presence of a monoclonal component (MC) of >3 g/dL and/or bone marrow plasma cell infiltration of >10%, and/or the presence of Bence-Jones proteinuria >1 g/dL in the absence of end-organ damage (CRAB). The MC components were as follows: IgGκ in 26 patients, IgGλ in7, IgAk in7, IgAλ in4, light chain λ in 1 and biclonal in 1. All patients performed PET/CT at our institution at the time of diagnosis together with WBXR and MRI of the spine and pelvis; the exam was repeated in 13 patients during follow-up (median 11.5 months, range 5-31). No patient received chemotherapy. Three authors (TZ,ER,VDS), blinded to each other, classified like "advantageous" or "not-advantageous" the use of PET/CT imaging for the overall management of the patients. Criteria of judgement were the influence of PET/CT in the identification of evolution to SMM to a symptomatic form to be treated, the confirmation of no evolution, the identification of other neoplastic diseases.
Results: In 12 cases PET/CT detected focal increased FDG uptake at diagnosis and upstaged the disease (26%). All the abnormal areas corresponded to bone lesions later identified by MRI (n=10) or TC (n=2) (100% sensitivity); in 1 patient bone lesion was also confirmed by a a novel detailed X-ray. In 3 patients the bone lesions were found outside the spine or the pelvis. In 2 additional cases PET/TC detected synchronous solid neoplasms (renal and prostatic). In 4 of the remaining 32 cases (25%) without FDG uptake at diagnosis, PET/CT allowed to classify as osteoporosis some doubtful lesions at standard imaging. In three cases PET/CT was considered falsely negative for bone lesions (91% specificity). In 1 cuneiform vertebra bone biopsy showed plasmocytoma; in 2 cases bone lesions detectable at MRI developed during the follow-up in X-rays areas suspected at diagnosis but PET/CT-negative. During the follow-up, PET/CT revealed evolution of disease in 4 of 13 patients (30.7%). PET/CT was considered advantageous in 14 SMM patients (30.4%), because of upstaging of MM (n=12) or detection of another neoplasm (n=2); notably, in all the upstaged patients the bone lesions were not identified by first WBXR and were outside the areas explored by first MRI in 3 patients (6.5% of the overall cohort and 25% of the PET/CT-positive patients).
Summary / Conclusion: PET/CT can upstage SMM at diagnosis or during the follow-up in at least one-quarter of cases and should be included in the imaging work-up of the patients with SMM.

B1493

INVESTIGATION OF THE RELATIONSHIP BETWEEN TREATMENT EFFECTS ON PROGRESSION FREE SURVIVAL AND OVERALL SURVIVAL IN CLINICAL TRIALS OF MULTIPLE MYELOMA BASED ON PUBLISHED LITERATURE

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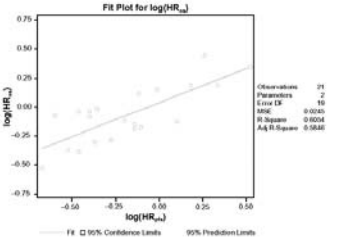
Background: A primary goal in the development of new oncology therapies is to improve overall survival (OS). In recent years, advances in the treatment of multiple myeloma (MM) have led to improvements in OS. However, with improved treatment options and multiple rounds of therapy it is becoming an increasing challenge to demonstrate OS improvements in the context of clinical trials. Thus, progression free survival (PFS) is seen as an important surrogate marker for OS. However, a clear relationship between PFS and OS in patients with MM has yet to be established.
Aims: Use published literature to test the hypothesis that treatment effects on PFS are positively associated with treatment effects on OS in MM.
Methods: An initial systematic literature review of MM identified 13 studies published between 2002 and March 2012 that reported hazard ratios (HRs) for the effects of treatment on PFS and separately on OS. The literature review and subsequent analysis were updated with HRs identified for 4 additional studies. Patients in 16 of the studies received first-line treatment (13 investigating induction and 3 maintenance), only 1 study was in the relapsed/refractory setting. The Pearson correlation coefficient (r) was used to estimate the relationship between the reported HRs (HR_{PFS} and HR_{OS}), and the log-transformed HRs ($\log(HR_{PFS})$ and $\log(HR_{OS})$). The log transformation was conducted to minimize the non-linearity between the two HR distributions and allow for values between positive and negative infinity. R^2 values were estimated from linear regression models of the HR and the log(HR) relationships, with 95% Confidence Intervals around the R^2 values estimated using Olkin and Finn's approximation of the standard error and Student's T distribution. Sensitivity analyses included the estimation of two weighted regression models to investigate the robustness of the R^2 estimates. One method weighted studies by sample size, and the second weighting method utilized the geometric mean of the variance of the HRs to account for the multi-directional error around the two HRs. The subgroup of studies investigating treatment effects for induction in newly treated MM patients was also investigated.
Results: Pearson r estimates for the treatment effects on PFS and OS showed a positive relationship between HR_{PFS} and HR_{OS} r=0.799(CI: 0.55-0.91; P<0.0001), and between $\log(HR_{PFS})$ and $\log(HR_{OS})$ r=0.778 (CI:0.51-0.90; P=0.0014). Linear regression models reflected that the treatment effect on PFS was significantly associated with the treatment effect on OS, and produced R^2 values of 0.638 (CI: 0.41-0.86) for the regression of HR_{OS} on HR_{PFS} and R^2 values of 0.605 (CI: 0.37-84) for the regression of $\log(HR_{OS})$ on $\log(HR_{PFS})$. (Figure 1) The sensitivity analysis results for the Pearson r estimates were generally consistent, ranging from 0.65 to 0.82. The weighted regression models produced extremes for the R^2 estimates ranging from 0.42 to 0.67; the lower R^2 values resulting from the weighting by geometric mean. The subgroup analysis of studies investigating treatment for induction produced similar values to that of the base case analysis, r=0.804 for $\log(HR_{OS})$ on $\log(HR_{PFS})$ and the R^2=0.65.



Summary / Conclusion: This analysis based on published data indicates that the treatment effects on PFS are positively associated with treatment effects on OS among patients with MM. Although this empirical evidence has shown a positive association, further investigation involving patient-level data are necessary to confirm PFS as a valid surrogate for OS.

B1494

C-MYC GENE RELATED ABNORMALITY AND ADDITIONAL CHROMOSOME 8 WERE UNIQUE CYTOGENETIC ABERRATION IN MULTIPLE MYELOMA

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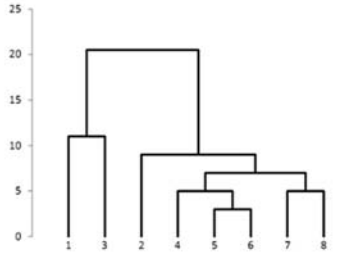
Background: Multiple myeloma (MM) is characterized by a significant heterogeneity and has multi-step genetic abnormalities. Worse prognostic factors (WPF) have been reported including non-hyperdiploidy (N-HD), and IgH/FGFR3, del17p, IgH/MAF1 by fluorescence *in-situ* hybridization (FISH) analysis.IgH/c-Myc has been widely accepted WPF in lymphoma, although the abnormality has not been fully studied in MM. Additional chromosome 8 was reported in WPF in 1990's. However, it has not been evaluated in the novel agent era.
Aims: To evaluate that c-Myc gene related abnormalities and additional chromosome 8 affected the prognosis.
Methods: Patients who were newly diagnosed with MM (NDMM) at the National Hospital Organization Disaster Medical Center (DMC) between June 2008 and June 2011 were enrolled in this study. As an initial therapy, cases received bortezomib and dexamethasone (BD) therapy was only evaluated. The Institutional Review Board of DMC approved this study, which was performed in accordance with the Declaration of Helsinki. Diagnostic criteria of MM in this study were according to the International Myeloma Working Group criteria. We

performed FISH analysis to detect major cytogenetic abnormalities including del13q, IgH/Bcl1, IgH/FGFR3, del 17p, and IgH/c-Myc. To clarify genetic aberrations, we additionally performed c-Myc translocation. All probe sets were from Abbott Laboratories. In the present study, WPF was defined as having at least one of following present; N-HD, IgH/FGFR3, and del 17p.Furthermore, to clarify the similarity of each aberrations, we performed hierarchical clustering analysis using Ward's method.

Results: Forty cases received BD therapy. The number of best response was as follows; sCR, CR, VGPR, PR, SD, and PD were1, 0, 12, 11, 15, and1, respectively. Fifteen cases had WPF. The number of IgH/c-Myc, c-Myc translocation (not with IgH), c-Myc gene amplification (GA), and additional chromosome 8 were2,1,5, and2, respectively. At the median follow-up duration was 399 days, fourteen cases were defined as relapsed or refractory MM (RRMM). Among 14 RRMM, 7 cases were available with cytogenetic results both at diagnosis and relapse. As additional aberrations, 3 of 7 cases gained WPF. In total, 8 of 12 cases had WPF in RRMM. On the other hand, 4 of 7 cases obtained additional chromosome8, and 2 of 7 cases gained c-Myc GA. In total, the number of IgH/c-Myc, c-Myc GA, and additional chromosome 8 were2,7, and 6 cases, and these aberrations were recognized in12 out of 14 RRMM. Hierarchical clustering analysis revealed del13q and IgH/FGFR3 made a cluster. Not only IgH/Bcl1, del17p, but also IgH/c-Myc and c-Myc gene translocation (not with IgH) were similar elements, and c-Myc GA and additional chromosome 8 genetic aberration were occurring closely together (See Image).



1; del13q, 2; IgH/Bcl1, 3; IgH/FGFR3, 4; del17p, 5; IgH/c-Myc, 6; c-Myc translocation (not with IgH), 7; c-Myc gene amplification, 8; additional chromosome 8

Summary / Conclusion: To the best of our knowledge, this is the first report focusing on c-Myc gene related abnormalities and additional chromosome 8 in NDMM and RRMM in the era of novel agents, and these aberrations might contribute to worse prognosis. In addition, hierarchical clustering analysis revealed that c-Myc gene related aberration and additional chromosome 8 were similar clusters in cytogenetic aberration.

B1495

PLASMA CELL PROLIFERATION FRACTION AND THE RISK OF PROGRESSION FROM SMOLDERING MULTIPLE MYELOMA (SMM) TO MULTIPLE MYELOMA (MM)

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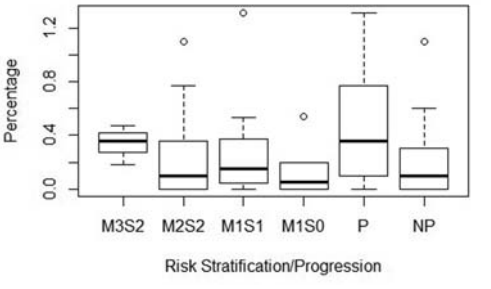
#contributed equally
**contributed equally*

Background: Increased plasma cell proliferation has been reported to be an adverse prognostic factor in patients with newly diagnosed MM; however, most previous studies were performed on marrow aspirates (which may not be fully representative of the marrow cellular content) and using assays that are either complex or lack plasma cell specificity. Furthermore, there is limited information on the significance of plasma cell proliferation in the setting of myeloma precursor disease. Using a double immunostaining technique with antibodies against Ki-67 and CD138, applied directly on bone marrow biopsy sections, we prospectively assessed the impact of plasma cell proliferation on patients with SMM who were low, intermediate and high risk for progression to MM based on previously published Mayo Clinic and PETHEMA group criteria.

Aims: To prospectively evaluate the significance of plasma cell proliferation in the progression of SMM to MM.

Methods: As per study protocol, 50 patients with SMM underwent a bone marrow biopsy and were classified according to risk of progression: 3 were high risk by both criteria (M3S2), 22 intermediate risk per Mayo and high risk per PETHEMA (M2S2), 15 intermediate risk by both systems (M1S1) and 10 were intermediate risk per Mayo and low risk per PETHEMA (M1S0). Bone marrow histological sections from each patient were doubled stained with antibodies against CD138/Syndecan (Cell Marque) and Ki-67 (Ventana) using an automated stainer (Ventana Benchmark ULTRA) and the plasma cell proliferation fraction was calculated in each case as the percentage of plasma cells (identified by the CD138 antibody) expressing Ki-67 (1056±139 plasma cells counted/slide). Descriptive statistics were used to analyze the basic features of the data in the study. The Wilcoxon signed ranked test was used to test for differences in the median percentage values of Ki67 staining between groups and in the percentage of positive Ki-67/CD138 stained cells among progressors and non-progressors to MM. An alpha error of <0.05 was considered to be significant.

Results: The median plasma cell proliferation fraction was 0.36% (0.18% - 0.47%) for M3S2, 0.1% (0% - 1.1%) for M2S2, 0.15% (0% - 1.3%) for M1S1 and 0.05% (0% - 0.54%) for M1S0 patients. Thus, the M3S2 patients had the highest median Ki-67 percentage, however this did not reach statistical significance when compared to groups considered at lower risk of progression by Mayo and PETHEMA prediction models, respectively. After a median follow up of 24 months (range 17-30 months), 9 of the 50 patients had progressed to MM (median time to progression 8 months): 3/3 (100%) in the M3S2 group, 4/22 (18%) in the M2S2, 2/15 (14%) in the M1S1 and 0/10 (0%) in the M1S0 group. The median percentage of positive Ki-67 stained plasma cells in patients that progressed (n=9) was 0.36% (0% - 1.3%) versus 0.1% (0% - 1.1%) in non progressors (P>0.05) (Figure 1. Percentage of Ki-67 positive plasma cells by risk stratification and progression to MM. P=progressors, NP=nonprogressors).



Summary / Conclusion: Based on prospectively evaluated SMM patients, we found the percentage of Ki-67 positive plasma cells to be less than 1% in 48/50 (96%) of patients. The plasma cell proliferation fraction using a double immunostaining technique on core biopsies demonstrated a trend towards progression to MM that did not reach statistical significance. This may be, at least, partly explained by the low numbers in the M3S2 (n=3) and progressor (n=9) subgroups. Per study protocol, continued comparison between groups is ongoing.

B1496

DOMESTIC SUBCUTANEOUS SELF-INJECTION OF BORTEZOMIB IN MULTIPLE MYELOMA

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Background: Bortezomib-based therapies are suggested as standards of care in management of patients with newly diagnosed and relapsed multiple myeloma. The recommended dose and schedule of Bortezomib is 1.3 mg/mq administred on days1,4,8, and 11 of 21-day cycles, a regimen active and well tolerated. Subcutaneous administration of bortezomib could be a good option for patients, particularly those with poor venous access.

Aims: We know that home intravenous administration of bortezomib is feasible to adequately informed patients, because we have recently demonstrated that a solution of bortezomib powder in normal saline stored at 4°C remains stable for nearly one month.

Since 2009, in our unit all patients requiring bortezomib for the treatment of multiple myeloma perform intravenous injection of the drug at home, after having been supplied with the exact dose in saline solution, in ready-to-use plastic syringes, appropriately prepared
under hood in sterile conditions. This procedure reduces the time spent by patients in hospital, improving convenience for patients and physicians. How-

ever, in some patients venous access may be difficult or sometimes unfeasible. As the drug is not histotoxic, subcutaneous administration is feasible, and this possibility is particularly attractive in domestic settings.
Methods: We have verified in 97 patients the safety and efficacy of subcutaneous injections
of bortezomib at the same dose as i.v. administrations (1mg/sm, days1,4,8,11), but dissolved in smaller saline volume (max. 1ml), in association with oral dexamethasone 20mg/dd. 1-2,4-5, 8-9, 11-12, in patients affected by multiple myeloma, with poor venous access.
In particular, the efficacy, evaluated as reduction of the monoclonal component during the i.v. period vs. the s.c. period was performed in a subgroup of 12 patients.
Results: Results indicated an equivalence between the two administration modalities, according to other larger controlled studies.
Based on these reports, 97 patients, requiring Bortezomib as part of anti Myeloma regimens, have been systematically treated by subcutaneous injections, performed at home.
No significant side effects have been reported so far. In particular, in 12 of them we have evaluated an equivalence between the subcutaneous and intravenous administration, according to other controlled studies.
Summary / Conclusion: The possibility to perform an antineoplastic regimen at home is particularly well accepted by all patients affected by multiple myeloma, with an achievement of a very good quality of life.

B1497

BORTEZOMIB (BTZ) RETREATMENT FOR RELAPSED/REFRACTORY (REL/REF) MULTIPLE MYELOMA (MM) IN REAL-WORLD MEDICAL PRACTICE: FINAL DATA FROM THE INTERNATIONAL, NON-INTERVENTIONAL, OBSERVATIONAL EVOBS STUDY

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Background: MM typically follows a relapsing course, with many patients (pts) requiring multiple lines of therapy. Previous studies have shown that Btz retreatment is effective in rel/ref MM; however, the efficacy and safety of Btz for rel/ref MM in everyday medical practice has rarely been studied prospectively, including its use in retreatment.
Aims: eVOBS is an observational study designed to prospectively assess Btz-based therapies for pts with MM in everydaypractice. Final data in pts receiving Btz retreatment for relapsing disease during eVOBS are reported.
Methods: Consenting adults scheduled to receive Btz for MM were enrolled at clinics in Belgium, Brazil, Canada, France, Greece, Spain, Sweden, and Turkey. Pts were also enrolled in Russia but are not included here; these data were presented at EHA 2012 (Abdulkadyrov et al, abs #1507). Therapies received in the 1 yr prior to pts' initial Btz were recorded retrospectively. Prospective observational data were collected over 3 yrs after Btz initiation, including data on subsequent Btz retreatments for relapsing disease received during this period. Data were electronically captured at baseline, after every Btz cycle, and every 12 wks after discontinuation or progression. Responses were investigator-assessed per EBMT, SWOG, M-protein reduction, or other (not specified) criteria. Adverse events (AEs) were graded per NCI-CTCAE v3.0.
Results: 96 of 873 pts enrolled to eVOBS received Btz as first retreatment for progressive disease during the prospective observation period. Median follow-up from start of retreatment was 11 mos. Median age was 62 yrs (range 34–80), 53% were male, 19%/58% had stage II/III disease (Durie-Salmon or ISS), and median number of prior therapies at initial Btz and retreatment was 2 and3, respectively. 30%/46% of pts initiated retreatment as 3rd/4th line therapy. 41% of pts initiated Btz retreatment in combination with dexamethasone, 21% had Btz monotherapy, 20% Btz in combination with lenalidomide, and 6% Btz with thalidomide, compared to 53%, 20%, 2%, and 7%, respectively, at initial Btz. Pts received a median of 6 Btz cycles at initial treatment and 4 at retreatment. 70% of pts started Btz at 1.3 mg/m^2 and 22% at ≤1.0 mg/m^2, vs 89% and 8% at initial Btz. ≥PR rate was 86% at initial Btz, including 49% CR/nCR (vs 69% and 37% for all 873 eVOBS pts). ≥PR rate at retreatment was 46%, including 15% CR/nCR. Median time to ≥PR was 68 days at initial Btz and 59 days at retreatment. ≥PR rate at retreatment was 68% vs 39% vs 20% in pts who had CR/nCR vs PR vs ≤MR at initial Btz (P=0.0022), and was 70% vs 48% vs 39% in pts with 1–2 vs 3 vs ≥4 prior therapies at retreatment (P=0.055). Median TTP/PFS with initial Btz was 11.4 mos (95% CI: 9.9–12.6) and median PFS from start of retreatment was 6.9 mos (95% CI: 4.6–8.2); median OS from start of retreatment was 17.6 mos (95% CI: 14.4–23.5). PFS and OS after retreatment were numerically longer in pts achieving CR/nCR vs PR vs ≤MR. Number of prior therapies at retreatment did not affect PFS; however, there was a trend towards longer OS in pts with fewer prior therapies. Discontinuations under Btz retreatment were mostly due to disease progression (34%), treatment completion (19%), and AEs (19%), compared to 16%, 34%, and 21% at initial Btz. PN during Btz retreatment occurred in 77% of pts, including 21% grade 3/4.
Summary / Conclusion: These data suggest that, in everyday medical practice, Btz retreatment is effective and tolerable in rel/ref MM, with approximately half of pts who responded to initial Btz achieving ≥PR at retreatment.

B1498

ELIMINATING THE NEED FOR 24-HOUR URINE STUDIES IN PHASE I MYELOMA PATIENTS

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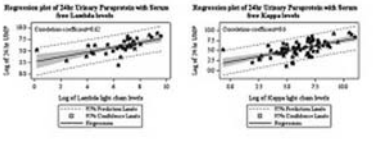
Background: Standard evaluation in myeloma patients for paraprotein estimation includes testing serum protein electrophoresis (SPEP), serum immunofixation (SIFE), serum free light chain (SFLC) ratio, and 24-hour urine collection for urine protein electrophoresis (UPEP) and urine immunofixation (UIFE). 24-hour urine collection is a cumbersome process. Prior studies evaluating SFLC ratio and SIFE/SPEP as substitute for 24-hour urine estimation of paraprotein have major limitations. Few studies included patients with a wide spectrum of plasma cell dyscrasias and others have included both newly diagnosed and relapsed/refractory myeloma patients making the conclusions uninterpretable. There are no prior studies examining the question in a homogenous patient population.
Aims: The aims of current study are to evaluate if SFLC ratio and SIFE/SPEP can substitute for 24-hour urine collection in a homogenous population of advanced myeloma patients enrolling in phase I trials, where a shift in secretion from intact immunoglobulin to SFLC, described as 'free light chain escape' is often observed.
Methods: We analyzed 116 patients that underwent pre-trial staging with SPEP, SIFE, SFLC ratios, UPEP and UIFE prior to enrollment in phase I trials between August 2006 and December 2012. Initially, we evaluated the sensitivity of testing SFLC ratios, SPEP and SIFE compared to UIFE. Subsequently, we assessed the linear relationship between SFLC dichotomized by abnormal ratio (<0.26 or >1.65) vs. 24-hr paraproteinuria to assess correlation. SAS 9.3 version was used for analysis
Results: The sensitivities of SPEP, SIFE and abnormal SFLC ratio assays were 77%, 95% and 96%, respectively, compared to sensitivity of 100% by UIFE. The sensitivity of SFLC+SPEP or SFLC+SIFE increased to 100% enabling detection of paraprotein in all patients (Table 1). In our second analysis, the quantification of SFLC correlated with 24-hr urinary paraprotein estimation. With every log increase in lambda and kappa light chains, 24-hr urinary paraprotein log increased by 0.52 times (P<0.0001) and 0.53 times (P<0.0001); correlation coefficients 0.62 and 0.6, respectively (Figure 1).

Table 1. Sensitivities of Urine IFE, SIFE, SPEP, SFLC ratio in phase I myeloma patients

Laboratory test	No. (% abnormal)
UIFE	116 (100)
SPEP	89 (76.73)
SIFE	110 (94.83)
Abnormal SFLC ratio	111 (95.69)
Serum IFE + abnormal SFLC ratio	116 (100)
Serum PEP + abnormal SFLC Ratio	116(100)

Figure 1. Correlation between 24-hr paraproteinuria and abnormal SFLC



Summary / Conclusion: In advanced myeloma patients in phase I trials, SFLC ratio in combination with SPEP or SIFE detected monoclonal paraprotein in 100% of patients. In addition, there was a linear correlation of SFLC with 24-hr urinary paraprotein estimation, questioning the need for the burdensome 24-hour collection. Further studies to monitor SFLC ratios and 24-hour urine continuously over a period of time may completely eliminate this cumbersome test in advanced myeloma patients.

B1499

CONTRIBUTION OF DIFFUSION AND ADC SEQUENCES IN WHOLE BODY MRI FOR MYELOMA STAGING

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Background: Treatment of Multiple myeloma (MM) is based on CRAB criteria including bone lesions. Prognostic criteria include Durie Salmon stage (DSS) based on radiography to detect bone lesions. With the advance in imaging, a new scoring was described: Durie Salmon stage PLUS (DSS+) where bone lesions are revealed by MRI or 18F-FDG PET/CT. This score includes 4 stages: IA (single focal lesion (FL) = plasmacytoma), IB (less than 5 FL or mild diffuse disease), II (5 to 20 FL or moderate diffuse disease) and III (more than 20 FL or severe diffuse disease).

Some studies have demonstrated the superiority of PET/CT compared to conventional MRI of the spine and pelvis (SP-MRI).The whole-body MRI with diffusion weighted imaging (WB-MRI) and ADC (apparent diffusion coefficient) mapping (DWI-MRI) seems to modify these arguments allowing to explore extraspinal bone and extraosseous lesions. The use of diffusion may improve sensibility and ADC mapping could avoid false positive. WB-MRI has a very low cost, high acceptability and lasts about 30 minutes with no radiation

Aims: To highlight the additive value of WB-MRI with diffusion and ADC compared to simple SP-MRI in one hand, and compared to WB-MRI with diffusion without ADC, in a second hand

Methods: 22 myeloma patients (median age 67 years, sex-ratio 0.6) were staged on DSS at diagnosis. Then, patients were classified before treatment by DSS+ with SP-MRI, DSS+ with WB-MRI and diffusion , DSS+ with WB-MRI combining diffusion and ADC. We used a 1.5T MRI system (Area, Siemens) to explore the spine with sagittal T1-weighted and coronal STIR sequences. The same coronal STIR sequence covered also the whole body from vertex to the middle thigh associated with axial diffusion-weighted images (two b-values, 50-800 s/mm2 with an axial ADC mapping).

Bone marrow infiltration (BI) pattern and the number of FL were assessed with the different imaging techniques by two radiologists. The staging levels of four systems (DSS, DSS+ SP-MRI, DSS+ WB-MRI with diffusion and DSS+ WB-MRI with both diffusion and ADC) were compared

Results: Among the 22 patients, 5 were classified in DSS I, 3 in DSS II and 14 in DSS III.

There were differences of concordance between DSS and DDS+ depending on the MRI protocol. Patients would have been staged differently according to DSS or DSS+ using: - SP-MRI: 14 of 22 patients (63%) (2 patients DSS I upstaged in DSS+ III, 11 patients down staged (Fleiss Weighted Kappa (FWK) 0.16 (95IC -0.19;0.51)) - WB-MRI with diffusion: 8 patients (36%) (1 patient DSS I upstaged, 7 down staged) (FWK 0.62 (95IC 035, 0.89) - WB-MRI with diffusion and ADC: 6 patients (27%) (FWK 0.63 (95IC 0.32;0.94) 9 patients were staged differently with DSS+ by SP-MRI and WB-MRI with diffusion: 2 were down staged and 7 upstaged. DWI-MRI showed 50 more FL than SP-MRI, including 8 sequelae lesions identified by ADC. DWI-MRI identified 2 extra medullary lesions. BI was upstaged for 8 patients, and down staged for 3 patients with diffusion sequence and ADC. For one patient, WB-MRI allowed to perform preventive femoral nailing on a bone lesion with cortical rupture. Three patients wouldn't have been treated without the discovery of bone lesions on MRI.

Summary / Conclusion: Compared to SP-MRI, WB-MRI detects extraspine and extraosseous lesion. DWI-MRI increases the number of visible lesions and therefore the sensitivity of WB-MRI. ADC improves the specificity distinguishing non-active lesions. Concordance between DSS and DSS+ is poor, each system providing different information, but diffusion and ADC in WB-MRI improve this concordance compared to classical SP-MRI

B1500

SUBCUTANEOUS ADMINISTRATION OF BORTEZOMIB REDUCES ADVERSE EVENTS

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Background: Bortezomib-based therapies are suggested as standards for patients with newly diagnosed and relapsed multiple myeloma. Until September 2012 the approved dose of bortezomib was 1.3 mg/m2 administered as a bolus intravenous (IV). This regimen is active and well tolerated in most patients. However, many of them usually develop adverse events (AE) short-medium term. As an alternative to the IV administration, subcutaneous (SC) administration of bortezomib is a good choice. It has been shown that SC bortezomib provides equal efficacy to IV bortezomib.

Aims: In this study, we compared adverse events of subcutaneous versus intravenous bortezomib.

Methods: We analyzed 78 patients diagnosed and treated in our Hospital from May 2007 to December 2012. None of them had received bortezomib previously. Bortezomib was administered as a part of a chemotherapy regimen: MPV, VD, VTD and VCD. 42 men and 36 women were included, with a median age at diagnosis of 69 years (43-82 years), 76 multiple myelomas (BJ 10, IgA 21, IgG 44) and 2 plasmacytomas. The route of administration was SC in 45 patients (57.7%) and IV in 33 patients (42.3%). 368 IV injections of bortezomib and 702 SC injections were administered.

Results: 31 (93.9%) of 33 patients, who received IV bortezomib, developed AE whereas 35 (77.8%) of 45 patients, who received SC bortezomib, developed AE. In patients with IV bortezomib, 33.3% of the AE appeared in the first cycle, while in patients with SC bortezomib only appeared 21.3%. The most common AE of IV bortezomib were gastrointestinal (GI) (30.5%), nervous system (NS) (29.1%), infectious (14.2%) inespecific (19.9%), hematological (5%) and cardiac (1.4%) disorders while in patients who received SC bortezomib were GI (23.4%), NS (22.7%), infectious (18.4%), inespecific (24.8%), hematologic (5.6%), skin (3.5%) and cardiac (1.4%) disorders (Table 1). If we compare the incidence of AE by route of administration with respect to total number of injections administered: 43 GI disorders in IV bortezomib vs 33 in SC ($p < 0.01$), 9 bacterial infections in IV vs 7 in SC (P=0.06) and 41 NS disorders in IV vs 32 in SC ($p < 0.01$) were reported. Peripheral neuropathy was reported in 23,4% of patients who received IV bortezomib (grade 1-2: 66.7%, grade 3-4: 33.3%) and in 17% of patients who received SC bortezomib (grade 1-2 : 70.8%, grade 3-4: 29.2%). The AE presented by treatment scheme of VD, MPV, VTD, VCD were respectively: cardiac (1%,2%,0%,0%), infectious (21.8%,13.7%, 7.7%, 13.3%); hematologic (2%,8.5%,0%,0%), inespecific (18.8%,22%,46.2%,26.7%), GI (26.7%,27.5%,15.4%,33.3%), NS (29.7%,23.5%,23.1,26.7%). Skin disorders in MPV and VTD 2.6% vs 7.7% respectively. 3-4 grades AE in those receiving IV bortezomib were NS (44%), hematologic (28%), infectious (24%) and GI (4%), while those receiving SC bortezomib grades 3-4 AE were NS (33.3%), infectious (33.4%), hematologic (28.6%) and GI (4.8%). If we compare the incidence of ≥ 3 AE per patient according to the route of administration, we only found statistical significance in peripheral neuropathy: 11 events in IV vs 7 events in SC (P=0.03). Patients who received IV bortezomib had to temporarily stop treatment in 24 cases (17%) and decrease the dose in 26 cases (18.5%), whereas patients receiving SC bortezomib treatment was discontinued in 8 cases (5.7%) and the dose was decreased in 7 cases (5%).

Summary / Conclusion: Patients receiving SC bortezomib have fewer AE and interruptions of treatment, getting safer administration of bortezomib and improving treatment adherence.

	IV Bortezomib		SC Bortezomib	
Adverse effects	**All grades**	**Grade≥3**	**All grades**	**Grade≥3**
Gastrointestinal	43 (30.5%)	1 (4%)	33 (23.4%)	1 (4.8%)
Cardiac	2 (1.4%)		2 (1.4%)	
Nervous system	41 (29.1%)	11 (44%)	32 (22.7%)	7 (33.3%)
Peripheral sensory neuropathy	33 (23.4%)	11 (44%)	24 (17%)	7 (33.3%)
Bacterial infections	9 (6.4%)	5 (24%)	9 (6.4%)	7 (33.4%)
Viral infection	11 (7.8%)		17 (12%)	2 (9.6%)
Herpes zoster	3 (2.1%)		1 (0.7%)	
Skin	-		5 (3.4%)	
Inespecific(*)	28 (19.9%)		35 (24.8%)	
Haematology	7 (5%)	7 (28%)	8 (5.6%)	6 (28.6%)
Thrombocytopenia	4 (2.8%)	4 (16%)	4 (2.8%)	2 (9.6%)
Neutropenia	3 (2.2%)	2 (12%)	4 (2.8%)	4 (19%)

(*) headache, asthenia, pyrexia, insomnia, edema, nervousness

B1501

COMORBIDITY IMPACT SURVIVAL IN MULTIPLE MYELOMA. A SINGLE INSTITUTION STUDY DEALING WITH VALIDATION OF ACE-27 SCORE

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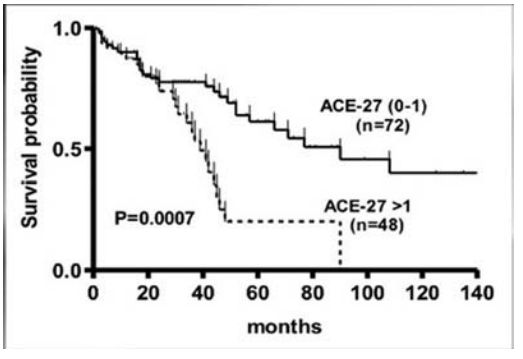
Background: Comorbidities increase in prevalence with age, but their impact on survival in patients with multiple myeloma (MM) is not known. The purpose of this study was to examine the impact of comorbidities on survival in multiple myeloma.

Aims: All patients (pts) with MM diagnosed and treated at the Department of Hematology-Oncology of Catanzaro General Hospital (Italy) from 2000 to 2010 were included in an observational database. The study was evaluated and approved by the local ethical committee.

Methods: Comorbidities were retrospectively graded as None (score=0), Mild (score=1), Moderate (score=2) or Severe (score=3) using the ACE-27 comorbidity index [Piccirillo et al. J Reg Mgmt 1999]. The primary endpoint was overall survival (OS), calculated from the date of diagnosis and censored at the time of last follow-up.

Results: 120 patients were identified in the database. Median age of patients was 69 years (range 41-86 years); 64 were male, 56 were female. Based on the ACE-27 comorbidity index, 24 pts (20%) had no comorbid medical conditions (score=0), 49 (40.8%) had mild comorbidities (score=1), 27 (22.5%) had moderate comorbidities (score=2), 20 (16.6%) had severe comorbidities (score=3). After a median follow-up time of 39 months (range, 2-160 months)53 patients died while median OS was 52 months. Survival curves by comorbidity category are presented in figure 1. As shown, median survival of patients with ACE-27 comorbidity index 0-1 was 90 months therefore significantly longer than median survival of patients with ACE-27 comorbidity index >1 (39 months)(HR=0.421;95% CI, 0.175-0.625; P=0.0007). As expected patients with ACE-27 comorbidity index 0-1were significantly younger than patients with ACE-27 comorbidity index >1 (67 yrs *vs* 70 yrs; P=0.04) whereas no association could be found between the degree of comorbidity and international staging system (ISS)(P=0.130). Finally, ACE-27 comorbidity index impacted therapeutic decisions. As matter of fact, patients with score index >1 less frequently underwent autologous bone transplantation (P=0.02).



Summary / Conclusion: The presence and severity of comorbidities confer a poorer prognosis to patients with MM. Further study is needed to determine to what extent comorbidities directly impact survival versus impacting therapeutic decision-making and tolerance of therapy.

B1502

EXPLORATION AND QUANTIFICATION OF THE DETERMINANTS OF BASELINE HEALTH-RELATED QUALITY OF LIFE (HRQOL) FOR PATIENTS IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: With the increased use of novel therapeutic agents and extended life expectancy of multiple myeloma (MM) patients, aspects of Health-Related Quality of Life (HRQoL) of these patients are gaining considerable importance. This is particularly so when considering the relapsed/refractory multiple myeloma (RRMM) setting.
Aims: To explore and quantify the baseline determinants of HRQoL in MM patients starting 2nd or 3rd line treatment.
Methods: A European, multicenter, observational study was conducted in RRMM patients starting 2nd or 3rd line treatment. Patients were asked to complete three EORTC questionnaires: 1) Quality-of-Life Core Questionnaire (QLQ-C30) including 15 domains (*Global Health Status/QoL, Physical, Role, Emotional, Cognitive and Social Functioning; Fatigue, Nausea and Vomiting, Pain, Dyspnea, Insomnia, Appetite Loss, Constipation, Diarrhea* and *Financial Difficulties*); 2) The EORTC QLQ-Multiple Myeloma (QLQ-MY20) including four domains (*Disease Symptoms, Side Effects of Treatment, Body Image* and *Future Perspective*); and 3) The EORTC QLQ-Chemotherapy-Induced Peripheral Neuropathy (QLQ-CIPN20) including three domains (*Sensory scale, Motor scale* and *Autonomic scale*). All EORTC questionnaires were adjusted according to robust international standards to be completed by physicians. Multivariate linear regressions were performed to explain the determinants of baseline HRQoL scores for MM patients starting 2nd or 3rd line treatment. All baseline EORTC scores were used as dependent variables; patients' socio-demographics, disease characteristics and treatment regimens were identified as explanatory variables via univariate testing.
Results: As of Dec 2012, 155 patients (mean age=69; 52.0% male) were enrolled in the study and included in this interim analysis, with an average time since diagnosis of 3 years. At baseline, 14.2% of patients were ECOG ≥2 in terms of their performance status. A total of 87.7% of patients started 2nd line treatment and 12.3% of patients started 3rd line treatment. A total of 33.5% of patients had been treated with prednisone and 40.0% of patients had been treated with bortezomib in their 1st line treatment. Among patients who started 3rd line treatment, all had been treated with prednisone and 52.6% had been treated with bortezomib. The majority of EORTC questionnaires were completed at baseline by physicians (n=152; 98.1%) and patients (n=151; 97.4%). Multivariate linear regression results indicate that some covariates appeared regularly as factors explaining MM patients' HRQoL (from the 7 functioning scores and Global Health Status/QoL), as well as patients' symptoms and side effects (from 17 symptom and side effect scores). Those most frequently showing statistically significant association with worse scores were: poorer baseline ECOG performance status (12 times), previous prednisone intake (10 times), presence of chronic heart failure (4 times), country (3 times) and cumulative dosage of prior bortezomib treatment (3 times) (Table 1).

Table 1: Statistically significant (p<0.05) covariates determining HRQoL per EORTC domain

EORTC questionnaires	Domains	Statistically significant covariates*				
		ECOG performance	Prednisone	Chronic heart failure	Cumulative bortezomib dosage**	Country
QLQ-C30	Global Health Status/QOL	X				X
	Physical Functioning	X	X			
	Role Functioning	X	X			
	Emotional Functioning					
	Cognitive Functioning					
	Social Functioning	X	X			
	Fatigue	X	X			
	Nausea and Vomiting		X			
	Pain	X				X
	Dyspnea		X			
	Insomnia					
	Appetite loss					
	Constipation		X			
	Diarrhea					
	Financial Difficulties	X				
QLQ-MY20	Disease Symptoms	X		X		
	Side Effects of Treatment	X	X			
	Body Image	X				
	Future Perspective					X
QLQ-CIPN20	Sensory scale		X		X	
	Motor scale	X	X	X	X	
	Motor scale: using the pedals	X		X		
	Autonomic scale			X		
	Autonomic scale: getting or maintain an erection				X	

* listed if reported at least three times
**In mg/m²

Summary / Conclusion: At the initiation of 2nd or 3rd line treatment, MM patients previously treated with prednisone showed worse scores than other patients on many domains of QLQ questionnaires. Similarly, a poorer ECOG performance status was associated with worse scores in the majority of QLQ domains. Among other factors found to be repeatedly associated with worse scores, the prominent ones were country, chronic heart failure and cumulative dosage of prior bortezomib treatment.

B1503

ALLOGENEIC STEM-CELL TRANSPLANTATION IN MULTIPLE MYELOMA IN REAL PRACTICE: LONG-TERM RESULTS FROM A SINGLE INSTITUTION.

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Background: Allogeneic stem-cell transplantation with myeloablative conditioning (MAC) in multiple myeloma (MM) is associated with a high transplant-relat-

ed mortality (TRM) with a 15% of long-term survivors. Allogeneic transplantation with reduced-intensity conditioning (alloRIC) results in a lower TRM with a higher relapse rate. When used in first line in a tandem transplant approach (auto/alloRIC), the incidence of acute graft-versus host disease (aGvHD) grade II-IV reported ranges between 20-43%, chronic GvHD between 50-75% and the TRM between 10-15%. The reported PFS was 25% beyond 7 years. Because of this high morbimortality and the higher rate of relapse, the role of allogeneic transplantation in MM remains controversial, especially as part of the frontlline therapy.

Aims: To analyze the results of allogeneic transplantation in patients with MM outside clinical trials at our institution over a period of 27 years.

Methods: : Between Feb 1986 and April 2009, 23 patients (17 M, 6 F, median age 41 –range 21-52 -) received a MAC from an HLA identical sibling donor. Disease status at the time of transplant was first response in 12 patients (53%) (3 CR, 9 PR), sensitive relapse in 3 (13%) (all PR) and refractory disease in 8 (35%). Conditioning regimen was heterogeneous (6 Cyclo/TBI, 2 Bu/cyclo, 3 BCNU/Mel/Cyclo/TBI, 4 Cyclo/Mel/TBI, 5 Mel/TBI, 2 Bu/Mel). GvHD prophylaxis consisted on cyclosporine/MTX (10), cyclosporine/PDN (8), cyclosporine (2) or other (3). Between April 2001 and Aug 2012, 31 patients (18 M, 13F, median age 48 –range 25-64) received an alloRIC: 25 of them (80%) from an identical sibling donor and 6 (20%) from an unrelated donor. Seven patients (13%) who did not achieve a CR after front-line autologous transplant received an alloRIC in a tandem strategy. 17 (31%) were in sensitive relapse (first relapse 14, second relapse 3). Seven patients (13%) had refractory disease at the time of alloRIC. Conditioning regimen consisted on Fluda/Mel (26 patients), Flu/TBI (3) and Fluda/Mel/bortezomib (2). GVHD prophylaxis consisted on cyclosporine/MTX (6) or cyclosporine/MMF (25). All patients in the alloRIC group had received a prior single autologous transplant.

Results: On an intention-to-treat analysis, the CR rate after MAC was 35%. The incidence of aGvHD grade II-IV and III-IV were 48% and 39%, respectively. The TRM at any time was 56%. The causes of death were GvHD in 7 patients, infection not related to GvHD in 5 patients and VOD in 1 patient. The relapse rate was 30%.There are 3 patients that remain in continued CR at 13, 23 and 27 years beyond transplantation. With alloRIC the CR rate was 45%. The incidence of aGvHD grade II-IV and III-IV were 55% and 22%, respectively. Nine patients develop chronic GvHD. The TRM at any time was 29% and the causes of death were GVHD in 7 patients and pulmonary hemorrhage and post-transplant lymphoma one patient each. Nine patients remain alive in continued CR from 5 months to 9 years of follow-up. After a median follow-up of 36.4 months, the median PFS was not significantly different between MAC and alloRIC and there was a trend towards a longer overall survival in the alloRIC group (4.6 vs 35 months, P=0.05).

Summary / Conclusion: Although a small fraction of patients with MM can be cured with MAC allogeneic transplantation, this procedure is associated with an extremely high TRM. Unfortunately, alloRIC was also associated with a high incidence of severe aGVHD resulting in a high TRM leading to a short PFS. New approaches aimed at decreasing the incidence of aGVHD are crucial.

B1504

BORTEZOMIB-BASED THERAPIES FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA IN REAL-WORLD MEDICAL PRACTICE: FINAL DATA FROM THE INTERNATIONAL, OBSERVATIONAL EVOBS STUDY

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Background: The efficacy of bortezomib-based therapies in patients with multiple myeloma (MM) has been demonstrated in several large, randomized controlled clinical trials; however, use of bortezomib for relapsed/refractory MM in real-world medical practice has rarely been studied prospectively.

Aims: eVOBS is a non-interventional, observational study designed to prospectively assess the efficacy and safety of bortezomib-based therapies for patients with MM in everyday practice. Final data are reported here.

Methods: Consenting adults scheduled to receive bortezomib for MM were enrolled between June 2006 and December 2010 at clinics in Belgium, Brazil, Canada, France, Greece, Spain, Sweden, and Turkey. Patients were also enrolled in Russia but are not included; these data were previously presented at EHA 2012 (Abdulkadyrov et al, abs #1507). Therapies received in the year prior to starting bortezomib were recorded retrospectively. Prospective observational data were collected over 3 years after bortezomib initiation. Data were electronically captured at baseline and after every bortezomib cycle. Upon discontinuation or progression, data on subsequent therapies, survival, and disease progression were collected every 12 weeks. Responses were investigator-assessed per EBMT, SWOG, M-protein reduction, or other (not specified) criteria. Adverse events (AEs) were graded per NCI-CTCAE v3.0.

Results: Overall, 873 pts were enrolled, with a median follow-up of 34.6 months; median age 65 years (range 27–88 years), 58% male, 28%/48% had stage II/III disease (Durie-Salmon or ISS), 25% had a creatinine clearance of <50 mL/min. Hypertension (47%) was the most frequent comorbidity. Median number of prior therapies received was 2. 41%/26% of patients initiated bortezomib in 2nd/3rd line therapy. In the year prior to starting bortezomib, 30% of patients had received thalidomide-based treatment, 13% melphalan-prednisone, and 10% had lenalidomide-based treatment. 30% of patients had no treatment in the year prior to starting bortezomib. 52% of patients initiated bortezomib with dexamethasone, and 17% received bortezomib monotherapy. 82% of patients started bortezomib at 1.3 mg/m^2. Median number of bortezomib cycles received was 5 (range 1–29). Discontinuations were mostly due to treatment completion (31%), AEs (25%), or disease progression (16%). 69% of patients had partial response or better (≥PR) as best response within the initiated line of therapy, including 37% complete response (CR)/near-CR. Median time to ≥PR was 56 days (95% CI: 49–63). Best response plateaued after 6 cycles. Median progression-free survival (PFS) and overall survival (OS) were 12.5 months (95% CI: 11.3–13.5) and 36.1 months (95% CI: 33.1–41.6), respectively. PFS and OS were significantly longer in pts with deeper response to bortezomib-based therapy, fewer prior therapies, better renal function, less advanced disease at first MM treatment, and age <65 years (P<0.001 for all parameters and endpoints). All-grade AE, grade ≥3 AE, and serious AE rates were 82%, 55%, and 45%, respectively. Common grade ≥3 AEs were thrombocytopenia (8%), anemia, and pneumonia (each 5%). 21% of patients had baseline peripheral neuropathy (PN) (14% grade1, 5% grade2, 2% grade 3/4). 51% of patients without baseline PN (n=689) had new-onset PN (10%/1% grade 3/4). The cumulative risk of developing PN plateaued after 6 cycles. In patients with vs without baseline PN, a lower starting dose of bortezomib (≤1 mg/m²) was more common (18% vs 11%, P=0.014). Median treatment duration was shorter in patients with grade ≥2 baseline PN vs no or grade 1 PN at baseline (4 cycles vs 5 cycles, P=0.04).

Summary / Conclusion: These data indicate that in everyday practice, bortezomib is active in the treatment of relapsed/refractory MM. AEs were consistent with the known safety profile for bortezomib.

B1505

ISOTYPE MATCHED IMMUNOGLOBULIN RECOVERY RATES IN IGG AND IGA MYELOMA PATIENTS AT MAXIMUM RESPONSE SUGGEST SUBTLE ISOTYPE SPECIFIC DIFFERENCES IN IMMUNE-RECONSTITUTION

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Background: The recent availability of immunoassays measuring Ig'κ and Ig'λ (HLC) isotypes for IgA and IgG immunoglobulins has allowed analysis of the impact of monoclonal plasma cell biology previously hidden from our view. These tests offer quantitative alternatives to immunofixation (IFE), and have been reported as an aid to monitoring IgA and IgG multiple myeloma (MM). Furthermore, the the Ig'κ / Ig'λ ratio (HLCr) has prognostic relevance at presentation and at maximum response. Intriguingly, the isotype matched immunoglobulin (uHLC) suppression (e.g. the level of IgGκ in an IgGλ monoclonal gammopathy patient) has also been shown to predict MGUS transformation to MM.

Aims: To assess the correlation between Ig'κ / Ig'λ ratios and IMWG response criteria in disparate group of MM patients and to analyse isotype matched immunoglobulin recoveries in different response categories.

Methods: Serial samples from 25 IgG (18 IgGκ; 7 IgGλ) and 21 IgA (14 IgAκ; 7 IgAλ) MM patients were analysed using commercially available immunoassays (The Binding Site, UK) to establish HLCr cutoffs for each isotype that classified patients as CR, VGPR, PR, SD and PD. These responses were validated in 131 IgG (90 IgGκ; 41 IgGλ) and 65 IgA (40 IgA κ; 25 IgA λ) MM patients. Responses were dichotomized into good response (VGPR and CR) v 0.81=near perfect agreement 0.81-0.61=substantial agreement,) in addition recovery of serum uHLC concentrations was calculated at maximum response (MR).

Results: Comparison of responses showed good agreement between HLCr and IMWG-assigned responses for both IgG and IgA MM patients at maximum response: IgG: good response v ≤PR (sensitivity: 0.93 (95% CI 0.85-0.98), specificity: 0.89 (95% CI 0.78-0.96); IgA: good response v ≤PR (sensitivity: 0.95 (95% CI 0.75-0.99), specificity: 0.89 (95% CI 0.76-0.96), and good overall agreement between individual assigned response criteria: IgG Weighted Kappa: 0.81 (95% CI 0.72-0.93); IgA Weighted Kappa: 0.86 (95% CI 0.79-0.95). HLCr ratios were abnormal in all MM presentation samples. Suppressed uHLC was noted in 98% IgGκ (88/90 (median 0.6g/L (range: 0.1-2.3g/L)) and

73% IgGλ (30/41 (median 0.8g/L, (0.1-4.0g/L)) MM patients. By contrast 75% IgAκ (30/40 (0.1g/L, (0.01-2.0g/L)) and 44% IgAλ (11/25 (0.3g/L, (0.01-1.22g/L)) MM patients had supressed uHLC. 20/20 (100%). IgG MM patients achieving a CR had normal uHLC serum concentrations (IgG kappa normal range: 2.19-10.70g/L; IgG lambda normal range: 0.90-6.74g/L) at maximum response, compared to 31/36 (86%) of patients achieving a VGPR and 21/74 (28%) of patients achieving ≤PR. However, there was a substantially lower incidence of uHLC recovery in IgA patients achieving CR (18/26; 69%), VGPR (10/15; 67%) or ≤PR (15/24; 63%).
Summary / Conclusion: There is good agreement between IMWG and HLCr assigned responses at maximum response in both IgA and IgG MM patients. IgG MM patients had a greater degree of uHLC suppression than IgA MM, which may be influenced by the concentration dependent, catalytic half-life of IgG and/or by differences in the biology of between IgG and IgA myelomas. Recovery of uHLC was high and correlated with response in IgG patients, but was lower and not associated with response in IgA MM. Further work is required to understand this phenomenon, particularly in the setting of immune-modulatory therapy.

B1506
DESCRIPTIVE STUDY CASES OF AMYLOIDOSIS: SUBANALYSIS OF CARDIAC INVOLVEMENT
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Background: Amyloidosis is a protein misfolding disorder in which soluble proteins aggregate as insoluble amyloid fibrils, that cause functional and structural organ damage. The most common form of systemic amyloidosis are light-chain amyloidosis, but it's the reactive AA amyloidosis the mostly detected in our area. Cardiac involvement may occur with or without clinical manifestations, and is considered as a major prognostic factor.
Aims: To review the cases of amiloidosis in our area, and obtain data on cardiac involvement
Methods: Retrospective case series, between January 1994 to March 2012, obtained from medical history of biopsy confirmed cases
Results: We identified 99 patients with mean age at diagnosis 70 years (range 25-84 years), 43 women and 56 men. All patients had biopsy-proven amyloidosis. There were 50 secondary amyloidosis, 41 light-chain amyloidosis, and 8 localized amyloidosis. Inflammatory diseases were presented in 9, blood diseases in 17, solid neoplasia in 5 and infectious diseases in 10. The main clinical manifestations were dysregulation of intestinal habit, heart failure, edemas and syncope. The affected systems were: kydney 61 (27 nephrotic syndrome), heart 43, digestive 31, autonomic nervous system 29, skin 8 and lung 1. Troponin I was elevated in 15. ECG was abnormal in 43 (atrial fibrillation or flutter 16, auricular-ventricular block and bundle-branch block auricular6, pseudoinfarct pattern8, low voltages8, repolarization changes5, and ventricular hypertrophy signs 1). Echocardiographic characteristics: 35 had left ventricular diastolic dysfunction, and 4 systolic dysfunction, ventricular hypertrophy 28, pericardial effusion 14, byrrefingency 16, auricular enlargement 16, valvulopathies8, and pulmonary hypertension 8. In AL amyloidosis 26 had monoclonal component, 18 IgG subtype, and 22 Lambda light chain. 13 had Bence-Jones proteinuria and 18 renal insufficiency. The preferred sites for biopsy were abdominal fat 45, rectum 14, kidney 17, skin8, endomiocardial biopsy2, bladder2, liver3, lung1, bone marrow7, tongue 1 and necropsy 4**.** The treatment was in 18 Prednisone and Melphalan, 4 Bortezomib-Dexametsone, 2 Lenalinomide, 1 polychemotherapy and 8 required hemodyalisis. 42 patients had more than 2-years overall survival
Summary / Conclusion: 1. On our environment amyloidosis is not exceptional, with an approximate incidence of 6 cases / year. 2. The most common type is A. secondary, unlike similar series, probably because of the absence of complete hematologic studies, essential in these patients. 3. Clinical involvement was the most common cardiac and renal failure. 4. The most common treatment for the AL, was prednisone plus melphalan-dexamethasone. 5. The 50% of total cardiac amyloidosis had involvement being a decisive factor in evolution, so it should perform echocardiography in all patients with suspected amyloidosis 6. The main features in the ECG were arrhythmias and right bundle branch block. Ultrasonography diastolic dysfunction was the most common finding with wall hypertrophy

B1507
THE IDEAL DOSE SETTING AND THE POSSIBILITY OF THERAPEUTIC DRUG MANAGEMENT OF LENALIDOMIDE ACCORDING TO PLASMA CONCENTRATION IN MULTIPLE MYELOMA
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Background: Lenalidomide, a novel therapeutic agent, has been found to be effective for myeloma. The initial lenalidomide dose can determined on the basis of renal function and peripheral blood cell counts, dose adjustments, particularly after drug administration, were necessary in some cases because of interindividual differences in pharmacokinetics.
Aims: We aimed to develop an equation for the predicted total area under the observed plasma concentration–time curve (AUC) of lenalidomide and to set the ideal lenalidomide dose in patients with multiple myelomas by using only 1 or 2 sampling points.
Methods: Twenty-one myeloma patients treated with lenalidomide were enrolled in this study after obtaining written informed consent. Plasma concentrations of lenalidomide from samples obtained just prior to and1,2,4,8, and 12 h after oral administration of lenalidomide were analyzed using high-performance liquid chromatography (HPLC). Pharmacokinetic analysis of lenalidomide was carried out with a standard non-compartmental method. The $AUC_{0\text{-}24}$ was calculated using the linear trapezoidal rule.
Results: The plasma concentrations of lenalidomide at 2 and 4 h (C_{2h} and C_{4h}, respectively) after its administration were highly correlated with the $AUC_{0–24}$ value for lenalidomide. The correlation were observed between the measured and predicted $AUC_{0\text{-}24}$ values for lenalidomide by using only the C_{2h} and C_{4h} sampling points in the equation (P<0.0001). Moreover, the results of receiver-operating characteristic curve (ROC) analysis of best sensitivity and specificity showed that the ideal $AUC_{0–24}$ of lenalidomide could be set below 940 ng· h/ml to avoid hematological toxicity.
Summary / Conclusion: The predicted $AUC_{0–24}$ value might be a new indicator for the management of lenalidomide therapy. In this study, the threshold predicted $AUC_{0–24}$ values suggested that 4 of 21 patients (19%) should have received a lower initial dose than that recommended by Dimopoulos, et al. It is possible to adjust the ideal dose by using the equation for $AUC_{0–24}$ with the C_{2h} and C_{4h} values in a test before lenalidomide therapy is actually initiated. Therapeutic drug management (TDM) of lenalidomide can be performed by establishing the minimum effective concentration and the minimum toxic concentration in a large prospective study in the future. These findings should provide economic benefits and facilitate personalized medicine in myeloma therapy with lenalidomide.

B1508
RENAL FAILURE IS REVERSIBLE AFTER THERAPY WITH BORTEZOMIB IN PATIENTS WITH MULTIPLE MYELOMA
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Background: The involvement of kidneys in patients with multiple myeloma (MM) is an adverse prognostic factor which influences the clinical course, therapeutic response and the survival of the patients. Conventional chemotherapy could not improve the outcome in this high risk group. After the introduction of Bortezomib in the therapeutical regimens, literature data report high rates of therapeutic response along with an improvement of the renal function. Nowadays Bortezomib containing regimens become a standard therapeutic approach in patients with MM and renal failure (RF).
Aims: To study the therapeutic response and the outcome of the RF after therapy with Bortezomib (Velcade) based regimens in patients with multiple myeloma MM.
Methods: For the period 2006-2011, 111 patients with MM, treated with Bortezomib – containing regimens were studied in two university clinics of hematology: University Hospital "Sv. Georgi", Plovdiv and Military Medical Academy, Sofia. In 33 (29.7%) RF was found. Mean age 57.8±9.1y M:F/ 1.1: 1. In the whole group patients in ISS-III – 42 (37.8%) are predominant, with IgGk variant 62 (55.9%). 7/33 were on chroniodyalisis. The therapeutic response, its duration and the median of survival (MS) were compared between the groups with and without RF. Statistical analyses were performed with SPSSv18.0.
Results: No significant differences between the two groups were found in sex and age distribution, levels of Hв and LDH. Patients with RF are predominantly in ISS - III (P<0.001, R=+0.659). In the group with RF, light-chain variants are significantly more frequent (P<0.001, r=0.292), the proportion of patients with level of β2M >3.5мсg/l is significantly higher (P<0.001, R=+0.614), so as patients with hypoalbuminemia <35g/l (P=0.014, r=-0.312) and advanced bone lesions (P=0.033, r=+0.413). Therapeutic response is achieved(CR+VGPR+PR) in 84 (76.4%) in the whole cohort and in 18 (75.0%) for the patients with RF (NS).Reversal to normal level of serum creatinin after therapy was registered in 15 (45.5%), reduction with > 50% was found in 12 (36.4%). Independence from hemodyalisis achieved 3/7 болни. ASCT was performed in 14 (17.9%) in the group without RF and in 6 (18.2%) in the group with RF (NS). Time to progression for the whole group was 14 mo; in the RF group - 11 mo, and 28 mo for the patients without RF (NS). MS in the RF group was 28 mo vs MS 58 mo for the patients without RF (P<0.005) and MS for the whole group - 51 mo.

Summary / Conclusion: The application of Bortezomib- based regimens in patients with MM results in similar outcomes in patents with standard risk and patients with RF. The high rate of therapeutic response (CR + VGPR) is combined with improved renal function in about 80%. Thus the first proteasome inhibitor is the only medicine by now which is applied without dose reduction and is therapy of choice in patients with MM and mild to moderate RF.

B1509

PROGNOSTIC VALUE OF IGM HEAVY IMMUNOGLOBULINS CHAIN ANALYSIS IN IGM MGUS AND WALDENSTRÖM MACROGLOBULINEMIA

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Background: Monoclonal IgM is the biomarker that characterize to Waldenstrom's macroglobulinemia (WM), a rare low grade B-cell lymphoma derived of the lymphoplasmacytic cell, but a serum IgM component also is present in MGUS. New determinations of heavy immunoglobulins chains have been developed as biomarkers to apply at clinical practice.

Aims: The aim of this study is present our experience in the use of free light chain assay (sFLC) and IgMk/IgMl ratios (HLCR) as biomarker at diagnostic in order to discriminate between MGUS and WM, and to evaluate their potential prognostic value during disease course.

Methods: 50 patients were examined following clinical protocol; serum samples were collected and kept frozen at -70ºC in the Biobank. Analysis of IgM was performed with the sFLC, (Freelite® test, the Binding Site, Birmingham, UK) and the HLCR, (Hevylite® immunoassay the Binding Site). Freelite® test is a nephelometric measurement of kappa and lambda light chains that circulate as light chain monomers or dimers and that are not bound to immunoglobulin heavy chain. Hevylite® immunoassay is based on specific polyclonal antibodies that recognize epitopes spanning the junction of the heavy and light chains of the individual immunoglobulin isotypes, it measures specifically IgMkappa and IgMlambda, separately. For ease of comparison IgM hevylite ratios were expressed as the involved monoclonal immunoglobulin/uninvolved polyclonal immunoglobulin (iHLC/uHLC).

Results: The study included a series of 29 WM, 21 IgM-MGUS. The median age was 67 years; M/F ratio 1.38. Median IgM HLCR was 381.8 in WM symptomatic, 75.84 in WM asymptomatic and 15.65 in IgM MGUS (P=0.001). Median IgM HLCR was higher in WM patients requiring treatment at diagnosis (370.7 v 43.897 P=0.026) and also it was higher at relapse/refractory (478.5 v 44.24 P=0.012). Median uHLC was higher in IgM-MGUS than WM patients to IgMk andIgMl: 0.39 g/L v 0.21 g/L, P=0.036; and 1.2 g/L v 0.32 g/L, P=0.019. Relapse/refractory patients had a mean uHLC lower than the patients who did not relapse (0.29 g/L v 0.52 g/L, P=0.04). Median sFLC level was 64 mg/L (10.88–993) in WM and 31.7 mg/L (6.08–141) in IgM MGUS (P=0.05). Median sFLC level was higher in WM patients requiring treatment at diagnosis than patients not requiring treatment (73.7 v 36.85 P=0.039). Median sFLC level was not significative in relapse/refractory patients (P=0.168) and it was not separate between WM asymptomatic and WM symptomatic (P=0.092).

There was a good correlation between IgM HLCR and sFLC ratio (r=0.3, P=0.044) but not with sFLC level; also IgM HLCR, sFLC level and sFLC ratio did not predict for overall survival (OS) and progression free survival (PFS) in our study. Mean estimated OS was 78.3 months (95% CI: 53.67-102.94) and PFS 69.7 months (95% CI: 47.28-92.13).

Summary / Conclusion: At diagnostic IgM HLCR and sFLC seems identifies patients WM / IgM MGUS and high levels of HLCR and sFLC were also seen in patients requiring treatment. IgM HLCR discriminates between WM symptomatic, WM smoldering and progressing patients. Uninvolved polyclonal immunoglobulin was significantly lower in Relapse/refractory patients, showing that these patients have a less robustimmune system. Further studies are needed to evaluate their value prognostic.

B1510

LENALIDOMIDE 25 MG + LOW-DOSE DEXAMETHASONE: EFFICACY & SAFETY IN CHINESE PATIENTS WITH RELAPSED/REFRACTORY MYELOMA

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Background: In China, patients (pts)with relapsed/refractory multiple myeloma (RRMM) have a clinical need for more effective treatments. In addition, multiple myeloma pts who cannot tolerate bortezomib and/or thalidomide (THAL) are left without effective treatment options. Previous studies performed in western countries have demonstrated that lenalidomide (LEN) is a more effective immunomodulatory drug than THAL, and is associated with less dose-limiting toxicity. In addition, LEN + low-dose dexamethasone (LoDEX) was associated with a response rate of 53% in heavily pretreated RRMM pts.[1] The MM-021 China Registration Trial assessed the safety, efficacy, and pharmacokinetic profile of LEN + LoDEX in Chinese pts with RRMM.

Aims: In this post-hoc analysis the starting dose, dose modifications, and duration of LEN treatment were assessed.

Methods: In this phase2, multicenter, open-label study,199 pts received LEN (25 mg/day on days 1–21) and LoDEX (40 mg on days1,8, 15, and 22; or 20 mg in pts aged > 75 yrs) in 28-day cycles until progression.LEN starting dose was adjusted per protocol according to creatinine clearance (CrCl): 25 mg/day for pts with none-to-mild renal insufficiency (RI) (CrCl ≥ 60 mL/min); 10 mg/day for moderate RI (CrCl ≥ 30 to < 60 mL/min); and 15 mg/every other day (eod) for pts with severe RI (CrCl < 30 mL/min). All pts provided informed consent.

Results: Pts had a median age of 59 yrs and 57% had received ≥ 4 prior therapies. Overall, 66% (n = 131) of pts had none-to-mild RI, 27% (n = 54) had moderate RI, and 7% (n = 14) had severe RI. Per protocol the majority of pts (n = 144; 72%) initiated LEN treatment according to the recommended starting dose of 25 mg/day; 45 (23%) pts started at 10 mg/day, and 10 (5%) pts at 15 mg/eod. The median number of LEN cycles administered was 9 (range 1–25). Dose reductions were required in 38 (19%) pts across all starting doses, with a median time to first dose reduction of 3.1 mos. The median average dose was 25 mg (range 6.3–25.0), indicating compliance with the planned treatment schedule. Overall clinical outcomes are shown in the Table.

Summary / Conclusion: Consistent with observations from other studies, LEN 25 mg was generally well tolerated in Chinese RRMM pts and provided clinical efficacy despite renal condition.

B1511

EFFICACY AND SAFETY OF PERCUTANEOUS VERTEBROPLASTY IN PATIENTS WITH SPINAL MYELOMA LESIONS.

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Background: About 80-90% of patients with multiple myeloma (MM) will develop skeletal related complications, including diffuse osteopenia, focal lytic lesions, pathological fractures, and bone pain, mainly located in vertebrae and skull. The therapeutic intervention for bone disease is based on analgesic medications, byphosphonates, radiation therapy and in some cases percutaneous vertebroplasty (PV) or balloon kyphoplasty. PV was described by Galibert and colleagues in 1987 as a minimally invasive procedure involving the injection of polymethylmetacrilate (PMM) associated to a radiopaque substance within a collapsed vertebral body via a percutaneous approach, under fluoroscopic computed tomography (CT) image guidance. PV seems to have a satisfactory efficacy in almost all patients (90-100%) with an improvement of pain. All studies reported small volume, local intradiscal or paravertebral cement leaks, usually being asymptomatic. Severe life-threatening complications are very uncommon (<1%) and in some reports have been related to a cement volume of greater than 4 mL.

Aims: To evaluate the efficacy and safety of percutaneous vertebroplasty (PV) in the treatment of spinal myeloma lesions (SML) refractory to analgesia, byphosphonates or radiation therapy.

Methods: Forty-two patients with SML, from 2 centers were eligible. Vertebral fracture or collapse was confirmed by computed tomography or magnetic resonance imaging. PV of more than one vertebra was performed if feasible. Pain response was evaluated by a qualitative scale at 24 h, one and six months after PV. Complications appearing during the follow of 30 days were considered secondary to PV .

Results: One hundred and ten PV were performed in 49 procedures. The number of vertebrae treated in each procedure was: 1 in 14 cases, 2 in 15 cases, 3 in 15 cases, 4 in 4 cases and 5 in 1 case, being the T12 the most frequent localization (16 cases). Cement leakage was observed in 46% of all patients. Six out of 42 patients (14%) experimented complications related to PV: two cases of psoas hematoma, one patient with pulmonary insufficiency, two patients suffered a pulmonary embolism (one died because of cement embolism) and one patient presented a subdural hematoma without neurological repercussion. Twenty-four hours after PV, 83% of patients referred a decrease in pain. The evaluation at 1 and 6 months later, showed an improvement of pain in 83% and 70%, respectively.

Summary / Conclusion: PV is effective for pain control in 70-80% of myeloma patients with SML at short and long-term. However, some patients may experience life-threatening effects. Further studies with more patients and more follow-up are needed to confirm the efficacy and the incidence of adverse effects.

B1512

"REAL WORLD" DATA FROM PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA WHO HAVE BEEN TREATED WITH LENALIDOMIDE AND DEXAMETHASONE, ACCORDING TO THE STANDARD CLINICAL PRACTICE

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Background: Lenalidomide is highly effective in the treatment of relapsed/refractory multiple myeloma (RRMM). However, there is limited published data about the efficacy and safety of lenalidomide treatment in a "Real World "(RW) patients setting outside clinical trials.

Aims: To assess the outcome and safety of lenalidomide treatment in a RW setting within the context of its approved indication through the analysis of retrospectively collected data from a registry in Greece.

Methods: This was an open label, retrospective, multicenter, non-interventional study on collecting and analyzing RW patient data in the treatment of RRMM in Greece, in accordance with the definitions of the International Society for Pharmacoeconomics and Outcomes Research (ISPOR). Patients signed an informed consent at the time of the data collection. The primary endpoints were: a) clinical improvement and complete response (CR) according to the EBMT criteria, b) dose modifications or treatment discontinuation. The secondary endpoints included a) time to first observed clinical improvement and CR, b) duration of treatment, c) duration of response (DOR), d) adverse events percentage requiring hospitalization, e) incidence of deep vein thrombosis (DVT), extramedullary relapse and improvement of humoral immunity.

Results: We collected data from followed 212 patient records (M/F: 120/91, mean age 68±9.8 years). Seventy-four patients (35.1%) received lenalidomide as 2nd line therapy and 137 (64.9%) as >2nd line. Sixty percent of patients received lenalidomide >24 months from diagnosis. The median number of cycles administered was 12 (range 1-57). Initial dose of lenalidomide was higher in patients <65 years compared to older patients (recommended dose of lenalidomide i.e. 25mg was administered in 84.5% of patients <65 vs 64.5% of elderly patients, *P*=0.02). Elderly patients received lenalidomide earlier in the course of the disease (*P*=0.04). Overall, objective response (≥partial response) was observed in 161 patients (77.4%); 42 patients (20.2%) achieved a CR. Median time to first observed clinical improvement and to best response was 2 and 5 months, respectively. Median time to CR for 2nd line or >2nd line was 4 and 11 months, respectively. Quality of response was independent of lines of therapies or previous treatment with thalidomide or bortezomib. Median DOR was 34.4 months. In patients who received lenalidomide until progression median DOR was not reached, whereas in patients who discontinued, it was 19 months (*P*<0.001). In the multivariate analysis, performance status and initial dose were predictive of DOR (*P*<0.05 for both parameters). Dose reductions were observed in 31% of patients (87% due to adverse events). Permanent treatment discontinuation occurred in 38.9% (adverse events: 26%, disease progression: 18.9%). Neutropenia was the most common adverse event leading to treatment discontinuation (6.6%). Median time to treatment discontinuation was 16.8 months. Performance status and initial dose of lenalidomide predicted for treatment discontinuation in the multivariate analysis (*P*<0.05 for both parameters). Adverse events occurred in 68.9% of patients (12.7% needed hospitalization). Myelosuppression was the most common adverse event (49.4%). Lenalidomide-related peripheral neuropathy was observed in 2.5% of patients. Extramedullary relapse occurred in 3.8% and DVT in 5.7% of patients. Improvement of humoral immunity between baseline and time to best response occurred in 50% of patients (P<0.001).

Summary / Conclusion: Our study on RW patient data confirms that lenalidomide is highly effective and safe in the treatment of RRMM, producing high objective responses and CR rates. Median duration of response was significantly longer in patients who did not discontinue therapy. Permanent treatment discontinuation was mainly due to adverse events. The latter finding addresses the need for suitable management of side-effects, according to guidelines, in order to avoid treatment discontinuation. Incidence of DVT and extramedullary relapse was low. Importantly, lenalidomide improved humoral immunity in 50% of patients.

B1513

BORTEZOMIB, NON-PEGYLATED LIPOSOMAL DOXORUBICIN, DEXAMETHASONE (PAD) REGIMEN AND AUTOLOGOUS STEM CELL TRANSPLANT IN NEWLY DIAGNOSED MULTIPLE MYELOMA: PRELIMINARY RESULTS OF A PILOT STUDY

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Background: Induction therapy followed by Autologous Stem Cell Transplant (ASCT) is considered the standard approach in newly diagnosed young patients with Multiple Myeloma (MM). It has been previously reported substantial activity in the front-line setting with Bortezomib, Doxorubicin and Dexamethasone (PAD) induction therapy, which was based on the scientific rationale of dual apoptotic signaling leading to in vitro synergy between bortezomib and doxorubicin (Ma et al, 2003) and additive activity with dexamethasone (Hideshima et al, 2001). In a phase II study, the combination of Bortezomib, Pegylated liposomal Doxorubicin and Dexamethasone was evaluated as induction before ASCT a high response rate was obtained: 58% of very good partial response (VGPR) including 13% of Complete Remission (CR). The treatment-related mortality was 3% and grade 3 to 4 adverse events (AEs) included thrombocytopenia (17%), neutropenia (10%), peripheral neuropathy (16%), and pneumonia (10%) (Palumbo A et al. 2010).

Aims: A phase IIb pilot open label trial in newly diagnosed MM patients (pts) was planned as Bortezomib, Non-pegylated Liposomal Doxorubicin and Dexamethasone induction therapy followed by consolidation with ASCT and maintenance with Bortezomib. Aims of our study were efficacy and safety in terms of ORR, toxicities and both overall and progression free survivals.

Methods: From January 2009 to February 2013, 29 pts (M/F: 15/14) with a median age of 63 years (range: 48–73) were enrolled in the study; twelve pts (41,4%) were more than 65 yrs. At diagnosis 55% of pts had Durie and Salmon staging II and 45% of them had the staging III. Patients showing ISS 1 were 7%, ISS 2 were 35% and ISS 3 were 58% of. Five pts had a renal impairment, extensive bone disease was documented in 19 cases and 3 patients showing extramedullary disease. Fourty-eight percent of pts had IgG, 21% IgA, 24% light chain and 7% non secretory MM. Unfavorable cytogenetic was recorded in 38% of cases. Planned treatment: Bortezomib$_{1,3}$ mg/mq iv d$_{1,4,8,11}$; Dexamethasone orally at the dose of 40 mg/d on days$_{1,4,8,11}$, in pts 65-70 yrs old and at the dose of 20 mg on d$_{1,4,8,11}$; Non-Pegylated Liposomal Doxorubicin 30 mg/mq on d 1 of a 28-day cycle up to 4 cycles. After PAD regimen pts underwent to high-dose cyclophosphamide (4 g/m2) with G-CSF support, peripheral stem cell harvest and ASCT (MEL 200 and 100 in pts over or above 65 years, respectively). After ASCT, all pts received maintenance with Bortezomib alone twice a month.

Results: Twenty-six pts were treated with PAD regimen and 22 of them underwent to ASCT. Majority of pts (93%) achieved more than a PR including 58% (15/26) of CR and 35% of VGPR (9/26 after 4 courses of PAD. Two pts (7%) achieved a PR. After ASCT all pts achieved at least VGPR including 86% (19/22) of CR. After a median follow-up of 22 months (range 3-50), PFS and OS are 91% and 100%, respectively. PAD regimen resulted well tolerated and WHO grade 1-3 AEs included neuropathy (19%), hematologic toxicities (42%), infections (9%), gastrointestinal toxicities (18%). No case of cardiac toxicity was observed

Summary / Conclusion: Sequential PAD, ASCT and Bortezomib as maintenance is an attractive regimen to maximize the efficacy of ASCT. PAD in frontline setting is a highly effective and well tolerated regimen. Our results from this promising pilot study need further testing in randomized phase III trials.

B1514

LIMITED SURVIVAL FOLLOWING SEQUENTIAL EXPOSURE TO CURRENTLY AVAILABLE NOVEL AGENTS IN MULTIPLE MYELOMA (MM): THE CASE FOR NEW THERAPEUTIC STRATEGIES

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Background: The treatment landscape of MM has significantly changed in the last decade, with a significant improvement in overall survival (OS) being demonstrated. Current treatment strategies involve sequential exposure to proteasome inhibitors (PI) and immunomodulatory drugs (IMiDs), athough the most effective sequencing of exposure has yet to be determined. Furthermore, the outcome for patients relapsing after exposure to the available novel agents remains unclear.

Aims: We have therefore examined the outcomes of patients who demonstrated progressive disease following exposure to IMiDs and Bortezomib in a "real-life" single centre setting, with the aim of describing progression-free and overall survival and identifying factors that may predict outcome.

Methods: Patients were considered eligible for this retrospective study if they have received sequentially Thalidomide-, Bortezomib- then Lenalidomide-

based combination therapy (LenCom) for MM. Case records were examined for key diagnostic details as well as depth and duration of response to PIs. Details of LenCom were recorded and T_0 was defined as the time point at which LenCom was discontinued, whether for disease progression or intolerance. Responses to therapy subsequent to T_0 and PFS/OS were defined for each patient.
Results: Between Jan'07-Sept'12, 56 patients were identified that met the inclusion criteria (28 Male and 28 Female). Median age at diagnosis was 59 yrs (range 33 to 87) with the distribution of ISS: 20% stage I, 28% stage II & 28% stage III (23% Unclassified). The median number of prior lines of therapy before LenCom was 3 (range 2-6). First line therapy was thalidomide-based in 64% with 36% having undergone HD melphalan/ASCT and 9% undergoing tandem ASCT/RIC AlloSCT. Second line therapy was bortezomib-based in 42% patients and all patients received novel agent-containing regimens in the following sequence: thalidomide/bortezomib/lenalidomide (with 53% patients receiving lenalidomide as 4th line or later). The median time from diagnosis to commencing LenCom was 52.5 months (mns) (4-146). At the last follow up, a median of 6.5 cycles of LenCom were administered to all patients and 7 cycles in those who had discontinued LenCom. 39 patients (70%) had reached T_0 (PD n=27, intolerance n=12). The median time from diagnosis and lenalidomide commencement were 64 mns (20-159) & 9 mns (0-32) respectively. Post-T_0, 23 patients received further therapy (thalidomide-based n=11, bortezomib-based n=3, clinical trial therapy n=4, HD Dex n=3, donor lymphocyte infusion n=2) and 7 patients received supportive care (unspecified n=9). 13 patients (57%) demonstrated PD as maximum response to first post-T_0 therapy. With a median follow-up of 4.3 mns (1-33), 28 patients have died (PD n=17, infection n=6, other n=5). The median OS from diagnosis and commencement of LenCom were 100 mns & 18.4 mns, respectively. The median PFS from commencement of LenCom was 16.2 mns. The median OS from T_0 was 6.8 mns, influenced by the beta2microglobulin at T_0 (< vs. ³ 3.0: 9 vs. 4.8 mns, P=0.027). The depth of response to LenCom affected the PFS (P<0.001) but not the post-T_0 OS (P=0.68).
Summary / Conclusion: This study demonstrates the poor outcomes for patients progressing from lenalidomide-based therapy after sequential exposure to all current available novel agents. This is an area of need for clinical development for both effective combination regimens and new biologically active agents if we are to truly extend the survivorship in MM.

B1516
ADDING CYCLOPHOSPHAMIDE TO LENALIDOMIDE-DEXAMETHASONE – A SAFE STATEGY TO IMPROVE RESPONSE IN RELAPSE/REFRACTORY MULTIPLE MYELOMA
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Background: Lenalidomide is an immunomodulator approved for relapsed/refractory Multiple Myeloma (rrMM). Cyclophosphamide is a safe alkylating agent with effect in MM. Combination strategies exploring synergistic effects aiming to achieve better responses are important in rrMM, with eventual impact on prolongation of these patients' survival.
Aims: We studied the efficacy and safety of adding cyclophosphamide to lenalidomide-dexamethasone (LenDex) in rrMM patients (pts) treated in two reference centers between May 2007 – Sep 2012, for at least 2 cycles.
Methods: We developed a multicentric, retrospective cohort study. Progression free survival (PFS) was evaluated from the start of treatment with LenDex.
Results: We reviewed 149 pts with rrMM treated with LenDex. Of these, 16 pts received intensification with cyclophosphamide per os in addition to LenDex (CRD) when they were clinically and/or biologically losing response. The analysis only included pts treated with more than 2 cycles of CRD: 50% males; median age at diagnosis 60 yo; median age at start of CRD 65 yo. The median number of previous lines was 2 (range: 1-6), and the main subtypes were IgG K 25%, IgA K 19% and light chain K 19%. 43% had high-risk cytogenetics; 31% extra-medular disease; 13% amyloidosis. Ten pts (63%) recieved lenalidomide 25 mg/d, four pts (25%) recieved 10 mg/d and two pts (12%) recieved 10 mg/d x 21 days (cycles of 28 days), 31% dexamethasone 20mg/week (range: 16-160mg/week) and cyclophosphamide was administrated at doses varying between 200 mg and 500 mg/week (median 300 mg/week). Overall, 7 pts (44%) improved their response again (2 pts (13%) to VGPR, 5 (31%) to PR) with impact in PFS when compared to patients who had SD/PD with LenDex and that were not intensified with CRD (median: 29 months vs 9 months); 4 pts (25%) achieved stable disease and 5 (31%) had progressive disease. The median overall survival, evaluated from start of CRD, was 15 months (median follow up 7 months; range: 2-38). Toxicity was mainly haematological (in 10 pts (63%), of grade III/IV in 8 pts). Three pts (19%) had infections. Thrombotic events were not found, prophylaxis was performed in all patients.
Summary / Conclusion: Adding cyclophosphamide to the regimen LenDex, in patients who are losing response, as a strategy to achieve a better biological and clinical response, seems to be a feasible, efficient and tolerable therapeutic option in patients with rrMM.

B1517
EVALUATION OF CD 45 EXPRESSION ON PATIENTS WITH MULTIPLE MYELOMA AND ITS EFFECT ON THE TIME TO PROGRESSION
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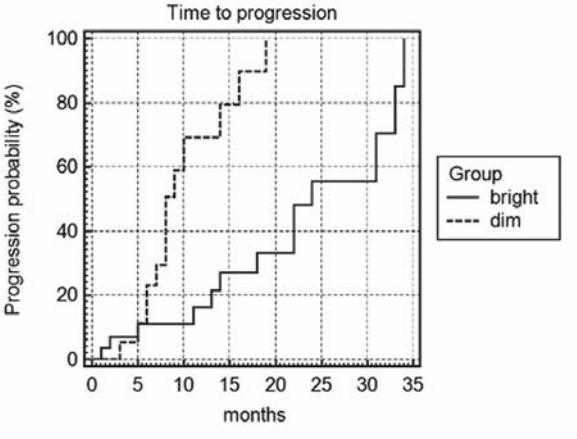
Background: Multiple myeloma is a disease characterized by the clonal proliferation of plasma cells with paraprotein production.Plasma cells usually have the specific immunophenotype CD38 +, 138 +, normal plasmocytes show a phenotype CD19+CD56-, while mature myeloma cells are CD19-CD56+. The surface antigen CD45 (protein tyrosin phosphatasis, receptor type C, PTPRC) normally expresses itself in young plasma cells. However, during the differenciation into mature plasma cells it goes down. The antigen-mediated signalization and activation of lymphocytes and plasma cells describe this character. CD45 negative cells are more resistant to apoptosis.According to the literature, there should be a low expression of the CD45 negative factor affecting the early progression of independent cytogenetic findings.
Aims: To compare the time to progression / relapse on patients with multiple myeloma with regard to CD 45 expression.
Methods: A retrospectiveanalysis. Evaluated were samples from 71 patients with myeloma from 5/2011 to 12/2012. Cytogenetic risk by Avet-Loiseau, 2005: negative = normal karyotype, changes on chromosome 13 and 17, t (4; 14) at (14, 16), positive = everything else and t(11;14).
Flowcytometric analysis: using 8-color flow cytometry which detected the presence of plasma cells to using cytometer FACS Canto II.
Identification of plasma cells with sequential gating using a combination of the antigens CD38, CD138, CD45 and the scatter properties of plasma cells, the expression of antigens on atypical, atypical and normal plasma cells, positive control by CD138 +, negative control by CD8- with adjustment of the sequential gates, for the detection of MRD limit sensitivity 0, 01% (10 e-4).
Results: According to theintensity of the flow cytometric expression of CD45 two groups were defined; low expression "dim" and higher expression "bright". Group parameters were compared to"dim"(n = 25) vs. "bright" (n = 46): median age 58 years (38-69) vs. 62 years (40-72) (P=0.30), male / female ratio 13/12 vs. 24/22 (P=1.0), unfavorable cytogenetics 16/21 (76%), r. 25/42 (60%) (P=0.26), not surveyed cytogenetics in 16% pts. vs 9% pts., the frequency of CR after the 1st line treatment 3/25 vs. 8/46 (P=0.73), the frequency of autologous SCT in 1st line 23/25 (92%) vs. 38/46 (83%) (P=0,48). Living patients 21/25 (84%) vs 43/46 (93%), median follow up by living patients was 11 months (1-40), vs. 20 months (1-98), median of progression 8 vs. 22 months (P=0,0003). The probability of progression at 24 months was 100% vs. 52% (P=0.0003).



Summary / Conclusion: The effect of CD45 expression in comparison to the risk of progression on patients with myeloma was evaluated. We compared a group with low and higher CD 45 expression. There was no difference in the demographic parameters, cytogenetics risk or 1st line treatment. The group with a low expression of CD45 had a significant lower TTP and therefore a higher probability of early progression. A research over a longer period and a larger group of patients is necessary.

B1518
FREQUENCY DYNAMICS AND PROGNOSTIC IMPACT OF WEIGHT LOSS AND BODY MASS INDEX IN MULTIPLE MYELOMA AT DIAGNOSIS
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Background: Several meta-analysis have shown that obesity [body mass index (BMI) ³ 30 Kg/m^2] is a risk factor for multiple myeloma (MM). MM behaves like many other cancers, and a general syndrome is frequently seen, including asthenia, anorexia and weight loss (WL). We hypothesized that the quantity and speed of WL, along with BMI, could have a prognostic impact in MM.
Aims: To assess the prognostic impact of WL and BMI at diagnosis in MM patients
Methods: We retrospectively analyzed clinical records of all symptomatic MM patients diagnosed from 2000 to 2013 in a population-based study, according to The Granada Cancer Registry. BMI was recorded at diagnosis. WL was measured in Kg during a given period of time in months (mo), as reported by patients. Patients with WL were divided in two groups: those with a well characterized phenomenon in time (mean WL in Kg/mo was calculated) and those that could no specify an amount of Kg or a period of time. We constructed overall survival (OS) curves according to Kaplan-Meier method. Log-rank test was used to compare OS curves. The statistical package used was SPSS v.20.
Results: 279 patients were diagnosed with MM during the thirteen years of the study, 128 males and 151 females (54,1%), median age 67 years (12-91). BMI was available in 173 patients: 54 (31,2 %) had obesity, 79 (45,7 %) overweight (BMI 25-29,9), 35 (20,2 %) normal range (BMI 18,5-24,9) and 5 (2,9 %) underweight (BMI < 18,5). WL data were available in 214 cases: 55 cases (25,7 %) had WL, 66 (30, 8 %) did no refer WL and was unknown in 93 (43,5 %). Median WL was2,5 Kg/mo (range: 0,83-7). Median OS was 18 mo (IC 95 %: 10,8-25,1), 40 mo (IC 95 %: 23-57) and 29 mo (IC 95 %: 18,6-39,4) for patients with, without or unknown WL, respectively (P=0,046). Median OS in patients with more o less intense WL (> o <2,5 Kg/mo) was similar. Likewise, OS between patients with measurable or unsettled WL was also similar. According to BMI categories, OS was 1 mo (IC 95 %: 0,1-1,8), 34 mo (IC 95 %: 11-57), 49 mo (IC 95 %: 0-102) and 61 mo (IC 95 %: 37,1-84,9) for underweight, obesity, overweight and normal weight, respectively (P<0,001).
Summary / Conclusion: At the moment of MM diagnosis, underweight is associated with a dreadful OS and obesity shows a tendency to shorter OS. At least one of every four patients shows WL. Even considering a potential bias in patient personal referral to WL, losing weight seems to be a negative prognostic factor that warrants a deeper study in bigger series; however, potential confounders of WL should be excluded.

B1519

PREDICTIVE VALUE OF LIGHT AND HEAVY CHAIN ANALYSIS IN MULTIPLE MYELOMA PATIENTS TREATED WITH BORTEZOMIB

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Background: The incorporation of new biomarkers useful to evaluate the immune production as free light chains (FLC) and Immunoglobulin heavy / light chain (HLC) ratios and the quantification of uninvolved isotype, could identify potentially risk factors to predict the response to therapies that included Bortezomib.
Aims: Present our experience in the use of new biomarkers to assess the response to treatment in patients diagnosed with Multiple myeloma.
Methods: We have analyzed these biomarkers in serum of 70 patients diagnosed with multiple myeloma (50 IgG, 20 IgA) treated with Bortezomib between 2004-2010. The analysis of FCL and HCL has been performed at diagnosis, after four cycles and at end of treatment with Bortezomib using FreeLyte and HeavyLite assay of The Binding Site Ltd. The evaluation of response has been defined according IMMWG criteria. The serum samples were collected and stored frozen at -70ºC in the Biobank at Miguel Servet University Hospital. The concentration of involved immunoglobulin has been compared with the M-component; the ratio of FCL and HCL and the degree of immunoparesis have been correlated with the intensity of response, the progression-free survival (PFS) and the overall survival (OS).
Results: A total of 28 females and 42 males with a mean age of 68 years has been included. In 11 patients an hematopoietic stem transplant were performed and were analyzed separately. Considering the original immunoglobulin isotype concentration, our results show that the follow-up of HCL ratio is more sensitive than monoclonal component to evaluate a strict response after Bortezomib therapy and the increase of HLC ratio of uninvolved isotype is associated with a longer PFS and OS.
Summary / Conclusion: Our results suggest that HLC ratio is a satisfactory marker of follow-up response and probably the uninvolved isotype concentration as marker of immunoparesis is correlated with the PFS and OS

B1520

LENALIDOMIDE/DEXAMETHASONE (RD) IN REFRACTORY/RELAPSED MULTIPLE MYELOMA: A RETROSPECTIVE MULTI-CENTER STUDY FROM "RETE EMATOLOGICA PUGLIESE" (REP).

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Background: Several data are available on the efficacy of the combination lenalidomide/dexamethasone in patients with relapsed Multiple Myeloma (MM).
Aims: We reported here a multi-center series of 290 patients with refractory/relapsed MM treated with lenalidomide/dexamethasone as salvage therapy.
Methods: Two hundred ninety patients (141 females and 155 males - median age 70 years [42-88]), were treated in 12 haematogical centers (REP). Median Hb value was 11 gr/dl (range 7-17,2), absolute neutrophil count 3.200/µl (range 700-14.360), PLT count 182.500/µl (range 3000-542.000).Median clearance creatinina value was 80 ml/min (range 1-225). In 18/45 analyzed patients karyotype had cytogenetic abnormalities; del 13q was observed in 12 patients, t(4;14) in 2 patients, t(11;14) in 2 patients, del 17p in 1 patient; complex karyotype was observed in 1 patient. Fifty-three % of patients presented with at least one severe comorbidity (diabetes mellitus, chronic obstructive bronchopneumonia, hypertension, atrial fibrillation or other cardiac arrhythmias, coronary heart disease, peptic ulcer, renal and hepatic dysfunction).The median number of previous lines of therapy was 3 (1-4). Ninety-nine patients had undergone autologous stem cell transplantation.The initial dose of lenalidomide was 25 mg/day in 170 patients, 15 mg/day in 56 patients, 10 mg/day in 54 patients and finally 5 mg/day in10 patients. In 14 cases lenalidomide/dexamethasone was associated to other drugs.
Results: Two hundred forty-six of 296 patients were evaluable for response. Patients received a median number of 7 cycles of lenalidomide/dexamethasone therapy (1-37). ORR was 68%: namely, according to IMWG uniform response criteria, 27 patients (16%) achieved a CR, 60 patients (36%) a VGPR, 81 patients (48%) a PR. Median time to best response was 4 months (1-11).Median duration of response to treatment received was 12,5 months (1-40). Median time to progression was 7 months (1-40).Grade 3/4 haematological toxicities occurred in 29 patients (17%), non-haematological toxicities in 59 patients (35%), causing interruptions of the treatment or reduction of daily dose. Quality of response correlates with number of previous treatments.
Summary / Conclusion: Our retrospective analysis confirms that lenalidomide/dexamethasone is effective in inducing significant responses in refractory/relapsed MM.

B1521

IMMUNOPHENOTYPIC ASSESSMENT AND CLINICAL OUTCOME IN PATIENTS AFFECTED BY MULTIPLE MYELOMA (MM)

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Background: The survival of patients affected by MM is variable depending upon the tumour mass at the diagnosis and by the intrinsic biological characteristics of tumour cells. Flow cytometry and immunological methods have allowed the characterization of a series of surface antigenic molecules expressed on either MM or normal cells. With this technique several molecules differentially expressed on normal and MM cells and correlated with the prognosis of MM patients have been identified. In details B-associated antigens, growth factor receptors, myeloid antigens and adhesion molecules can be found on pathological plasma cells.
Aims: Some studies have demonstrated that in about 50% of MGUS patients and 33% of MM patients the plasma cells express CD117 (c-kit), while normal plasma cells are CD117 negative. Moreover, both the normal plasma cells and those of patients with MGUS are usually positive for the CD43.
Methods: In the last 5 years we have analyzed, at diagnosis, the bone marrow blood of 71 patients affected by MM. 49 out of 71 presented a IgG component and the remaining 22 patients were IgA. On the basis of the staging criteria (ISS), 38/71 pts. were in stage II and 33/71 in stage III; the clinical stage (remission, progression or stable disease) was defined with clinical re-evaluation after chemotherapy and/or re-staging at 6 months from diagnosis.
Results: The immunophenotype of bone marrow plasma cells demonstrated the expression of CD38 (very bright) and of CD138 while CD19 was absent; 49/71 were CD43+(dim) and 20/71 CD117+(dim). 15 out of 20 CD117-positive

patients showed a specific immunophenotypic pattern (CD117+/CD43-). These patients were in stage II and showed a favorable clinical outcome, as demonstrated by a higher DFS and OS than the remaining patients, regardless of the treatment administered.
Summary / Conclusion: The possible prognostic role of CD117 and CD43 in MM warrants further clinical investigation on a larger series of patients even on the basis of new therapeutic strategies.

B1522
LONG-TERM USE OF LENALIDOMIDE IN PATIENTS WITH RELAPSED MYELOMA IN A SINGLE CENTRE: RESPONSE, TOXICITY AND FACTORS AFFECTING SURVIVAL.
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Background: *Background.* Lenalidomide is licensed for the treatment of relapsed multiple myeloma after ≥ 1 therapy, although reimbursement is available for patients in the UK after ≥ 2 therapies. Treatment (combined with Dexamethasone) is recommended until disease progression but data on its efficacy and toxicity is limited outside of clinical trials. This study looks at Lenalidomide usage at a single centre: Nottingham University Hospital (NUH), which has the most patients on the drug in the UK.
Aims: *Aim:* The aim of this study is to evaluate the response, toxicity and survival of patients with relapsed myeloma on Lenalidomide, and factors impacting on these.
Methods: *Methods:* All patients initiated on Lenalidomide between 1st June 2009 and 31st December 2010 at NUH were included in this study. Using retrospectively collected data we performed univariate and multivariate Cox regression analysis to calculate the likelihood of death (hazard ratios) based on several patient features (sex, age, complications) as well as starting dose and dose modification of Lenalidomide. The cut-off date for our cohort was 31st January 2013 to allow for at least 2 years of follow-up. Response was evaluated using modified IMWG/EBMT criteria.

	Univariate analysis				Cox regression	
	Pts	Events	EFS (SE)	p	HR (95% CI)	p
Age				0,0002		
< 6 months	40	26	32% (7)		2,70 (1,21 – 6,04)	0,01
> 6 months	43	11	72% (7)		Reference	
Group				0,007		
Group 2	24	16	28% (9)		2,64 (1,30 – 5,35)	0,007
Group 1	59	21	62% (6)		Reference	
MLL Status				0,001		
MLL (+)	58	32	42% (6)		2,92 (0,90 – 8,42)	0,07
MLL (-)	24	4	81% (8)		Reference	
Day 8 response				0,01		
≥ 1000 blasts	12	9	20% (12)		1,25 (0,74 – 3,63)	0,58
< 1000 blasts	69	27	59% (6)		Reference	

Results: *Results:* A total of 83 patients were included in the analysis of whom 44 (53%) were male. The median age was 71 yrs (range 30 –87); 25 (30%), 32 (39%) and 26 (31%) were aged <65 yrs, 65-75yrs and >75yrs, respectively. The median number of lines of previous therapy was 3 (range 1-10). Median number of cycles of Lenalidomide received was 15 (range 1-45) with 24 patients (29%) reaching ≥ 24 cycles. 55 patients (66%) started at 25mg and dose modifications occurred in 52 patients (63%) in the whole cohort. Of the 83 patients, maximal response on Lenalidomide therapy was assessable in 81 patients, with an ORR (CR+VGPR+PR) of 69 % with 14 (17%), 8 (10%) and 34 (42%) achieving a CR, VGPR or PR, respectively. In those who achieved ≥ PR, mean time to maximum response was 7.3 cycles (range 1-37). Of the 83 patients, 56 (67%) had at least 1 in-patient hospital stay during their time on Lenalidomide, the most frequent cause being infection. Overall, 56 patients (67%) have stopped Lenalidomide; of these 68% were due to progressive disease and 32% due to toxicity. The median OS of the whole cohort from time of commencing Lenalidomide is 730 days (IQR 255-989 days). Factors affecting survival were examined and the adjusted hazard ratios revealed that women have better survival (HR 0.36, 95%CI 0.18 – 0.73) compared with men when adjusted for confounders (P=0.003). Factors significantly associated with poor survival on multivariate analysis were no dose modifications of Lenalidomide, grade IV thrombocytopenia, grade IV neutropenia and eGFR <30, with p-values of 0.002, 0.0018, 0.026 and 0.0013, respectively. Starting dose, grade III anaemia or presence of VTE were not significantly associated with survival.
Summary / Conclusion: *Conclusions:* This study shows that nearly 70% of patients with relapsed myeloma, despite being heavily pre-treated, can achieve a partial response or better to Lenalidomide and many may remain on the drug long-term. Response can continue to deepen up to 3 years into receiving treatment. A median OS of 2 years after starting Lenalidomide is reported in this study, with dose modifications of the drug significantly improving survival. The development of certain drug related toxicities can significantly shorten survival.

B1523
THE CHOICE OF REGIMENS BASED OF BORTEZOMIB FOR PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: EXPERIENCES FROM CHINA
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Background: Novel drugs, such as Bortezomib, have significantly improved the response rates in multiple myeloma (MM). But how to chose the bortezomib based regimens as the initial therapy of MM while there few prospective randomized trials even the retrospective reports that compared modern combinations with bortezomib. Here, we report on four bortezomib-based therapies in newly diagnosed MM patients in the Chinese population from three blood treatment centers.
Aims: To evaluate the effect and adverse events of four bortezomib-based therapies in newly diagnosed MM patients in the Chinese population from three blood treatment centers.
Methods: In the initial eight 28-day cycles, newly diagnosed symptomatic patients were treated with combination therapy including bortezomib plus dexamethasone (PD) and the triplet combinations of PD with adriamycin (PAD), cyclophosphamide (PCD), thalidomide (PDT) between February1, 2006 and May 31, 2012. Among the above regimens, bortezomib (1. 3 mg/m^2) was given intravenously on days1,4,8, 11, while dexamethasone (20 mg/m^2/day) was given intravenously on days 1–2, 4–5, 8–9, 11–12, adriamycin (10 mg/m^2) was given intravenously on days 1–4, cyclophosphamide (200 mg/m^2) was given intravenously on days 1–4 and thalidomide (100 mg) was administered orally each day.
Results: The overall response rate (ORR) (≥ partial response, PR) of all the 164 eligible patients was 89.6% (including 29. 3% very good partial response (VGPR) and 26. 8% complete response/near complete response (CR/nCR). The ORR of patients in PTD, PCD, PAD and PD groups are 86.7%, 95.4%, 91.4 and 78.8%, respectively. The rate of VGPR and better in these groups are 53.3%, 65.2%, 62.9% and 33.3%, respectively. The ORR and the rate of VGPR and better in PCD groups were superior to PD□*P*=0.025, 0.003□.
The median PFS was 16.0months (95% CI: 11.9 –20.1 months) in the patients who received PDT, and 23.0 months (95% CI: 7.5-32.5 months), 23.9 months (95% CI: 10.3-37.5 months) and 21.8 months (95% CI: 7.7-36.0 months) in PCD, PAD and PD respectively with no significant differences between. The 2-year respective PFS was 31.9±9.0%, 46.0±9.0%, 44.0±12.0%, 40.0±11.0% in PTD, PCD, PAD and PD regimens, respectively. At the time of analysis, 33 (20.1%) patients had died, including 10 patients who had received PTD, 14 patients in PD groups, 7 in PCD groups and 2 in PAD groups with the causes of death consisted of treatment-related, myeloma, and others, respectively.
The median OS of the 164 patients was not reached, while 5-year respective OS was 53.8±8.0%, and there was significant differences between the groups (*P* =0.007).The median OS for PD arm was 41,8 months (95% CI: 20.2-63.4 months) while other arms were not reached, but the median OS for PTD, PCD and PAD was significant longer than PD (*P*=0.043, 0.028, 0.009).The 3-year respective OS was 64.0±9.0%, 69.0±12.0%, 76.0±17.0%, 58.0±10.0% in PTD, PCD, PAD and PD regimens. The frequently observed toxicities were neutropenia, thrombocytopenia, fatigue, infection, herpes zoster, and peripheral neuropathy. Peripheral neuropathy was more frequently reported in PTD group without routine anti-viral therapy, but much more lower in other groups with anti-viral therapy. The incidence of neuropathy was extremely higher in PTD regimen than other groups, especially grade 2 and 3.
Summary / Conclusion: Our experience indicated that bortezomib-based regimens were active and well-tolerated in the Chinese population, triplet combinations PCD, PAD regimens are superior to PTD or doublets, especially the PCD regimen.

B1524
THERAPEUTIC RESPONSE IN PATIENTS WITH MACROFOCAL FORM OF MULTUPLE MYELOMA , TREATED WITH BORTEZOMIB - CONTAINING REGIMENS
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Background: There is a small but well recognized in the clinical practice group of patients with multiple myeloma (MM), characterized by multiple bone lesions and small tumour burden – the so called "macro-focal form" of MM.
Aims: To analyze the incidence, clinical manifestation, therapeutic response and prognosis of the patients with macro-focal form of MM, treated with Bortezomib – containing regimens

Methods: For the period 2006-2011, 111 patients with MM, treated with Bortezomib – containing regimens were studied in two university clinics of hematology: University Hospital "Sv. Georgi", Plovdiv and Military Medical Academy, Sofia. Mean age was 58.2±8.8y, M: F / 1.05: 1. Clinical stage was defined according to ISS. In 12 (10.8%) macro-focal form of MM was found. Response rates in the groups with macro-focal and classic form were compared and a comparison of major parameters of the disease activity and bone metabolism in the two groups was performed. For statistical analysis SPSSv18.0 was used, the assessment of median survival was done by Kaplan-Maier method with log rank test.
Results: The patients with macro-focal form of MM were predominantly in an early clinical stage acc to ISS (P=0.002, r=-0.323), more frequent were the non-secretory and light - chain variants (P<0.001, r=+0.441). There were no patients > 65y, no severe anaemia, no renal impairment, no hypoalbuminemia were registered in the patients with macro-focal form. Hypercalciemia was found in 2 (16.7%). The share of patients with macro-focal form with elevated LDH was significantly higher 6 (50%) vs 11(11.1%), P=0.036 r=+0.409. All the patients had generalized bone lesions, grade II+III (P<0.001; R=+ 0.658). Patients with macro-focal form received therapy with VelDex as 2 and 3 line. In all of the them paraprotein disaapeared or was found only by IF, plasma cells in bone marrow were 1-2%. In 10 (83,3%) bone lesions did not progress in size and alleviation of bone pain was found. In 2 (16.7%) there were X-ray data of significant reduction of the size of the bone lesions; 3 (25%) underwent ASCT. After therapy a significant increase of AP level and decrease of Ca, LDH and β2M was found. By the time of analysis all the patients are in remission, no median of survival is formed.
Summary / Conclusion: MRI and PET are very helpful in the diagnostics and monitoring of the macrofocal form of MM. They distinguish between the plasma cell infiltration of the bone marrow and osteolytic foci in the structure of the bone. Although this is a small group of patients, data confirm the findings from similar studies: the application of Bortezomib results in suppressing not only the activity of the disease, but also bone resorbtion.

B1525
LENALIDOMIDE CONSOLIDATION THERAPY FOLLOWED BY LENALIDOMIDE MAINTENANCE AFTER FIRST LINE THERAPY FOR MULTIPLE MYELOMA
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Background: Multiple myeloma is still incurable disease. However, the prognosis of this disease has been improving according to the administration of novel agents. Among of these novel agents, lenalidomide is confirmed the validity of maintenance setting by a randomized controlled study. In addition, the immunoregulatory function of lenalidomide is not clear.
Aims: This phase 2 study investigated the efficacy of lenalidomide maintenance therapy after induction therapy and clarified the immunoregulatory function of lenalidomide.

	Pre-Consolidation (n=35)	Consolidation (n=35)	Maintenance (Best response) (n=35)
sCR	0	9	15
CR	0	2	0
VGPR	15	16	13
PR	19	8	7
SD	1	0	0

Methods: We assigned 35 patients younger than 75 years of age, who were not achieved complete response (CR) after first line therapy, to receive consolidation treatment with reduced lenalidomide (at a dose of 15 mg per day, on days 1 to 21 of each 28-day cycle, for four cycles) and dexamethasone (at a dose of 20 mg per day, on days 1 to4, days 8 to 11, and days 15 to 18, every four weeks, for four cycles) followed by maintenance therapy with lenalidomide (5 mg per day for two years). The primary end point was best response during maintenance therapy. The secondly end points was to clarify the changing of the cell in peripheral blood during therapy using flow cytometry.
Results: Not only consolidation therapy, but also maintenance therapy improved response rate (CR 0, Very Good Partial Response (VGPR) 15, Partial Response 19 before consolidation therapy; stringent Complete Response (sCR) 9, CR 2 VGPR 16, PR 8 after consolidation therapy; sCR 15, CR 0, VGPR 13, PR 7 best response during maintenance therapy). After all, 80% of patients achieved VGPR and more. Thirty-there patients (94%) are still on the study, while two patients discontinued because of progressive diseases. Median progression free survival is not reached. The rates of grade 3 or 4 peripheral neuropathy were not high (3 cases). However, grade 4 skin rush was observed in three patients whose CCL17 level were higher than 1000 pg/ml. Cytotoxic T lymphocytes which were positive $CD3^+$, $CD8^+$, and $CD57^+$ increased in almost patients' peripheral blood during maintenance therapy (31 patients). In addition, Natural killer cells, which were CD3-, $CD16^+$, and CD56+, increased in many patients during maintenance therapy (28 patients). γδ-T cells did not increase during maintenance period.
Summary / Conclusion: Lenalidomide plus dexamethasone consolidation therapy followed by low dose lenalidomide maintenance therapy after first line therapy significantly improved response rates among patients with multiple myeloma. However, some patients were suffered from severe skin rush, so we must invest which type of patient is suffered from critical skin adverse event in the future.

B1526
SECOND AUTOLOGOUS TRANSPLANTATION FOR PATIENTS WITH RELAPSED MULTIPLE MYELOMA
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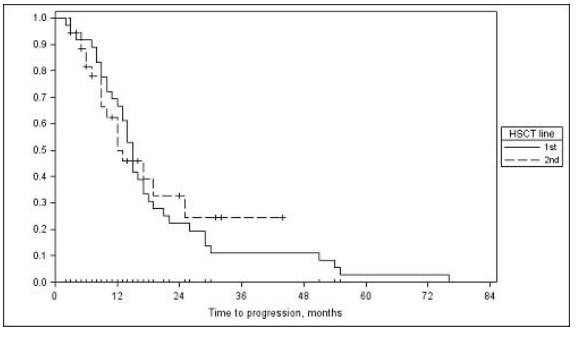
Background: currently the best treatment option for multiple myeloma (MM) patients relapsing after autologous stem cell transplantation (ASCT) remains to be defined. A second ASCT is one of the available options. There are few published data about the safety and effectivenes of this treatment.
Aims: to compare time to progression (TTP) between the first-line ASCT (1ASCT) and the second ASCT (2ASCT) performed at disease relapse. To compare the safety of 1ASCT and 2ASCT.
Methods: we retrospectively reviewed MM patients' recordswho received multiple ASCT at Vilnius University Hospital in 2001-2102. Patients who received 2ASCT for progressive disease were selected for the analysis. Patients who received their second ASCT due to suboptimal response or as planed multigraft procedure were excluded from this analysis.



Results: we indentified 36 patients who received 2ASCT at disease relapse. The median age of patients at 1ASCT was 57 years (32-69) and at 2ASCT was 59 (39-70) years. The median overall survival for the whole group was 123 months . The median time between 1ASCT and 2ASCT was 23 months (4-87). All patients received induction chemotherapy before 1ASCT. Only one third of the patients (N12, 33.3%) received induction containing novel agents (thalidomide – 8 and bortezomib – 4)before the first transplant. 66.6% (24/36) of patients received induction chemotherapy before 2ASCT. All of these regimens contained novel agents (bortezomib – 19 and thalidomide – 5). 12 patients were retransplanted without induction chemotherapy. Conditioning regimen was mainly Mel200 and dose intensity was not different between 1ASCT and 2ASCT. All patients engrafted. There were no transplant related deaths. There were significantly lower mucositis scores and fewer febrile neutropenia episodes after 2ASCT as compared to 1ASCT (p values 0.007 and 0.019, respectively). Median TTP (calculated since the date of the respective ASCT) was not different after 1 ASCT and 2ASCT (15 and 12 months respectively, P=0.783) (Figure 1).
Summary / Conclusion: The second ASCT is safe and effective treatment option for MM patients relapsing after their first ASCT.

B1527

EARLY ACHIEVEMENT OF A GOOD QUALITY RESPONSE PREDICTS BETTER OUTCOME IN MULTIPLE MYELOMA

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Background: Prolongation of progression free survival (PFS) and overall survival (OS) remains the ultimate goal of cancer therapy. Response criteria for MM elaborated by the International Myeloma Working Group have been widely used to facilitate precise comparison between new treatment strategies and to better define the exact magnitude of response. The importance to achieve CR after completion of a therapeutic program remains a goal in MM treatment; nevertheless association between CR and survival outcome has been noted in several analyses, whereas in a number of clinical trials CR is often, but not consistently, associated with better OS. Furthermore analysis of OS in MM is becoming increasingly complicated due to the impact of novel agents as rescue therapies. Little is known about the relationship between the speed of achievement of a substantial response and survival end points in MM
Aims: We report a retrospective analysis of 48 MM patients treated at diagnosis by our institution with bortezomib based regimens in order to evaluate whether early acquisition of a good quality response impacts on outcome. To this aim, responses obtained after 2 courses of therapy were analyzed for the impact on PFS and OS.
Methods: From January 2009 to December 2012, 48 MM patients were treated by our institution with bortezomib based regimen, as induction therapy at diagnosis. Median age was 59 years (range 39-78); males were 28; bone lesions were present in 40 patients (83%); 31 patients were in III stage (65%); median number of administered courses was 4 (range 2-10). Median follow up was 23 months (range 2-75). PFS was defined according to IMWG criteria. A CM reduction ≥75% after 2 courses of therapy was defined as Early Good Response (EGR). Survival curves were calculated for PFS and OS by the Kaplan Meier method and were compared using a 2-sided log-rank test. Patients undergoing autologous stem cell transplantation were censored at the time of transplant. Cox regression analysis was performed using various parameters as covariates.
Results: All patients were evaluable for response. After 2 courses of therapy 38 overall responses were observed (CR +nCR =4, VGPR =9, PR=25). At the end of therapy 41 patients reached at least a PR (CR +nCR =10, VGPR =11, PR=20). At the time of analysis 41 out of 48 patients are alive. At univariate analysis EGR (P=0.006) and a response ≥PR at the end of therapy (P=0.005) significantly impact on PFS. At multivariate analysis the only independent factor influencing PFS was EGR (P=0.041). The projected PFS at 60 months for EGR and non-EGR patients is 53% and 19% respectively (P=0.006). OS was affected only by age and poor response to therapy (PD).

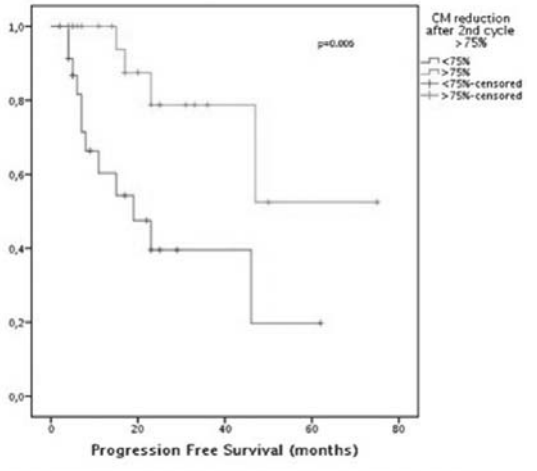

Fig.1 : PFS after 2 courses of therapy according to achievement of CM reduction ≥75%.

Summary / Conclusion: Achievement of CR has been considered as the first primary end point of new therapeutic strategies in MM. Recently several large clinical studies have demonstrated that not only the acquisition of CR, but also the maintenance of durable CR appears to influence outcome. Our data suggest that, in addition to the achievement and maintenance of CR, the early acquisition of a good quality response, even if not complete, is a significant predictor of PFS. As reported in other hematological malignancies (CML,HD), also in MM early evaluation of response could become the driver to address subsequent therapeutic approaches. Consequently, as Rajkumar suggests, CR could be considered a reflection of underlying disease biology, rather than a primary end point of therapy, functioning as a prognostic marker for those patients with inherently favorable disease.

B1528

EFFICACY OF LONG-TERM LENALIDOMIDE TREATMENT OF MM PATIENTS WITH AT LEAST ONE PRIOR THERAPY BASED ON CZECH LOCAL DATA

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Background: Multiple myeloma is a hematological malignancy that usually relapses in spite of the clinical response to initial therapy. In two randomized phase III trials (MM-009 and MM-010), lenalidomide and dexamethasone significantly prolonged time to progression and overall survival (OS) in patients with relapsed/refractory multiple myeloma (rrMM) compared with dexamethasone alone. Lenalidomide with dexamethasone is now accepted as valid and effective treatment option for most patients with rrMM. We conducted analysis on Czech local data from the Registry of monoclonal gammopathies conpared the results of continuous lenalidomide plus dexamethasone therapy in clinical trial patients and patients treated according the health insurance coverage.
Aims: Our aim was to demonstrate the clinical value of continuous lenalidomide treatment until disease progression in the absence of reimbursement restrictions on Czech local data.
Methods: Totally, we collected and analyzed data from 250 MM patients with at least one prior therapy, all patients signed informed consent form of their data collection to Czech Registry of monoclonal gammopathies. 46 patients were treated by continuous lenalidomide plus dexamethasone within standard arm of some clinical trial and 204 patients obtained the same therapy, but maximally 8-10 cycles according the health insurance companies rules based on Czech Myeloma Group guidelines. We analyzed therapy response in both group of patients and also Kaplan-Meier survival estimates were compared between patients on continued treatment versus the patients treated with local reimbursement restrictions.
Results: Overall, patient basic characteristics were comparable in both groups, with no significant p-values for any of the variables documented. Median of previous lines of therapy was 2 in both patient groups. Overall response rate was statistically significantly better in group of patients with continuous lenalidomide therapy (58.8 versus 38.9 %, P=0.014). Overall survival (OS), time to progression (TTP), disease free survival (DFS) and duration of response (DOR) were all found to be statistically significantly longer (with p values between 0.001 and 0.005) in population treated in clinical trials. As with other therapies using combination of lenalidomide plus dexamethasone at first relapse is more effective regarding response rate and durability than using it after multiple salvage therapies. The results of continuous lenalidomide treatment within clinical trials was similar to those obtained in registration studies including observed side effects of therapy.
Summary / Conclusion: Czech myeloma group confirmed on local data obtained from Registry of monoclonal gammopathies benefits of continuous lenalidomide plus dexamethasone treatment until disease progression. Continued lenalidomide treatment is associated with better therapy response results and also with a statistically clearly significant survival advantage. Our findings so confirmed results of some other studies dealing with similar themes.

B1529

MATHEMATICAL MODEL OF SERUM FREE LIGHT CHAIN DECLINE IDENTIFIES A KINETIC PREDICTIVE MARKER OF SURVIVAL IN PATIENTS WITH MYELOMA TREATED WITH BORTEZOMIB, DEXAMETHASONE AND STEM CELL TRANSPLANTATION

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Background: According to 2011 International Myeloma Working Group recommandations, serum-free light chain (sFLC) assessment is indicated in non secretory Multiple Myeloma (MM). sFLC monitoring is now routinely used for diagnosis and early response treatment assesment but its actual prognosis values regarding survival is not clear.
Aims: Based on mathematical modeling, this study aimed at evaluate the predictive value on progression free survival (PFS) of modeled sFLC kinetics during frontline tratment.
Methods: Model-based analysis of sFLC kinetics from 28 newly diagnosed MM patients treated at a single institution with 4 cycles of bortezomib and dexamethasone plus autologous stem cell transplantation (ASCT). MONOLIX program was used to fit Box-Cox transformed [sFLC] concentration-time profiles during the4 induction cycles to the equation : [sFLC(t) =[sFLC1*exp(-KDEC1*t)]+[sFLC2*exp(-KDEC2*t)]+[sFLC3*exp(KPROD*t)]] where $KDEC_1$ and $KDEC_2$

represent the decline rates of sFLC while KPROD is the involved light chain production rate observed after the end of the decrease.The predictive values of the modeled kinetic parameters , regarding PFS were tested using logistic regression, and survival analysis.
Results: Median PFS was 17.9 months . Three modeled kinetic parameters categorized by their medians had strong predictive values regarding PFS using univariate tests: sFLC decline rate $KDEC_1$ (median PFS = 38.6 months if $KDEC_1 \geq$ median vs 14 months if $KDEC_1 <$ median, P=0.01); light chain production rate KPROD (median PFS = 24.9 months if KPROD < median vs 7.8 months if KPROD ≥ median, P=0.01); and initial SFLC production slope sFLC3 (median PFS = 24.9 months if sFLC3 < median vs 14 months if sFLC3 ≥ median, P=0.03). The same model based analysis for M-protein decline was not significant (P=0.53). Using multivariate analysis, the onlyremaining significant predictive factor of PFS was: $KDEC_1$ (HR, 0.11; 95% CI 0.01-0.88; P=0.04).
Summary / Conclusion: The modeled kinetic parameter of SFLC decline, $KDEC_1$, may be easily used as an early predictor of survival after frontline ASCT in MM. Validation in larger studies is warranted.

B1530
STUDY OF THE PROGNOSTIC VALUE OF SERUM FREE LIGHT CHAINS RATIO AT DIAGNOSIS IN SPANISH POPULATION WITH MULTIPLE MYELOMA
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Background: Monoclonal gammopathies are a group of disorders characterized by clonal expansion of B cells that usually secrete intact monoclonal immunoglobulin, monoclonal free light chains or both.The quantification of serum free light chains (FLCs) is used in the diagnosis, monitoring and prognosis of monoclonal gammopathies.
Aims: The aim of this study is to evaluate the prognostic value of serum FLCs ratio at baseline in newly diagnosed multiple myeloma (MM) in a Spanish population.
Methods: We studied 73 patients with newly diagnosed multiple myeloma (56 intact immunoglobulin MM (IIMM) and 17 light chains MM (LCMM)) during a period of five years (2008-2012). Serum free light chains were measured by turbidimetry (Freelite™, The Binding Site, Birmingham, UK). Survival was defined as the time from initial diagnosis to death or the last follow-up and was calculated by the method of Kaplan and Meier. The survival curves were compared using the log-rank test. A p value <0.05 was considered to be significant. Statistical analysis was performed using IBM SPSS Statistics 20.
Results: FLCs ratio was calculated as k/lratio and we used the median FLCs ratio like cut-off with a FLCs ratio of >26 and <0.24 for kappa and lambda MM respectively for assessing survival. The FLCs ratio was categorized in two groups: "low" (sFLC ratio <26 and >0.24) or "high" (sFLC ratio ³ 26 and £ 0.04). There were 37 patients in group "low" and 36 patients in group "high". Of the 73 patients studied, 17 died and 56 survived during five year follow-up. In group "low" died 4 patients (10.81%) whereas in group "high" died 13 patients (36.11%). The percentage of deceases in each isotype of MM was the following: 50% (IgD IIMM), 33% (IgA IIMM), 29% (Lambda LCMM), 22% (Kappa LCMM), 14% (IgG IIMM), 0% (IgM IIMM). The five years survival was 59% and 88% in patients in groups "high" (with an abnormal FLCs ratio ³ 26 and £ 0.04) and "low" (with a FLCs ratio between <26 and >0.24) respectively (P=0.007). The estimated relative risk of the event (death of the patient) occurring in group "high" was 4.11 (1.57 – 10.71 95% IC) higher than in group "low".
Summary / Conclusion: An altered baseline serum FLCs ratio at diagnosis is an important predictor of poor prognosis in patients with multiple myeloma in our population. So, serum FLCs ratio can be used as survival predictor. The patients with IgA and IgD isotypes have a poor prognostic.

B1531
DIFFERENCES AMONG MGUS AND MGUS WITH ASSOCIATED DISEASES AT DIAGNOSIS AND FOLLOW UP
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Background: Monoclonal gammopathy of undetermined significance (MGUS) is an asymptomatic premalignant clonal plasma cell or lymphoplasmacytic proliferative disorder. MGUS has been reported in association with several non-malignant disorders (including autoimmuine disorders). Nevertheless, it is not clear whether these conditions are pathogenetically related or merely represent coincidental associations.
Aims: Compare the characteristics at diagnosis and follow-up of patients with MGUS with those MGUS associated with other disorders (MGUSad).
Methods: A total of 327 MGUS with at least 3 years of follow-up were identified in our hospital. Medical history was revised in order to identify disorders associated with MGUS according to Bida et al. (*). Recorded data from each MGUS were: presence of immunoparesis (defined as a decreased below 25% the lower normal limit in the level of 1 o 2 immunoglobulin (Ig), of the uninvolved Ig), evolving type (defined as an increase in the level of serum M –MC- protein of at last 10% with respect previous year determination) and cuantification of free light chains (FLC). Additionally, sustained immunoparesis we consider in those patients with immunoparesis at diagnosis and at last in a second determination in the follow-up.
Results: From 2007 to 2011, 327 patients with MGUS were identified in Hospital Txagorritxu. 201 (61.5%) were consider associated to other entities while on 126 (38,5%) no other disease could be identified.
Associated conditions with MGUS were: hypertension 50.6%, osteoporosis 10.6%, polyarthrosis 18,2%, chronic liver diseases 15.4%, autoimmune diseases 21.7%, malignant diseases 13.1% and tuberculosis 3.1%).
No different in median age at diagnosis was observed (64 vs. 65 p: 0,782). Regarding the presence of paresis and FLC ratio at diagnosis, no differences were observed between MGUS and MGUSad (18,9 vs 12,4; p:0.05 and 17,8% vs 18,2; p: 0.615, respectively). Considering MC, 25% of MGUS were considered as evolving type vs 24% of the MGUSad (p:0.570). Significatively, sustained immunoparesis were observed in 18,5% of MGUS vs. only 9,7% of MGUSad (p:0.013). We also could identified 6 multiple myeloma patients progressed from a MGUS. According to their medical history, 3 of them would be considered as MGUSad. Sustained immunoparesis and evolving type was documented in 2 of them.
Summary / Conclusion: It has been suggested that the reduction of uninvolved immunoglobulins can be considered a predictive marker of progression. In our study, MGUS with associated disease show lower rates of sustained immunoparesis. It could be hypothesized that pathogenesis of MGUSad has different origin than a primary neoplastic entity.
**"Disease Associations With Monoclonal Gammopathy of Undetermined Significance: A Population-Based Study of 17,398 Patients"*

B1532
FAMILIAL MULTIPLE MYELOMA
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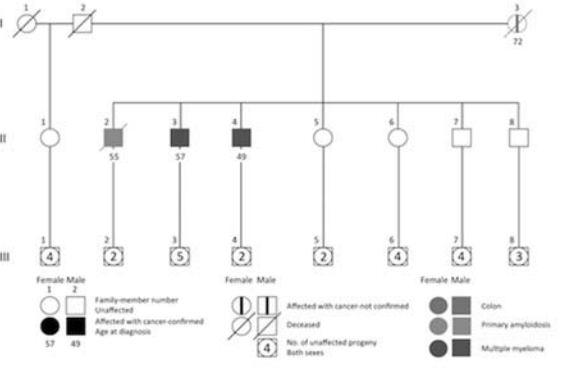
Background: A recent data derived from 239 cases with multiple myeloma (MM) and 220 controls disclosed an increased cancer risk among individuals with first-degree relatives with MM (RR = 5.64; CI, 1.16-27.51). An increased risk for MM was also seen if the first-degree relatives had experienced another tumor disease (RR = 1.21; CI, 0.86-1.17). The medical literature contains reports of around 130 families with two or more cases of MM, MGUS, or WM, about 80 of these contain ≥ 2 cases of MM.
Aims: We describe here a family with familial multiple myeloma and associated cancer. We aimed to screen three generation for plasma cell disorders, to get information about the associated cancers, and impact of environmental factors.
Methods: Two brothers with multiple myeloma and all their siblings and progeny were invited. Subjects were informed about the screening program. Serum protein end immunofixation electrophoresis analyses were performed in all. In addition, all persons were asked to complete a questionnaire containing items questioning the environmental exposure to several factors.



Results: Three siblings in one generation were affected with plasma cell disorders, two with multiple myeloma and one with primary amyloidosis. The patient with primary amyloidosis was 55-year-old at diagnosis. He had kappa light chain disease involving liver, kidney, bone marrow, spleen, and heart. Stage IV kidney disease with low level proteinuria progressed to end-stage renal disease while he was receiving bortezomib-cyclophosphamide-dexamethasone treatment. He had Mayo Clinic stage III cardiac involvement at the time of diagnosis. He died almost two years after the diagnosis because of dialysis-related problems. He had complete response except organ response. Two

other siblings were diagnosed with MM, seven and six years ago, respectively. They were 57 and 49-year-old at diagnosis. Former underwent two autologous hematopoietic stem cell transplantation (ASCT) for IgG kappa MM. He has complete remission. The latter underwent ASCT for IgG lambda disease. He has a stable disease for the last three years. All affected siblings lived in a village located in a damp and rainy geographic area during the first two decades. All were in close contact with plants and livestock. They were exposed to pesticides/insecticides. Only the patient with amyloidosis was a smoker. Their mother has died due to mass on her liver and colon. However her diagnosis was not confirmed.
Summary / Conclusion: Summary/conclusions: We note that familial MM patients were more likely to report exposure to farming, damp, and pesticides, compared with unaffected family members. Genetic and environmental factors that may influence development of MM should be elucidated.

B1533
HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA. A SINGLE CENTER EXPERIENCE FROM EHU1ST NOVEMBER OF ORAN.
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Background: Autologous stem cell transplantation (ASCT) after a high dose conditioning is an established treatment modality with definitive indications for multiple myeloma (MM). However, this line of treatment requires many expensive resources, such as freezing of the harvest product in order to maintain cell viability until stem-cell reinfusion. The storage of harvested stem cells, in standard refrigerators at +4°C, is an alternative to cryopreservation.
Aims: We evaluated the efficacy and safety of autologous hematopoietic stem cells in adult patients undergoing autologous stem cell transplantation (ASCT) for multiple myeloma (MM), with non-cryopreserved hematopoietic stem cell.
Methods: Autologous stem cell were mobilized using G-CSF (15µg/kg/day) alone. Leukapheresis to harvest stem cells was performed on day -2 and -1. The grafts were kept in a conventional blood bank refrigerator at 4°C until reinfusion on day 0. The conditioning regimen consisted of melphalan 200 mg/m^2 in all patients. The post chemotherapy myeloablative phase was managed without growth factors.
Results: Between May 2009 to 31nd December 2012, 109 adults with MM were treated in our center in Oran. The median age at ASCT was 55 years (range, 27-67). There were 65 males and 44 females. The median harvested CD34$^+$ cell count was 3.37×10^6/kg (range, 1.22 to 13.22). All patients had neutrophil engraftment on the median of day 10 (range, 7-17) and platelet transfusion independence on the median of day 13 (range, 9-24). In the 107 evaluable patients, the median post-transplant overall survival had not been reached; the estimated overall survival at 5 years was 71% and the median disease-free survival was 35 months .One hundred days transplant-related mortality was 1.8%.
Summary / Conclusion: High-dose chemotherapy and autologous transplantation without cryopreservation is an effective and safe method which simplifies the procedure and is feasible and cost saving in the treatment of MM in developing countries.

B1534
PREVALENCE OF OLIGOSECRETARY MYELOMA AND ITS CLINICAL IMPLICATION: AN ASIAN SINGLE-CENTER EXPERIENCE
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Background: Oligosecretary myeloma is a subgroup of multiple myeloma (MM) characterized by low levels of serum and urine monoclonal(M) protein below thresholds of measurable disease (a serum M protein ≥ 1g/dL, a urine M protein ≥ 200 mg/day). Clinical data about oligosecretary myeloma is still scarce.
Aims: In this study, we aimed to investigate prevalence of oligosecretary myeloma, incidence of oligosecretary myeloma during disease progression, and its prognostic implication.
Methods: We retrospectively identified MM patients in Samsung Medical Center. We reviewed the serum/urine electrophoresis studies and serum free light chain (FLC) assays of the patients at the time of initial diagnosis and at the time of disease progression.
Results: A total of 390 patients with MM were included in the analysis. At initial diagnosis, 38 among 390 patients (9.7%) had oligosecretary myeloma. Among 352 patients with MM at initial diagnosis, 32 patients (9.1%) changed to oligosecretary myeloma during disease progression. Oligosecretary myeloma at initial diagnosis and during disease progression had measurable disease on serum FLC assay in 62.5% (no./total no, 15/24) and 55.6% (no./total no, 15/27) of the patients, respectively. Median time from the the diagnosis of MM to the onset of oligosecretary myeloma was 22.3 months (range, 8.1-74.8). Median OS from the onset of oligosecretary myeloma to death is 28 months (95% CI; 10.6-45.4). Common clinical presentations of oligosecretary myeloma at disease progression were progression of bone lesions (n=14, 43.8%), hypercalcemia and/or azotemia (n=11, 34.4%), and plasmacytoma (n=4, 12.5%). Median overall survival of MM (n=320), oligosecretary myeloma at initial diagnosis (n=38), oligosecretary myeloma during disease progression (n=32) were 49.4 months (95% CI, 37.3-61.5), 55.2 months (95% CI, 35.8-74.6), and 62.1 months (95% CI, 34.8-89.4), respectively (P=0.41).
Summary / Conclusion: In the current study, we found that 9.7% of newly diagnosed MM had oligosecretary myeloma and additional 9.1% of MM changed to oligosecretary myeloma during disease progression. It seemed that there was no significant differences in overall survival between MM and oligosecretary myeloma. Further studies are anticipated regarding feasibility of FLC assay for the prediction of response and progression on oligosecretary myeloma.

B1535
THE CONTRIBUTION OF SERUM FREE LIGHT CHAINS ASSAY TO THE DIAGNOSIS AND FOLLOW - UP OF PATIENTS WITH MULTIPLE MTELOMA- CLINICAL AND LABORATORY CORRELATION (A SINGLE MEDICAL CENTER EXPERIENCE
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Background: Measurement of serum free light chains (sFLC) has recently become available for the diagnosis and monitoring of patients with plasma cell dyscrasias. The International Myeloma Working Group has considered the use of the sFLC for screening in combination with serum protein electrophoresis and serum immunofixation essay. The baseline measurement would serve as a prognostic value, as well as for monitoring patients with oligosecretory myeloma and establishing a stringent complete response.
Aims: The aim of our study was to evaluate the contribution of the sFLC assay to the diagnosis and follow up of patients with multiple myeloma in a single center. We studied the correlation between (sFLCs) levels and clinical and laboratory indices that are widely used in the diagnosis and follow-up of patients with multiple myeloma.
Methods: We performed a retrospective study of 50 patients with multiple myeloma who were treated in Carmel Medical Center Hematology Department in Haifa between 2006 and 2012. The study was approved by the hospital's Helsinki committee. Four patients were excluded from the study due to lack of full medical information. The clinical and laboratory data of remaining 46 patients were collected from the electronic medical files. The laboratory data included levels of sFLC, hemoglobin, creatinine, calcium, serum protein electrophoresis (SPEP), albumin, beta2-microglobulin (B2M), bone marrow aspirate and biopsy, as well as bone disease status at the time of diagnosis, before and after each treatment cycle, when possible. Statistical analysis was performed on 70 out of 144 treatment cycles in which full information on the sFLC levels before and after each treatment cycle was available. The proportion of sFLC change before and after each treatment cycle was compared to the proportion of change in hemoglobin, creatinine, calcium, M-protein, albumin, and B2M.
Results: The statistical analysis of the clinical and laboratory findings showed that a decrease in sFLC levels was associated with a decrease in creatinine levels (P=0.048), and an increase in hemoglobin levels (P<0.0001) following the treatment. The decrease of sFLC was associated with a decrease in M-protein levels following each treatment cycle. A statistically significant correlation was found between the percentage of decrease in SFLC levels and B2M (P<0.0001) and the percentage of increase in levels of albumin (P=0.001)). These findings are important as they are correlated with the response to treatment as defined by the International Staging System (ISS). Furthermore, a return of sFLC to levels of <=30 was associated with a good treatment response as defined by the International Myeloma Working Group uniform response criteria. However we found that three of our patients had an active extramedullary disease despite of normal levels of sFLC. We did not find correlation between the improvement of sFLC levels (P=0.922) in patients responding to the treatment and various types of multiple myeloma.
Summary / Conclusion: Our findings are in concert with previous findings in literature which stress the importance of serum FLC analysis at diagnosis, follow up and evaluation of response to treatment in patients with multiple myeloma. However, the results of our study raise the question of the value of sFLC analysis in evaluating the response to treatment in patients with extensive bone and extramedullary disease, as three of our patients with such a presentation had near normal levels of sFLC. We assume that further prospective studies on contribution of sFLC in larger groups of myeloma patients are warranted.

B1536
MULTIPLE MYELOMA: A TEN YEAR EXPERIENCE OF A CENTRAL HOSPITAL
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Background: In spite of the introduction of novel drugs (Bortezomib or Lenalidomide) for the treatment of Multiple Myeloma (MM), this is still an incurable malignant disease. The introduction of new drugs, has been advocated as

improving patients´ outcomes but it also implies a consequent increasing cost for Health Services, sometimes with scarce evidence for a real benefit. Nevertheless, benefits from high cost treatments are expected if the overall survival of patients can be improved.
Aims: The primary objective of our study was to compare the overall survival(OS) of Multiple Myeloma treated with and without the new drugs in our institution for a ten years period. The survival outcome among groups of patients according to treatment options and age were also compared.
Methods: All MM patients diagnosed and treated at our Hospital between January 2000 and December 2011 were retrospectively analysed. Details of age, stage of the disease, and treatment regimens were performed. The Kaplan-Meir method was used for survival outcome estimates.
Results: The population included a total of 189 patients (94 males and 97 females) with a median age of 66 years (range 31-95 years) and staged: Stage IA 10% (19), Stage IB 1%(2),Stage IIA 31,4% (60), Stage IIB7,3% (14) Stage IIIA 27,2% (52) and Stage IIIB 18,8% (36). Six patients were not staged. Twenty nine patients (15%) died before any treatment or only received dexamethasone. Thirty nine patients (21%), aged under 65 years, were treated with Idarubicine+dexamethasone (dexa), or thalidomide+dexa or bortezomib+dexa before being submitted to high dose melphalan(HDM). Thirty nine patients (21%), aged under 65 years failed the aim of HDM. Patients aged equal or above 65 years (n=111; 58%) were treated with different chemotherapeutics regimens, without introducing the new drugs (bortezomib or lenalidomide) on first line. The Kaplan-Meir method was used for survival outcome estimates. The overall survival (OS) at five years for the total population studied was 40%. Patients under 65 submitted to HDM had a OS at five years of 65%. In contrast, the OS of patients not submitted to HDM was 40%. When we analysed the population treated with the new drugs (submitted or not to HDM) the OS was 55%, in comparison of 45% of OS in the population not treated with bortezomib or lenalidomide.The overall survival (OS) at five years for the total population studied was 40%. Patients under 65 submitted to HDM had a OS at five years of 65%. In contrast, the OS of patients not submitted to HDM was 40%. When we analysed the population treated with the new drugs (submitted or not to HDM) the OS was 55%, in comparison of 45% of OS in the population not treated with bortezomib or lenalidomide.
Summary / Conclusion: The proportion of newly diagnosed patients that didn´t start treatment or only had dexamethasone was very high, contributing to 15% of all of our population. The overall survival at five years was better than it was years ago. The population treated with the new drugs seems to have a better outcome. As expected, patients submitted to HDM have the best performance. We may expect that the introduction of the new drugs in first line also in the patients older than 65 years will have a positive impact on the final general outcome of MM patients.

B1537

HEMOSTASIS IN PATIENTS WITH MULTIPLE MYELOMA BEFORE AND DURING HIGH-DOSE CHEMOTHERAPY.

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Background: The incidence of thrombosis in patients(pts) with multiple myeloma(MM) is about 10% and it increase in the presence of additional risk factors, such as central venous catheter and chemotherapy. This fact underline the importance of preventive anticoagulant therapy.
Aims: The aim of the study was to investigate the blood coagulation status in patients with MM before and after heparin administration
Methods: 17 pts: 11 males, 6 females at the age of 40-60 years (median 52years) were included in the study. Immunochemistry variant was IgG in 7 pts, Ig A in3, Bense-Jones in6, and 1 case of non-secretory MM. Induction therapy included bortezomib + dexametazone±cyclophosphomide±adriamycin and after it all pts achieved at least VGPR. All pts received heparin-sulphate in continuous infusion 500 IE/hour as thromboprophylaxis before and during high-dose chemotherapy. Hemostasis analisis was performed before and 24 hours after heparin administration. Routine tests were used: Activated Partial Thromboplastin Time (APTT). D-dimer level and also integral methods: thromboelastography (TEG) and thrombodinamics. TEG characterized by parameters of reaction time (R), clot formation time (K), angle and maximal amplitude (MA). Thrombodinamics is a new method based on a spatial fibrin clot growth registration. Hemostasis status was estimated by the clot growth rate (Vst, norm at range 20-30 mkm/min).
Results: Before heparin administration APTT study showed there was normal coagulation in 15 pts (88%) and 2 patients had hypocoagulation. The TEG data confirmed mild hypocoagulation (K – 11.2min, angel – 18.4 degrees, MA – 43.6mm) in 1 pt with APTT elongation (45 sec). Other pt with APTT elongation had normal TEG. Mild hypercoagulation was detected with TEG in 3 (18%) pts. One of them had decreased R (5.4 min) and K (1.5 min), and two had increased MA (64.3mm and 68 mm). In 2 pts D-dimer levels were elevated (754 and 1907ng/ml). TEG data of other pts showed normocoagulation. Vst was elevated (31-49 mkm /min) in 8 pts (47%) and was normal in other 9 (53%). Maximal Vst (49 mkm /min) was registered in the case with minimal APTT (26 sec) and increased R and K (5,4 and1,5 min). Mild hypocoagulation was detected in 7 pts (41%) a day after heparin administration with APTT (38-53 seconds) and TEG (according 1 or 3 parameters: R – 28,4 – 33,8 min, K – 9,2 – 16,8 min, angel – 13,1 – 20,6 degrees, MA – 42,3mm). In other 11 pts (59%) these parameters were normal. In 1 pts with elevated D-dimer level it became normal, in other – remained elevated (881 ng/ml). Thrombodinamics showed hypocoagulation in 10 pts (59%, 11-19 mkm /min). Vst was normal in 3 pts (18%), increased in 4 pts (23%, 33-36 mkm/min). Among all 17 pts during high-dose chemotherapy only in 1 case catheter-associated thrombosis was diagnosed. In that case hypercoagulation was confirmed by thrombodinamics and D-dimers, but not with APTT and TEG before and after heparin administration.
Summary / Conclusion: The results of our study confirm the possibility of thrombotic complications during high-dose chemotherapy. thrombodinamics seems to be more sensitive vs. routine tests to reveal hypercoagulation condition.

B1538

BENDAMUSTINE : A NEW OPTION FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: Bendamustine is a bifunctional alkylating agent, with low toxicity, that produces both single- and double-strand breaks in DNA, and shows incomplete cross resistance with other alkylating drugs. proved to be effective either in relapsed/refractory and in new diagnosed MM as single agent or combined with steroid and has also additive/synergistic activity with bortezomib. Here we evaluate the efficacy and tolerance of bendamustine in combination with bortezomib-dexamethasone in patients with relapsed/refractory multiple myeloma and focus on individual factors associated with outcome. Prognosis of relapse is severe in relapsed and refractory multiple myeloma, and we need new options for managing these patients.
Aims: Our aim is to investigate the role of Bendamustine in patients affected by relapsed and refractory multiple myeloma, considering safety and efficacy of this treatment option. We performed a retrospective analysis of patients of our division with relapsed/refractory MM, who had received bendamustine as salvage therapy.
Methods: 16 patients, 9 males, 7 females, with advanced relapsed/refractory multiple myeloma, received a chemotherapy schedule containing Bendamustine. Median age at diagnosis was 62.6 years (range 39-82) while age at start of treatment was 67 years (range 48-83), and median number of prior lines of treatment was 5.7 (range 4-8). ISS was equally distributed, and cytogenetic characteristics were evaluated only in 6 patients, and only 2 of them had cytogenetic abnormalities, and in particular one of them had del13q and in the other one was observed t(11;14). All the patients had previously been treated with schedule containing bortezomib and lenalidomide, while 90% of them had been treated with melphalan, 75% with cyclophosphamide and 38% with anthracyclines, and 30% had also received radiotherapy. 69% of patients had undergone single autologous stem cell transplantation. The last treatment before bendamustine was a bortezomib-based regimen in 30%, an IMIDs-based regimen in 53%, a combined bortezomib/IMIDs-based regimen in 23%, while 15% of patients had received other chemotherapies. All the patients were relapsed/refractory to last therapy received.
Results: We considered in our study patients who had completed at least two courses of Bendamustine schedule. A total of 47 cycles was administered (median3, range 2-6). In 91% of patients bendamustine was variously associated to bortezomib (66%), or IMIDs (25%) and only in 8% it was combined only with dexametasone. In our schedule, Bendamustine was given, at a median dose of 142 mg/sqm (range 100-200) on day +1 and +8 every 28 days = After a median follow-up of 3 months, median OS from diagnosis was 60.2 months, while OS from start of Bendamustine was 3.6 months (range 2-6 months). 2/16 patients died for other causes (one for cardiovascular disease and the other one had a gastric cancer). Grade 3 transfusion-dependent anemia occurred in 42% while in 57% grade 3 neutropenia occurred. However, only 1 patients interrupted the schedule due to hematologic toxicity (it was the one affected by gastric cancer). We observed no serious extrahematologic toxicity, only grade 1 gastrointestinal side effect, treated by common antiemetic drugs. According to IMWG uniform response criteria, 11 out of 16 evaluable patients achieved a partial response after a median time of 3 months with an overall response rate of 68%. In particular, for 3 patients of this study, Bendamustine schedule was a bridge to second autologous autologous stem cell transplantation, after having achieved a PR.
Summary / Conclusion: In our study we have considered a group of patient with refractory/relapsed multiple myeloma, and it confirms that Bendamustine can be considered an effective option in advanced patients and moreover it could be considered as a bridge to pre-autologous stem cell transplantation. However, these results have to be validated by controlled studies involving larger number of patients.

B1539

TWENTY-FIVE MG LENALIDOMIDE EVERY OTHER DAY IN PATIENTS AFFECTED BY MULTIPLE MYELOMA AND RENAL FAILURE

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Background: Lenalidomide is a derivative of Thalidomide, with tumoricidal and immunomodulatory activity. It is available as oral agent, which is effective in the management of newly diagnosed, relapsed or refractory multiple myeloma and as maintenance therapy after autologous stem-cell transplantation.
Aims: With normal renal function, Lenalidomide administered orally reaches its maximal plasma concentration after a median time of 0.6-1.5 h, and it is eliminated through glomerular filtration and active tubular secretion in 3 to 4 hours. Serum half life increases up to 9 hours if moderate/severe renal impairment is present (creatinine clearance <50 or <30 mL/min, respectively). In the latter cases a reduction of the daily dose is recommended. However, there is no theoretical assumption that protracting the full standard doses could be equally effective and tolerated in patients requiring reduced doses.
Methods: Twelve patients, 7 female and 5 male, with a median age of 61.2 years (range: 49-81) affected by advanced resistant and progressive multiple myeloma (median number of previous treatment lines:3, range : 0-5, all including bortezomib) with concomitant renal failure (median calculated creatinine clearance 48.3 ml/min, range : 18-119) were treated with monthly 21-day courses of 25 mg lenalidomide every other day and dexamethasone.
Results: Disappearance of urinary light chain and reduction of serum creatinine were detected in four patients (33%); four patients (33%) had a partial response, and two of them (16%) were in stable disease, whereas two patients (16%) had signs of progressive disease. No patient experienced significant myelotoxicity; four patients required red cell transfusions. No SAE occurred during treatment.
Summary / Conclusion: These preliminary observations point to a significant therapeutic effect in more than half of a small population of patients (66%) with advanced disease. However, these results have to be validated by controlled studies involving larger number of patients.

B1540

SERUM FREE LIGHT CHAIN QUANTIFICATION IN MONOCLONAL GAMMOPATHIES AND MULTIPLE MYELOMA PATIENTS

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Background: Identifying patients with optimal response and long term survival is important for clinical guidance because patients with these features are likely not need further therapy. Serum Free Light Chains (sFLC) are used for better assessment of treatment response, thus patients are considered to achieve stringent Complete Response (sCR) by having CR criteria plus normal serum Free Light Chains Ratio (sFLCR) and absent clonal cells in bone marrow.
Aims: sFLC are commonly assessed in patients with monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM) as indicators of disease progression.
Methods: In the last two years, we have examinated 40 patients with Monoclonal Gammopathy (MGUS) and 30 patients with Multiple Myeloma (MM) in course of therapy. We have assessed serum Free Light Chains (sFLC) and serum Free Light Chains Ratio (sFLCR) for evaluation of progression disease and treatment response. Serum FLC concentrations were measured by nephelometry, using particle-enhanced, high-specificity, homogeneous immunoassays.
Results: We observed an increase of sFLC in patients with monoclonal gammopathy in evolution, with simultaneous progression of monoclonal component (M-spike). In MM patients sFLC and sFLCR were evaluated for assessing the response to treatment, and we observed a strict correlation with disease status. Moreover, in MM patients sFLC monitoring proved to be the earliest indicator of disease relapse.
Summary / Conclusion: Our results confirm the role of sFLC in the monitoring of MGUS and MM patients. Specifically in MM patients sFLC and sFLCR evaluation seems to be very useful in identifying patients response and early relapse.

B1541

THE PREVALENCE OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS) AND ASYMPTOMATIC MULTIPLE MYELOMA (AMM) FOR HIGH RISK GROUPS

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Background: To determine the frequency of monoclonal gammopathy of undetermined significance (MGUS) among elderly patients presented to outpatient clinics with bone and diffuse bodily pain with elevated erythrocyte sedimentation rate (ESR). In addition, it is also aimedto compare the results of the Immunofixation Electrophoresis (IFE) and Serum Free Light Chain (FLC kappa/lambda) assays used in the diagnosis of monoclonal gammopathies and to determine which method is more sensitive in terms of the diagnosing MGUS.
Aims: To compare the results of the Immunofixation Electrophoresis and Serum Free Light Chain assays used in the diagnosis of monoclonal gammopathies and to determine which method is more sensitive in terms of the diagnosing monoclonal gammopathy of undetermined significance.
Methods: One hundred and sixty patients older than 50 years of age with ESR≥50 mm/h were included to the study. In these patients, IFE, FLC kappa/lambda, immunoglobulin levels (IgG, IgM, IgA, and IgE), bone marrow aspiration and biopsy, complete blood count, biochemical tests (serum calcium, phosphorus, total protein, albumin, alkaline phosphatase, and creatinine, etc.) and ß-2 microglobulin levels were studied. Patients were compared in terms of IFE and FLC values by stratifying according to diagnosis made including MGUS, AMM or MM.
Results: Of the 160 patients evaluated, monoclonal gammopathy was detected in 44 patients (27.5%). Monoclonal gammopathy was detected by IFE in 36 patients (22.5%), whereas it was detected by FLC in 30 patients (18.7%). There were 22 patients (13.75%) in whom monoclonal gammopathy was detected by both methods (IFE and FLC). In our study, MGUS incidence was found as 20% in the high-risk patient group.
Summary / Conclusion: MGUS was detected in the 20% of the patients older than 50 years of age presented with ESR elevation and diffuse bone pain. In the present study, it was found that both methods had similar sensitivity; however, IFE was found to have slightly higher sensitivity, when FLC and IFE methods were compared regarding detection of monoclonal gammopathy. It was concluded that, among the elder population, patients presented with diffuse bone pain, especially those with elevated ESR, should have to be evaluated in terms of MGUS.

B1542

EPIDEMIOLOGICAL DATA OF MULTIPLE MYELOMA IN ALBANIA

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Background: Multiple Myeloma includes a maximum of 10% of hematological diseases. Frequency constantly increases with the age of the general population. Currently about 35% of patients with Multiple Myeloma are younger than 65 years old, 28% are 65-74 years old, and 37% are older than 75 years. The data of recent demographic curve, probably in the nearest future, will increase the incidence of elderly patients.
Aims: To present some epidemiological data for patients with Multiple Myeloma in Albania.
Methods: A retrospective study includes patients with Multiple Myeloma in the Hematology Service for 17 years. We studied the annual dynamics incidence, the dynamics of the distribution of the cases based on age, sex, geographic distribution in 36 districts of our country.
Results: We evaluated 428 patients with Multiple Myeloma, hospitalized during the period 1995-2011, 57.5% male and 42.5% female. Mean age 60.6±10 years, 29.6-83.5 years age variation. Emergency hospitalizations in a year for MM are 15.49%. Annual incidence dynamics analysis gives 19 cases in 1995, 10 cases in 1996, 6 cases in 1997, 14 cases in 1998, 13 cases in 1999, 19 cases in 2000, 13 cases in 2001, 26 cases in 2002, 21 cases in 2003, 25 cases in 2004, 45 cases in 2005, 37 cases in 2006, 34 cases in 2007, 31 cases in 2008, 42 cases in 2009, 41 cases in 2010, 32 cases in 2011. Annual incidence per 100 000 inhabitants: 1995: 1.06, 1996: 0.56, 1997: 0.34, 1998: 0.77, 1999: 0.73, 2000: 1.05, 2001: 0.79, 2002: 1.56, 2003: 1.25, 2004: 1.46, 2005: 2.74 , 2006: 2.25, 2007: 2.07, 2008: 1.88, 2009: 2.55, 2010: 2.49, 2011: 1.95. Higher incidence per 100 000 inhabitants was observed in the age group 55-64 years old (62.32), and in some cities (27.9) compared to some others (2.8). Died in hospital an average 2.7 cases per year, from all the patients with MM.
Summary / Conclusion: Multiple myeloma is a disease with increase of new cases per year. The sheer number of new cases with MM per year is 25 compared with another previous study that was 17 new cases per year, as well as the increase in the annual incidence / 100 000. MM incidence increases with age, but the youngest average age of patients with Multiple myeloma in our study relates to the youngest average age of the Albanian population. Males are more affected than females in the ratio 1.3 /1, in comparison with previous

studies we see that there is an increase of the number of women with Multiple Myeloma in Albania.

B1543

A NEW OPPORTUNITY IN RELAPLSED AND REFRACTORY MULTIPLE MYELOMA : PEGYLATED LIPOSOMAL DOXORUBICIN, CYCLOPHOSPHAMIDE AND DEXAMETHASONE (CED)

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Background: Patients affected by multiple myeloma almost invariably become chemo-resistant: hence, one of the main topics in clinical research is the quest for therapeutic alternatives to overcome refractory disease.
Aims: Since 2009, we have treated patients affected by Multiple Myeloma, relapsed and refractory to most of the therapeutic options available, with a chemotherapy based on a combination of pegylated liposomal doxorubicin, cyclophosphamide and dexamethasone (CED), at monthly doses respectively of 35 mg/m^2 on day1, 800 mg/m^2 on day 1 and 20 mg/d on days 1-4, with very interesting results.
Methods: Eighteen patients (11 women, 7 men), with a median age of 60 years, (range: 43-75) affected by advanced, relaplsed and progressive multiple myeloma, whose median number of previous treatments was 3 lines (range 2-6) were treated with monthly CED courses (median number of courses: 3; range: 1-17).
Results: One patient (5%) had a complete remission (CR), in nine patients (50%) there was a partial response (PR); the disease remained stable in two patients (11%) and six patients (33%) did not benefit from CED treatment. Median response duration was 6 months (range: 2-20). The toxicity profile of CED was satisfactory: hematological toxicity (WHO grade 2) was observed in 7 patients (38.8%) and there were extra-hematologic side effects only in two cases (11%) : an episode of acute renal failure in one patient and bradycardia in another.
Summary / Conclusion: In conclusion, we believe strongly that these results can encourage further studies as they have been obtained in patients with severely advanced disease stage, previously not exposed to anthracyclines. These preliminary data point to a significant result in 55% of patients with particularly advanced disease. However, these results have to be validated by controlled studies envolving larger number of patients.

B1544

TREATMENT RESPONSE IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA PATIENTS

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Background: Multiple myeloma is a malignant plasma cell disorder. It is the second most frequent haematological malignancy and characterized by malignant plasma infiltration or the bone marrow and is associated with an increased level of monoclonal protein in the blood and/or urine. The treatment of multiple myeloma (MM) has undergone significant developments in recent years. The development of new agents with potent anti-tumor activity has considerably improved the survival of MM patients.
Aims: Retrospective evaluation of the effect and safety of combination of bortezomib, doxorubicin and dexamethasone (PAD) in the treatment of relapsed/refractory myeloma patients.
Methods: 38 patients were treated for median of four 28-day PAD cycles (1-8). Bortezomib was given at 1.3 mg/m^2 (days1,4,8,11), doxorubicin at 9 mg/m2 (days 1-4) and dexamethasone 20 mg po (days 1-4, 8-11).
Results: 38 patients were evaluable for efficacy and safety, 61% had refractory disease and 39% were relapsed. The median age was 58 years (37-75), 52% were male, 48% female. Median time from diagnosis was 14 months (2-110) and median number of prior therapy lines was 1 (1-4): 72% had undergone conventional chemotherapy, 15% Alkerane and Dexamethasone and 13% were autografted. Overall response rate of 62% was observed, 30% of patients achieved a complete response (CR), 24% a very good partial response(VGPR), 32% a partial response (PR). Stable disease (SD) was observed in 14%. The median progression free survival (PFS) was 17,2 months. The most common grade 3-4 toxic effects were neutropenia 11%, thrombocytopenia 13%, anemia 7%, infections 9%, peripheral neuropathy 4.3% and gastrointestinal disturbances 2.1%. One toxic death (1.1%) due to sepsis was noted.
Summary / Conclusion: The combination of bortezomib, doxorubicin and dexamethasone (PAD) is well tolerated and induced clinically signifiant responses and prolonged remission duration in patients with relapsed and refractory MM

B1545

SIX YEAR RESULTS OF THE THERAPY OF MULTIPLE MYELOMA INTRACELLULAR PROTEASOME INHIBITOR

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Background: Advances in basic research on the biology of MM have led to the emergence of new effective drugs acting specifically on the mechanisms that regulate the growth of myeloma cells, their apoptotical death, the state of the bone marrow microenvironment, which has opened up new possibilities in the treatment of this incurable disease. Of these, the most promising was the intracellular proteasome inhibitor - bortezomib.
Aims: To evaluate the results of the six-year treatment of multiple myeloma (MM) intracellular proteasome inhibitors.
Methods: From December 2006. to December 2012 - 125 patients with MM were treated (55 men and 70 women) (aged 36 to 81 years (median 65 years)). The majority of patients were diagnosed with stage IIIA process, I stage on the ISS was found in 83 patients, II stage - in 30 and III stage - in 12 patients. Among the immunological types of MM prevailed myeloma G (75%), a myeloma A - was diagnosed in 20% of patients, myeloma Bence-Jones in 5% of patients. Each patient was held from 4 to 10 courses of therapy. As first line Bortezomib therapy was appointed in conjunction with MP in 35 (58,3%) and PAD in 25 (41,7%) patients. As second lines Bortezomib was given to 65 patients with relapsed and refractory MM . Bortezomib monotherapy was used at the dose of1,3 mg/m^2 in 20 patients (30,7%), in combination with dexamethasone in 25 patients (38,6%) and in the protocol CVD in 20 (30,7%) patients. The effect of treatment was assessed using standardized international criteria EBMT. Toxicity assessment was performed using the criteria of the National Cancer Institute of the United States, version 3.0.
Results: In the group of patients treated with Bortezomib as first line therapy - overall response rate was achieved in 82% patients (CR + nCR - in 24% and PR- in 58%). When using bortezomib in second lineas therapy - a complete response (CR and nCR) was achieved in 13 patients (20%), partial response (PR) – in 29 (45%), minimal response (MR) - in 8 patients (11, 9%). Thus, the overall clinical response rate was 76,9% (CR + nCR + PR + MR). The median time to achieve response was 3 months. The main side effects of bortezomib therapy were peripheral neuropathy in 58%, fever in 25%, fatigue in 45% of patients respectively. Infectious complications (herpes zoster, conjunctivitis) were noted in 33% of patients, thrombocytopenia and symptoms of gastrointestinal toxicity were reported in 42% and 35%, respectively. A statistical method of calculating the cumulative fraction of survivals (Kaplan-Meier) was used to evaluate survival, with P<0,05 established as the reliability criterion. In the group of patients who hed received bortezomib therapy, the median survival has not been reached, the 6-year survival rate - 74%.
Summary / Conclusion: Bortezomib is a highly effective therapeutic drug, which plays an important role in the treatment of MM, as I line and the subsequent lines.

B1546

LENALIDOMIDE IN COMBINATION WITH LOW-DOSE DEXAMETHASONE IN RELAPSE/REFRACTORY MULTIPLE MYELOMA: A RETROSPECTIVE STUDY

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Background: Two randomized controlled trials, MM009 and MM010, demonstrated that lenalidomide in combination with dexamethasone was superior to placebo plus dexamethasone in relapse/refractory multiple myeloma (rrMM).
Aims: We aimed to investigate the efficacy and safety profile of this combination in rrMM in a small-sized post-marketing analysis.
Methods: Primary end-point was overall response rate (ORR); secondary endpoints included progression-free survival (PFS) and overall survival (OS). Treatment (Rd) consisted of lenalidomide (25 mg on days 1 to 21) and dexamethasone (40 mg on days1,8, 15, 22) in repeating 4-week cycles. Dose modifications were implemented accordingly, if needed.

Table. Response to lenalidomide according to baseline characteristics

Response, N (%)	Overall	Age, years		Age, years		Previous thalidomide		Plasmocytoma		Previous ASCT	
		≥65	<65	≥75	<75	Yes	No	Yes	No	Yes	No
CR	16 (35.6)	13 (43.3)	3 (18.8)	5 (45.5)	11 (31.4)	8 (38.1)	8 (32.0)	3 (33.3)	13 (35.1)	4 (26.7)	12 (38.7)
VGPR	8 (17.8)	5 (16.7)	3 (18.8)	2 (18.3)	6 (17.1)	5 (23.8)	3 (12.0)	2 (22.2)	6 (16.2)	3 (20.0)	5 (16.1)
PR	11 (24.4)	8 (26.7)	4 (25.0)	3 (27.3)	9 (25.7)	4 (19.1)	8 (32.0)	2 (22.2)	10 (27.0)	5 (33.3)	7 (22.6)
SD	10 (22.2)	4 (13.3)	6 (37.5)	1 (9.1)	9 (25.7)	4 (19.1)	6 (24.0)	2 (22.2)	8 (21.6)	3 (20.0)	7 (22.6)
ORR	35 (77.8)	26 (86.7)	10 (62.5)	10 (90.9)	26 (74.3)	17 (80.9)	19 (76.0)	7 (77.8)	29 (78.4)	12 (80.0)	24 (77.4)
P			0.20		0.67		0.59		0.97		0.80

Results: A total of 46 (31 male) patients were included into the study. Median age was 67.5 (range: 44-86) years. Only 11 patients were older than 75 years of age. Median number of prior therapies was 3 (3-6). Proportions of patients with prior thalidomide use, autologous hematopoietic stem cell transplantation (ASCT), and plasmocytoma were 45.7%, 32.6`%, and 19.6%, respectively. ORR and complete response rates were 77.8% and 35.6%, respectively. Older age, prior thalidomide use, ASCT and plasmocytoma did not have an impact on response rates (Table). Median follow-up, PFS, and OS (95% CI) were 7 (7-12), 17 (8-30), and 18 (14-not reached) months. Grade III-IV adverse events included neutropenia (23.9%), anemia (19.5%), thrombocytopenia (17.4%), deep vein thrombosis (2.2%), infections (10.9%), and fatigue (6.5%).
Summary / Conclusion: Rd is highly active in patients with rrMM.

B1547

TREATMENT OF BENCE-JONES MULTIPLE MYELOMA WITH CHEMOTHERAPY WITH/WITHOUT STEM CELL TRANSPLANTATION-

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Background: Bence-Jones myeloma multiplex is a progressive disease characterized by excessive numbers of abnormal plasma cells in the bone marrow and overproduction of incomplete immunoglobulins,containing only the light chain portition of the immunoglobulini.This type of myeloma occurs 15-20%.The median overall survival is approximately 4 years.Pts outcome in Bence-Jones myeloma has been remarkably improved due to the use of combination therapies including chemotherapy and stemm cell transplantation.High dose melphalan with autologus stem cell support has been an integral part of myeloma therapy for more than 25 years.
Aims: A retrospective analysis of outcome of treatment Bence-Jones myeloma.
Methods: Since 1995. until 2011. we were treated 54 pts (37 men and 17 female), average age 56 years (range 36-85). According to the clinical stage, patients were divided as follows: 3 pts ICS, 13 pts IICS, 38 pts IIICS. Regarding ISS score, the group included: ISS1 16 pts, ISS2 9 pts, ISS3 29 pts. Renal insufficiency was present in 30 pts. High risik pts was defined by the presence of following factors: b2M >5,5 mg/l, albumine<3,5gr/dl, CRP>6, high lactat dehydrogenase, renal failure, stage III. Patients treated with induction, consolidation and maintenance therapy.Conventional induction treatments were applied as following regimens: VAD (40), MP (9), CTD (1), PAD(2) and TAD (2).
Results: Conventional chemiotherapy introductory clinical response was achieved in 35pts (65%) (MR-4 pts, PR- 14 pts, VGPR- 17 pts), while in 19 pts (35%) established disease was resistant. Transplantation had been done with 30 pts (56%), while 24 pts (44%) were treated with conventional chemotherapy adjusted to the vital age and comorbiditity. In the group of pts with transplantation done tandem had been carried out with 10 pts and secondary SCT had been done in 4 relapsed pts. With 1 pts with tandem SCT allogenic(singen) SCT had been done. TRM is3,4%. Maintenance therapy with Thalidomid had been done in 25 pts for 4-40 months. Impact of high risk factors on outcome/TTP/OS was of no significance, but the transplanted patients had significantly longer TTP (mediana 8 months vs 6 months, P=o,o11) and longer OS (mediana 44 months vs 21 months ,P=0,0003). 16 pts (30%) of treated pts are living, while 38 pts (70%) died. Univariate log. regres. analysis showed that non-transplant patients are 10,41 times more likely to terminate lethal compared to transplant patients (RR 10,41(95%C.I.43,47-2,52),P<0,001).
Summary / Conclusion: Our study showed ASCT is a more effective method of treatment of patients with BenceJones myeloma compared to the conventional chemotherapy, but the results are still unsatisfactory. One of the major efforts to improve the results of intensive therapy and ASCT involves the integration of novel agents (proteasome inhibitors and immunomodulatory drugs) into the transplantation sequence.

Myeloproliferative neoplasms - Biology

B1548

A DATA INTEGRATION STRATEGY FOR HIGH-THROUGHPUT OMICS ANALYSES ALLOWS THE PREDICTION OF NOVEL TRANSCRIPTION FACTOR TARGET GENES

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Background: We have demonstrated that expression of the transcription factor “Nuclear Factor Erythroid-2” (NF-E2) is aberrantly increased in patients with Myeloproliferative Neoplasms (MPN). Moreover, NF-E2 overexpression in a transgenic mouse model elicits a MPN-like phenotype, that includes the development of thrombocytosis and, most notably, transformation to acute myeloid leukemia (AML). However, since only a small number of NF-E2 target genes are known, the downstream mechanisms by which transcription factor overexpression promotes a MPN phenotype are not known.
During recent years, the reduced cost and subsequent increase in the use of high throughput methods has increased the public availability of datasets resulting from omics analyses. However, few published studies rely on multiple datasets concomitantly, especially not on data generated by other investigators. Here we provide a strategy for combing several publically available data sets for the prediction of novel transcription factor target genes. This strategy increases the predictive power of the results, thereby providing a more efficient use of resources, time and money. We demonstrate the efficacy of this approach using NF-E2 in the context of MPN as an example.
Aims: To combine available high-throughput datasets to generate highly promising candidates for further analysis as NF-E2 target gene.
Methods: NF-E2-ChIPseq data of K562 cells (ENCODE project) were downloaded both as raw data and as processed files. The peak information representing potential NF-E2 binding sites was used to link these peaks to the closest transcription start site (TTS) using the “ChIP-Seq Tool Set” from UCDavis. In the resulting list of genes, the GeneID was used as a unique identifier. In order to improve the predictive power and decrease the false positive rate of the ChIP-seq experiment, we correlated the results from various expression profiling experiments detailed below with the identified peaks. The following datasets were used: gene expression analysis of peripheral blood granulocytes from PV patients and healthy controls (Görttler et al., 2005), gene expression following NF-E2 modulation in CD34+-HSC (Wehrle et al., 2013) as well as several publically available datasets stored at the ArrayExpress repository, including a set of gene expression analyses from MPN-patients (E-GEOD-26049). In order to combine these datasets, the BioMart database was used to convert each of the different, dataset specific identifiers to GeneIDs. Subsequently, the BioMart database was used to obtain further annotation data such as gene description and chromosomal position. These datasets were merged into a Microsoft Access database, and subjected to further filter steps and analyses.
Results: The strategy detailed above was successfully used to identify novel candidate NF-E2 target genes. A large proportion of these have been subsequently verified by functional analyses including luciferase reporter gene assays of the identified promoter regions, site directed mutagenesis of the identified NF-E2 binding sites as well as induction of target gene expression by reconstitution of NF-E2 in NF-E2-deficient CB3 cells and repression of protein expression by shRNA against NF-E2. (Wehrle et al., 2013 / Peeken et al., unpublished / Reihnemann et al., unpublished / Siegwart et al., unpublished).
Summary / Conclusion: The combination of several high-throughput datasets from different techniques reprocessed to incorporate the same identifier allows the stringent prediction of novel transcription factor target genes. Compared to more conventional approaches, these analyses save a substantial amount of time and resources and result in a higher proportion of verifiable targets.

B1549

PROANGIOGENETIC MOLECULES VEGF, HIF-1ALPHA AND ENOS IN MYELOPROLIFERATIVE NEOPLASMS

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Background: Angiogenesis is important for the progression of hematological malignancies, including myeloproliferative neoplasm (MPN). Hypoxia-inducible factor-1alpha (HIF-1alpha) regulates the transcription of vascular endothelial growth factor (VEGF), a major gene responsible for angiogenesis. Several studies have demonstrated that a nonhypoxic pathway via nitric oxide (NO) is involved in the activation of HIF-1alpha and that NO enhances VEGF produc-

tion through stabilization of HIF-1alpha.
Aims: A target of this study is to correlate the expression of major angiogenic molecules VEGF, HIF-1alpha and endothelial NO synthase (eNOS) in bone marrow and peripheral blood of MPNs. Using this approach, we describe angiogenic factors in polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) according to JAK2V617F mutation status.
Methods: The expression of angiogenicfactors VEGF, HIF-1alpha and eNOS are examined in the bone marrow and granulocytes using immunohistochemistry and Western blot, respectively. Microvessel density is assessed by immunostaining with CD34 and CD105 antibodies. VEGF and HIF-1alpha mRNA levels are measured by real time quantitative PCR. ELISA-based assay with fluorogenic substrates is used to determine total HIF-1alpha in the context of a whole cell as a HIF-1alpha /Cytochrome C ratio.
Results: Using DNA sequencing, JAK2V617F mutation is detected in 90% of PV and per 60% of both ET and PMF patients, out of total 198 MPN patients. By immunohistochemistry, we show that $CD34^+$ cells are generally increased in bone marrow biopsies of PMF patients despite of JAK2 mutation, as well as in JAK2 negative ET patients. The angiogenic marker CD105 demonstrates elevation in the bone marrow of all forms of MPN patients. The highest percentage of both $CD34^+$ and $CD105^+$ cells is observed in PMF patients, not influenced by JAK2 mutant allele burden. VEGF mRNA levels are increased in hematopoietic progenitor $CD34^+$ cells and granulocytes of JAK2 mutation positive PV and PMF patients. VEGF protein expression is double increased in the granulocytes of JAK2 mutation positive ET patients, and also demonstrates an increase in homozygous JAK2 mutation forms of PV patients. Immunohistochemical analyses reveal that the percentage of VEGF-positive cells is increased in the bone marrow of JAK2 mutation positive ET patients. eNOS protein expression is decreased in granulocytes of JAK2 mutation positive ET and PMF patients. Also, eNOS protein expression is generally decreased in the bone marrow of MPNs, except JAK2 mutation negative PMF patients which demonstrates 30% increase. HIF-1alpha gene expression is increased in hematopoietic progenitor $CD34^+$ cells of JAK2 mutation positive PV and PMF patients. HIF-1alpha protein expression is decreased both in granulocytes and the bone marrow of PMF patients, as well as in the bone marrow of JAK2 mutation positive ET patients. HIF-1alpha is reduced in the granulocytes of MPN in comparison to healthy controls, while was diminished in peripheral mononuclear cells. Presence of JAK2 mutation augments HIF-1alpha protein level in PV patients.
Summary / Conclusion: CD105 expression, being highest in PMF, is a better marker of microvessel density compared to CD34. VEGF protein level is generally increased in ET patients, while HIF-1alpha and eNOS protein levels are reduced in MPNs. Presented variations in angiogenic factors expression among certain types of MPNs and its JAK2 mutant allele burden can be of predictive significance.

B1550

EXPRESSION ANALYSIS OF JAK-STAT DEPENDENT S100 CALCIUM BINDING PROTEINS A4 AND A12 IN MYELOPROLIFERATIVE NEOPLASMS

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Background: S100 calcium binding proteins are generally up-regulated in tumors and play essential roles in both tumor progression and suppression. S100A4 positively regulates tumor cell proliferation, invasion and metastasis, while proinflammatory S100A12 is mostly expressed in human granulocytes.
Aims: S100A4 and S100A12 are produced via activation of the JAK-STAT kinase pathway, while JAK2 mutation induces constitutive activation of JAK/STAT signaling in myeloproliferative neoplasms (MPN). Therefore, determination of S100A4/12 mRNA and protein levels in peripheral blood and bone marrow can represent the potential markers of development and prognosis in MPN.
Methods: Using immunohistochemistry and proteinarray we analyzed S100A4 and S100A12 protein expression in bone marrow and granulocytes of MPN, respectively, as well as S100A4 and S100A12 mRNA levels in hematopoietic progenitor $CD34^+$ cells and granulocytes of MPN by microarray analysis.
Results: S100A4 gene expression is significantly increased ($P<0.01$) in hematopoietic progenitor cells of ET and PV patients despite to JAK2 mutation, while in PMF do not reach a statistical significance compared to healthy controls. S100A4 gene expression is generally reduced in granulocytes of MPN patients. The level of S100A4 protein was decreased in bone marrow of MPN patients compared to healthy controls, while JAK2 mutant allele burden increased protein expression only in bone marrow of PMF patients. S100A4 protein expression is decreased in granulocytes of PV patients compared to other MPN, regardless of JAK2 mutation. Moreover, S100A4 protein expression is increased in JAK2 positive PMF patients in comparison to MPN patients without JAK2 mutation. In contrary, proinflammatory S100A12 protein expression is increased in JAK2 mutation negative MPN patients compared to mutation positive MPN patients, where PMF patients demonstrated the most increased S100A12 protein expression in granulocytes. S100A12 protein expression was slightly decreased in bone marrow of PV and PMF patients, while was significantly increased in JAK2 heterozygous ET patients, compared to healthy controls. S100A12 gene expression, absent in controls, is increased in hematopoietic progenitor cells of PV patients.
Summary / Conclusion: Overexpression of S100A4 gene in hematopoietic progenitors of MPN is not associated with the presence of JAK2 mutation, while S100A4 protein has decreased expression in bone marrow of MPN. Interestingly, JAK2 mutant allele burden only increased S100A4 protein expression in bone marrow and granulocytes of patients with PMF. S100A12 protein expression was in favor of MPN without JAK2 mutation, but still PMF demonstrates its dominance in granulocytes of JAK2 positive MPN. It is possible that these two members of the S100 family may be a target for therapy or have a role in staging of MPN clinical setting and support in treatment conclusion.

B1551

MICRORNA EXPRESSION ANALYSIS IN PATIENTS WITH PRIMARY MYELOFIBROSIS, POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTOSIS

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Background: MicroRNAs (miRNA) are 19-22 nucleotides in length, non-coding small RNA molecules. These small molecules are thought as non-functioning molecules in the past, but today it was understood that they have critical roles in cell differentiation, proliferation and apoptosis. A miRNA can be specific for a large number of target genes and it is thought that they have critical roles in regulating gene expression.
Aims: In this study, we aimed to open a new door for the development of targeted therapies in Philadelphia chromosome (-) myeloproliferative disorders consisting of primary myelofibrosis (PMF), polycythemia vera (PV) and essential thrombocytosis (ET) by researching the roles of miRNAs in the pathogenesis of these diseases.
Methods: Forty-nine patients with ET, 33 patients with PV, 22 patients with PMF (46 males, 58 females) and 40 healthy volunteers were included in the study. The co-morbidities and drugs used by patients (hydroxyurea, anagrelide, interferon) were noted. *JAK2* positivity was determined. The expression levels of mir155, mir181a, mir221, mir222, mir223, and mir451 were determined by RT-PCR and analyzed by $\Delta\Delta$CT method. The statistical analysis of the data was performed by using Kruskal Wallis and Dunn tests.

		mir155	mir181a	mir221	mir222	mir223	mir451
Control	n	40	40	40	40	40	40
	%25	0,00019	0,11933	0,86799	0,01627	5,149E-7	1490195,13331
	median	0,00037	0,25122	2,42670	0,02892	22,23952	5620942,30299
	%75	0,00064	0,82503	8,97562	0,07619	65,72211	42569759,79780
PV	n	33	31	26	25	27	33
	%25	0,00036	0,13490	1,75430	0,00263	2,73277	7017,45068
	median	0,00126	0,30166	10,32460	0,01912	12,00535	88532,38002
	%75	0,00305	0,04200	71,95002	0,00350	35,50030	519404,01602
ET	n	49	46	42	43	44	46
	%25	0,00273	0,00216	1,40992	0,0314-7	28,03377	7680,33057
	median	0,00187	0,30681	22,33815	0,10972	87,81531	73275,66169
	%75	0,00762	1,62979	140,62741	1,89121	1669,01267	879232,88400
PMF	n	19	19	15	17	18	17
	%25	0,00361	0,09929	3,49429	0,0114-7	42,00257	27084,65632
	median	0,00358	0,45470	16,98277	0,04694	335,39857	538554,24585
	%75	0,05001	1,31850	51,96862	2,63616	22752,6+884	4404235,53575

Results: Differences were determined in the expression levels of mir155, mir221, mir222, mir223, mir451 among the groups (table 1). It was determined that mir155 was expressed in higher levels in all 3 disorders compared to the control group ($P<0.05$). Mir221 was found in higher levels especially in ET and PMF group ($P<0.05$). Mir222 expression was found lower in PV patients ($P<0.05$) and higher in ET, and PMF patients compared to control group. Mir223 expression was found higher in ET, and PMF group than the control group ($P>0.05$). Mir451 levels were lower in all 3 groups compared to the control group ($P<0.05$). Between the groups, there was no difference in expression levels of mir181a. It was also determined that *JAK2* positivity, co-morbid diseases, drugs used by the patients, and gender did not affect the miRNA expressions.
Summary / Conclusion: In this study, we showed the different expression levels of stated miRNAs. In the future, these molecules can be used for the differential diagnosis and as the targeted molecules in the treatment of myeloproliferative disorders.

B1552

APOPTOSIS-RELATED GENES METHYLATION PROFILE IS ALTERED IN MYELOPROLIFERATIVE NEOPLASMS PHILADELPHIA NEGATIVE PATIENTS

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Background: Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF) are chronic Myeloproliferative Neoplasms chromosome Philadelphia negative (MPN Ph-) characterized by a myeloaccumulation and apoptosis deregulation. Epigenetic regulation, such as altered DNA methylation profile of apoptosis-related genes promoters CpG islands may be involved in this process.

Aims: (1) To investigate the apoptosis-related genes promoters CpG island methylation status in MPN Ph- patients and SET-2 JAK2 V617F positive cell line; (2) To compare apoptosis-related gene methylation profile among PV, ET and PMF patients and (3) To associate JAK2 V617F mutation status with methylation results.

Methods: This study enrolled six patients: 2 PV, 2 ET and 2 PMF (4 males and 2 females, mean age(ma)=60 years). For each disorder, one patient was JAK2 V617F positive and the other was JAK2 V617F negative. For the control group, 3 health subject were studied (1 male and 2 female, ma=55.3y). This research was approved by the local ethics committees (protocol n. 247) and the consent form was signed by the patients and volunteer controls. The DNA was isolated from leukocytes and SET-2 cells by phenol-chloroform protocol, treated with RNAse and purified using MiniElute Purification kit®. The methylation profile of *APAF1*, *BAD*, *BAG1*, *BAX*, *BCL2*, *BCL2L11*, *BCLAF1*, *BID*, *BIK*, *BIRC2, BNIP3L, CASP3, CASP9, CIDEB, CRADD, DAPK1, DFFA, FADD, GADD45A, HRK, LTBR, TNFRSF21, TNFRSF25, WRAP53* genes was analyzed by *Epi-Tect Methyl qPCR Array System*®. The results were expressed as hypermethylated (HY) or unmethylated (UM) percentage of the gene.

Results: JAK2 V617F positive PV patients showed higher HY percentage of *BCL2* (8.42%), *CASP3* (16.16%), *CASP9* (14.17%), *CRADD* (33.58%), *DFFA* (13.93%), *TNFRSF25* (9.66%) and *WRAP53* (10.16%) than controls (mean percentage of HY: 0.74%, 1.23%, 6.25%, 3.59%, 1.48%, 1.21% and 1.77%, respectively). JAK2 V617F negative PV patient showed increased HY percentage of *CRADD* (23.6%), *DFFA* (8.77%) and *WRAP53* (10.61%) in comparison to controls, as for JAK2 V617F positive PV, but *CIDEB* HY percentage was higher in JAK2 V617F negative PV patient (34.68%) compared to controls (mean: 4.10%) and JAK2 V617F positive PV (1.88). Furthermore, JAK2 V617F positive PMF patients showed *CRADD* (18.7%), *DFFA* (13.20%) and *WRAP53* (7.64%) with higher HY percentage than controls and JAK2 V617F negative PMF patients. In contrast, *LTBR* gene HY percentage was lower in JAK2 V617F positive PMF (6.66%) compared to controls (mean: 27.68%), to JAK2 V617F positive PV (30.76%), JAK2 V617F negative PV (19.10%) and JAK2 V617F negative PMF (30.09%). ET patients, SET-2 cells and controls showed a very similar apoptosis-related gene methylation profile. *LTBR* gene HY percentage was lower in JAK2 V617F positive ET (11.57%), JAK2 V617F negative ET (11.08%) and SET-2 cells (1.03%) than in controls (mean: 27.68%).

Summary / Conclusion: Increased methylation may be involved in apoptosis-related genes deregulated expression in MPN, as highlighted by *BCL2*, *CASP3*, *CASP9* and *CRADD* hypermethylation percentage results in JAK2 V617F positive PV patients. Supported by FAPESP (2011/20135-2, 2011/51616-6).

B1553

HUMAN PLATELET LYSATE AS A POTENTIAL SUBSTRATE FOR STUDYING MECHANISMS OF MYELOFIBROSIS DEVELOPMENT

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Background: Mechanisms of myelofibrosis development in chronic myeloproliferative disorders are a subject of current research interest, because despite lots of new therapeutic options this phenomenon remains to be hardly treated. Human platelet lysate (HPL) is a good substrate to explore the inner mechanism of influence of megakaryocytic system on bone marrow stromal cells in laboratory experiments.

Aims: Characterization of human platelet lysate (HPL) from patients with primary myelofibrosis (PMF) and post-polycythaemia vera myelofibrosis (post-PV MF). Exploration of how HPL influence proliferation rate of mesenchymal stromal cells (MSC). Evaluation of collagen expression, as a main component of extracellular matrix, in MSC cultured in presence of HPL.

Methods: HPL was produced from pooled platelet concentrate of healthy donors and platelet-rich plasma of patients with PMF and post-PV MF; patients' HPL was normalized according to protein concentration. MSC were isolated from bone marrow of healthy donors and cultured in presence of varying concentrations of HPL (5, 10, 20%) or 10% fetal bovine serum (FBS) as negative control. Proliferation of MSC was quantified using MTT assay. Concentration of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor – beta (TGFβ) and hepatocyte growth factor (HGF) was determined in HPL from fourteen patients with PMF and three patients with post-PV MF using specific ELISA kits. Immunofluorescence was used to estimate collagen type 1 and type 3 expression in MSC in different culture conditions.

Results: We observed a significant increase in VEGF and bFGF concentration (2,5 and2,4 fold, respectively, compared to the age-matched healthy controls, P<0.01) in HPL from patients with myelofibrosis. For TGFβ and HGF we found a tendency to increase in concentration (1,2 and1,7 fold, respectively), but the difference was statistically insignificant (P=0,2). In case of culturing MSC with HPL from patients with myelofibrosis, we found that cells maintained their proliferative activity at the same level as with HPL from age-matched healthy controls (optical density – 0,155 and 0,145, accordingly, P=0,17). Using immunofluorescence with specific antibodies, we showed a constant high expression of collagen type 1 in MSC regardless of culture conditions, whereas expression of collagen type 3 increased when cells were cultured in higher concentration of HPL (20%).

Summary / Conclusion: To the best of our knowledge, this is a first attempt to reproduce cellular events in myelofibrosis using HPL from patients with chronic myeloproliferative disorders. Our data demonstrate that patients' HPL can be used as a substrate for culturing of mesenchymal stromal cells in order to study the mechanisms of myelofibrosis, as it contains increased concentration of growth factors and maintains MSC proliferation rate. We also showed that composition of extracellular matrix proteins depends on the HPL concentration. This is an ongoing study and we are working further to evaluate HPL from patients with myelofibrosis.

B1554

JAK2 46/1 HAPLOTYPE, JAK2V617F MUTATION AND CLINICAL CARACERISTICS OF THE MACEDONIAN PATIENTS WITH CLASSICAL MYELOPROLIFERATIVE NEOPLASMS

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Background: It is predicted that the inherited genetic background in the individual patients with classical myeloproliferative neoplasm (MPN) influences the disease susceptibility and the phenotype expression of the MPN. Recently, several groups suggested that JAK2V617F positive MPN are acquired preferentially on a specific constitutional germline JAK2 46/1 haplotype which is tagged by the "C" allele of single nucleotide polymorphism (SNP)rs12343867 (C/T), and indicate the genetic basis for predisposition to MPN. But, subsequent data, showed equal distribution of this SNP among JAK2V617F negative MPN, indicating that it is a potential common inherited susceptibility factor for MPN. Moreover, only few studies investigated the potential role of the JAK2 46/1 haplotype at the MPN phenotype in context of the clinical presentation and the complication of the diseases.

Aims: In order to extend further those observations we conduct a retrospective study to assess the frequency of JAK2 46/1 haplotype in a group of patients with MPN in comparison with population controls. Furthermore, we evaluate the association of 46/1 with the JAK2V617F mutational status and the clinical characteristics in the series of patients with MPN that were diagnosed and treated at the University Clinic of hematology-Skopje, Republic of Macedonia.

Methods: The study group consisted of 212 adult (>15 years) patients with MPNs that were diagnosed and followed at our Institution. According to the 2008 WHO criteria 79 patients were classified as Polycythemia vera (PV), 95 as Essential thrombocythemia (ET), 10 as Myelofibrosis primaria (MP) and 28 were classified as atypical (a) MPNs.The 46/1 tag SNP rs12343867 (C/T) was genotyped using the TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The JAK2 V617F mutation was analyzed by fluorescent allele-specific PCR followed by CE on ABI 310 Genetic analyzer .

Results: The incidence of 46/1-linked C allele was significantly higher in all MPN entities [PRV (0.538), ET (0.437), MF (0,464), and in aMPN (0.55} in comparison with healthy controls (0.290) (P<0,01 for all comparisons); The frequency of the JAK2V617F mutation ranged from 89%in PV, 67% in ET, 60% in MP to _46,4% in the aMPN. The frequency of the JAK246/1 C allele was significantly higher in the JAK2V617F positive patients with PV, MP and aMPN(P<0,01 for all comparisons) except for ET patients, in which genotype distributions were similar among JAK2V617F positive and negative patients (genotype: CC 7/14%, CT 22/29% , TT 67/57%; C-allele frequency 41/43%; P=0,76).
Correlations of the clinical features at diagnosis and long-term prognosis between the two JAK2 46/1 different MPNs groups revealed comparability regarding all tested parameters such as blood counts, NAP score, rate of thrombotic and hemorrhagic complications, disease transformation and survival.

Summary / Conclusion: Our results confirmed latest observations that JAK2 46/1 haplotype is a susceptibility factor for developing ET independent of

JAK2V617F mutational status and does not further affect the clinical course and prognosis of the disease .Our findings indicate that JAK2 46/1 haplotype predispose for development of MPN trough "the fertile ground hypothesis" which suggest that cells that are carrying the haplotype gain selective advantages in situations when oncogenic mutations occur.

B1555

GENETIC ABNORMALITIES IN DIAGNOSTICS OF BCR-ABL-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background: Genetic mutations result in abmormalities of myelopoietic proteins and lie in the basis of myeloproliferative neoplasms (MPNs) development and its subsequent progression.
Aims: The aim of our study was to assess frequencies of JAK2, MPL mutations and cytogenetic aberrations in patients with BCR-ABL-negative MPNs.
Methods: Blood samples from 547 patients with BCR-ABL negative MPNs were selected. The investigated group included 203 cases of Polycythaemia vera (PV), 102 cases of Essential thrombocythemia (ET), 102 cases of Primary myelofibrosis (PMF) and 140 cases of Chronic myeloproliferative disease, unclassified (CMPD-U). The average age of the patients group was 52 years (18-80 years). The peak of the incidence of diseases was at the age between 50 and 60 years. Polymorphism of JAK2 (V617F) was defined by PCR-RFLP assay. Mutations in exon 12 of JAK2 were detected by sequence analysis in 69 PV patients. Polymorphisms of MPL (W515L; W515K) were defined by Realtime PCR in two replications in 44 ET patients and 51 patients with PMF. Conventional cytogenetics of bone marrow (BM) with Chromosome banding analyses were performed for 129 patients.
Results: The frequencies of JAK2 617F allele have been detected as follows: 99,0% (201/203) in PV, 56,4% (58/102) in ET and 49,7% (50/101) in PMF. The frequency of 617F allele JAK2 in CMPD-U was8,6% (12 of 140 cases) and confirmed MPN diagnosis for these 12 patients. The frequency of mutations in exon 12 of JAK2 was2,9% (2/69) in group of patients with CMPD-U. The frequencies of MPL 515L allele were2,3% (1/44) in ET and 2% (1/51) in PMF. Cytogenetic analysis of the BM cells stratified 129 patients into different prognostic groups. Normal karyotypes were defined in 85,3% cases (110 of 129). Aberrant karyotypes were defined in 14,7% cases (19/129) and included3,9% (5 cases) karyotypes with isolated chromosomal aberrations (del(20q), del(13q) (favourable prognosis),6,2% (8 cases) karyotypes (intermediate risk), and4,7% (6 cases) karyotypes with compelx karyotypes (unfavourable prognosis). Isolated chromosomal aberrations which cause favourable prognosis were defined reliably more often in PV cases than in PMF cases (p<0,0000). Moreover, the frequency of the complex karyotypes was statistically higher in PMF cases as compared to PV and ET cases (p<0,0000). 2 of 6 patients with complex karyotypes in karyotypes had transformation from MPN to AML.
Summary / Conclusion: Point mutations in JAK2 and MPL genes are specific markers for patients with BCR-ABL-negative MPNs. The integration of molecular genetics with cytogenetics helps to stratify patients into different risk groups and optimize treatment strategy.

Myeloproliferative neoplasms - Clinical

B1556

RECURRENT CEP85L-PDGFRB FUSION IN A PATIENT WITH A TRANSLOCATION T(5;6) AND AN IMATINIB-RESPONSIVE MYELOPROLIFERATIVE NEOPLASM WITH EOSINOPHILIA

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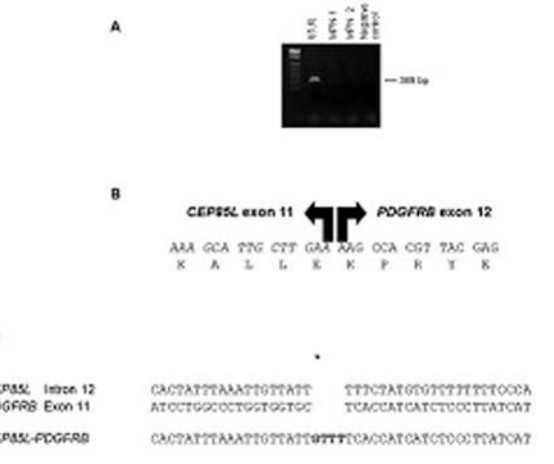
Background: Fusion genes involving the catalytic domain of tyrosine kinases (TKs) play an important role in the pathogenesis of hematological malignancies and solid tumors. In *BCR-ABL1*-negative myeloproliferative neoplasms (MPNs) several different tyrosine kinase fusion events have been described, most commonly involving the genes encoding the platelet-derived growth factor receptor alpha (*PDGFRA*) or beta (*PDGFRB*). Since the introduction of small molecule kinase inhibitors, TK fusions have emerged as prime therapeutic targets. Here, we present the case of a 45 year old male with persistent unexplained eosinophilia. A bone marrow aspirate and biopsy showed increased cellularity with myeloid expansion and marked eosinophilia without signs of monoclonality. Cytogenetic analysis on a bone marrow aspirate revealed a 46,XY,t(5;6)(q3?3;q2?2).
Aims: In this patient our objective was to investigate the underlying fusion gene of his translocation t(5;6) and if possible use it as a molecular marker during treatment.
Methods: Break apart FISH using previously described in house probes demonstrated that *PDGFRB* at 5q33 was disrupted but *ETV6-PDGFRB,* the most common fusion involving this gene, was not detected by RT-PCR.Standard Gold Taq Polymerase based PCRs were performed on cDNA and gDNA extracted from peripheral blood leukocytes. Sanger Sequencing was performed on cDNA and gDNA in forward and reverse. Peripheral blood samples were received from this patient at 7 different time points before and after treatment.



Results: At the time of initial analysis no similar translocation had been reported and therefore the diagnostic investigations suggested the likely presence of a novel fusion involving *PDGFRB* and an unknown partner gene on chromosome 6. Initial attempts at RACE-PCR were unsuccessful and hampered by limited availability of suitable pre-treatment material. Subsequently, a novel *C6orf204-PDGFRB* (now known as *CEP85L-PDGFRB*) fusion was reported in a patient with T-cell acute lymphoblastic leukemia (T-ALL). Thus, we hypothesized that our patient may harbour the same or similar genetic defect. For the RT-PCR, a product was amplified from the patient with the t(5;6) but not controls which upon sequencing revealed an in frame cDNA fusion between exon 11 of *CEP85L* and exon 12 of *PDGFRB.* For gDNA, Sequencing showed the genomic fusion was between intron 12 of *CEP85L* and exonic sequence of *PDGFRB* exon 11 (Figure 1)**.**Five months after the start of imatinib treatment neither the genomic nor the mRNA fusion was detectable by nested PCR or RT-PCR. *CEP85L-PDGFRB* remained undetectable in all subsequent samples with a follow up of over 3 years. Imatinib was continued without interruption during this time The sensitivity of the PCR assay to detect the *CEP85L-PDGFRB*-fusion gene was 10^{-4} .
Summary / Conclusion: Here, we report a recurrent *CEP85L-PDGFRB* fusion in a patient with eosinophilia and an MPN. The fusion was confirmed by spe-

cific amplification of the genomic breakpoints and reverse transcription polymerase chain reaction (PCR).The patient was treated with imatinib and achieved hematologic and cytogenetic remission. Minimal residual disease screening over 3 years with nested PCR failed to detect *CEP85L-PDGFRB* mRNA or genomic DNA, confirming a long term molecular remission on imatinib. In our view, the detection of the exact gene fusion is clinically relevant for effective long term management of these neoplasms as it enables specific follow up by sensitive molecular analysis.

B1557

CLINICAL SIGNIFICANCE OF IMMATURE PLATELET FRACTION IN BCR-ABL1-NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Background: Platelet activation plays a pivotal role in the pathogenesis of BCR-ABL1-negative myeloproliferative neoplasms (MPN)-associated thrombosis. Evidence is mounting to support a potential usefulness of immature platelet fraction (IPF) measurement for vascular risk stratification in various thrombotic disorders.
Aims: The aim of this study was to characterize the clinical and laboratory determinants of IPF in patients with BCR-ABL1-negative MPN. In addition, we investigated the association between IPF and previous thrombosis.
Methods: One-hundred thirty-five patients have been studied. Sixty-one patients (45.2%) had ET, 25 (18.5%) PV, and 21 (15.6%) MPN-U. Among the 28 (20.7%) patients with myelofibrosis, 25 patients had PMF, while 3 patients had post-TE or post–PV myelofibrosis. Forty-eight patients had a history of previous thrombotic event, including arterial thrombosis (n = 31), venous thrombosis (n = 14), or both (n = 3) events. Complete blood counts, including the measurement of IPF were performed in whole blood by the fully automated hematology analyzer XE-2100 (Sysmex).
Results: In patients on cytoreductive therapy but not in untreated patients, IPF% was significantly higher in those with previous thrombosis than in non-thrombotic patients [2.4 (1.7-3.4) vs. 3.3 (2.4-5.1) %, P=0.011]. Similarly, in patients aged ≥ 60 years but not in younger patients, IPF% was significantly higher in those with previous thrombosis than in nonthrombotic patients [2.6 (1.8 -3.7) vs. 3.6 (2.4 - 5.2)]. In the entire population, a significant inverse correlation has been observed between platelet count and IPF% (Rho = - 0.23, P=0.008). In addition, in non-PV patients, IPF% was not significantly different between JAK2 V617F positive vs. negative patients. Multivariate logistic regression showed that only male gender (odds ratio, 3.3; 95% CI, 1.4 to 8.0; P=0.007) and the upper tertile of IPF% (odds ratio, 3.7; 95% CI, 1.2 to 10.7; P=0.018) are independently associated with a history of previous thrombosis, after adjusting for age, hematocrit, white blood cell count, platelet count, cardiovascular risk factors, underlining diagnosis, JAK2 mutational status and cytoreductive therapy.
Summary / Conclusion: We found that increased platelet turnover, as reflected by high IPF%, is associated with a history of thrombotic events in patients with MPN. In addition, our data support the hypothesis that current antithrombotic therapy might not specifically address this mechanism of thrombogenesis. New prospective studies are warranted to evaluate the usefulness of incorporating IPF% in risk stratification models to better identify patients at increased risk for thrombotic complications and/or treatment failure.

B1558

DISEASE CHARACTERISTICS AND PERIPHERAL BLOOD CD34+ CELLS IN IDIOPATHIC MYELOFIBROSIS

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Background: Idiopathic Myelofibrosis (IMF) is chronic myeloproliferative neoplasm characterized by constitutive mobilization of hematopoietic stem cells (HSC) and progenitor cells (HPC) into the peripheral blood (PB). The interaction between the chemokine CXCL12 and its receptor CXCR4 plays a pivotal role in determining the trafficking of CD34+ cells between the bone marrow (BM) and the PB.
Aims: IMF is associated with downregulation of CXCR4 by CD34+ cells due to epigenetic events. Altered gene expression was corroborated by the detection of abnormally high CD9 or CD164, and low CXCR4, membrane protein expression in IMF CD34+ cells. Moreover, endothelial precursor cells (CD34+/CD133+) are increased in the blood of a subset of patients with IMF, and peripheral endothelial cells bear the same molecular markers as hematopoietic cells, suggesting a primary role of pathological endothelial cells in this disease.
Methods: We evaluated, by flow cytometry, the number of CD34 positive cells in peripheral blood and the expression of CXCR4, CD9, CD117 and CD133 on these cells. In our institution we are following 31 patients affected by IMF, according to WHO criteria (M: 18, F: 13; median age: 57 years, range: 48-68 years).
Results: In all patients, at diagnosis, we found a high count of CD34+ cells in PB (greater than $15x10^6$/l; median:2,$4x10^6$/l, range:1,8-3,$2x10^6$/l) compared with normal controls and other Philadelphia-negative chronic myeloproliferative neoplasms. In all cases CD34+ cells were negative for CXCR4 while expressing high intensity CD9. About 40% of CD34+ cells expressed CD133, while 20% expressed CD117 at low intensity. In no case was detected coexpression of CD133 and CD117, suggesting a simultaneous presence of two distinct hematopoietic progenitors, endothelial progenitors and myeloid progenitors. We monitored every 6 months the phenotypic pattern of CD34+ cells, and after 36-48 months we observed an increase of myeloid precursors (CD34+/CD117+: 45,7%) compared with a reduction of endothelial precursors (CD34+/CD133+: 15,3%) in patients who showed clinical and laboratory signs of disease progression.
Summary / Conclusion: By comparing these findings with other clinical data, our results seem to confirm that, according to the natural history of disease from an initial stage towards a fibrotic phase (pancytopenia and/or splenomegaly), there was a change in PB CD34+ cells. Immunophenotypic profile of PB CD34+ cells is associated in IMF with patients' clinical characteristics and may have potential prognostic application.

B1559

THROMBOTIC CEREBRAL EVENTS IN ESSENTIAL THROMBOCYTHEMIA

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Background: Patients with Essential Thrombocythemia (ET) are frequently asymptomatic and many remain so. Symptomatic patients tend to present with thrombotic manifestations in distinct locations. The thromboses are more commonly arterial than venous. The thrombotic cerebral events (TCE) are rare, also if ET patients may present a wide spectrum of neurologic symptoms (NS) at onset or during the course of the disease, secondary to microvascular involvement
Aims: We retrospectively described CTE occurred in 310 patients affected by ET referred in our centre from 1990 to 2012 in order to identify clinical and biological features associated to these complications
Methods: We analysed the incidence of TCE; in particular we evaluated the prevalence of JAK2V617F mutation in this setting and the possible role of JAK2 mutation in the management of CTE
Results: 33/310 patients (10.6%) presented TCE. The average age of TCE patients was 71 years, with prevalence of female sex (21 vs. 12 patients). Only 4 patients were high risk for 2 o more cardiovascular factors (hypertension, diabetes, obesity, dyslipidemia, smoking, thrombophilia). 54,5% of patients developed CTE before diagnosis of myeloproliferative neoplasm (MPN), with a median time of 16 months of latent phase of myeloproliferative disease prior to confirmed diagnosis of ET. Of the 22 TCE preceding diagnosis, 18 were arterial events (8 transient ischemic attack, 9 strokes), only 2 events were venous (2 ocular thrombosis). 7 patients (21,2%) developed CTE as presentation of disease. All these patients received anti-platelets treatment starting from thrombotic event. At time of diagnosis, cytoreductive therapy, mainly with hydroxyurea, was started. Therefore, 11 patients (33,3%) developed TCE after diagnosis of ET, despite of anti-platelet and cytoreduction, with 15% of patients (5/33) with recurrent neurological thrombosis. The blood count at diagnosis and at time of TCE was similar, in particular no leucocytosis or extreme thombocythemia were present. The median value of hematocrit, white blood cell count and platelets at time of CTE were 41%, 7.720/mm3 and 650.000/mm3, respectively. JAK2V617F mutation was evaluated in 30/33 patients, with significant prevalence of JAK2 positive versus negative patients (83,3% vs. 16,7%). All patients with recurrent CTE were JAK2 mutated. Overall, 91% of patients (30/33) are alive, with an average follow-up of 57,6 months
Summary / Conclusion: we observed that 10,6% of ET patients presented CTE, with high prevalence (75,7%) of cerebral events as first presentation at time of diagnosis or as first sign of latent disease, for CTE antecedent to diagnosis. The majority of cerebral events were arterial. Mostly of patientswere females and presented higher prevalence of JAK2V617F mutation. This may lead to the fact that, in selected cases of CTE, where there is evidence of laboratory signs suggestive of MPN, the detection of the *JAK2*V617F mutation provides an early diagnosis of MPN. Moreover, a subgroup of patients (33,3%) developed CTE also after ET diagnosis and during antiplatelet and cytoreductive treatment, with high risk of thrombotic CTE recurrence (15%). This seems to suggest that in this selected setting, an enhancement of thrombotic prophylaxis could be proposed, for example with association of cytoreduction and anti-coagulant oral therapy, in presence of JAK2 mutation or other significant cardiovascular factors or thrombophilia predisposition

B1560

CHARACTERIZATION OF DIFFERENT REGIMENS FOR INTRODUCING SECOND-LINE ANAGRELIDE: RESULTS FROM A MULTICENTER STUDY OF 177 PATIENTS IN FRANCE

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Background: Anagrelide (ANA) is indicated in the EU for at-risk patients (pts) with essential thrombocythemia (ET) and at least one of: >60 years; platelet count >1000x10^9/L; history of thrombo-hemorrhagic events; in whom prior therapy (PT) is not sufficiently effective or well tolerated. The Summary of Product Characteristics (SPC) recommends starting ANA at 1.0mg/day, in two divided doses and maintaining this dose for ≥1 week. Then the dose may be individually titrated to achieve the lowest effective dose required to reduce and/or maintain platelet count <600x10^9/L, ideally at 150–400x10^9/L. The dose increment must not exceed 0.5mg/day per week and 2.5mg is the maximum single dose. There is no recommendation on how to transition from PT to ANA.
Aims: This observational study (NCT01192347) aimed to identify switch modalities used when introducing ANA and determine their influence on 6-month (mo) outcomes (including efficacy, tolerability and maintenance on ANA at 6 mo) in 44 clinical sites across France.
Methods: Pts were enrolled within 1 mo of switching to ANA; up to 1 mo of retrospective data were collected. As this was a non-interventional study, dosing schedule and follow-up visits were at the investigator's discretion. Pts were followed up for 6 mos. All relevant data were collected and recorded from pt records at the end of the follow-up.
Results: In total, 177 pts were enrolled (safety set n=175), the majority were female (62%) and aged >60 years (76%). Median age was 70 years. Median baseline platelet count was 553x10^9/L. Intolerance to therapy (65%) and inefficacy (41%) were the most frequent reasons for treatment switch (factors not mutually exclusive). ANA starting doses ranged from 0.3–1.5mg/day. The SPC□recommended starting dose was used most frequently (53%). However a notable proportion of pts started on 0.5mg/day (41%). The median ANA dose at study end was 1.5mg/day (range 0.3–4.0mg/day). The method of ANA introduction was consistent with the SPC in 76% of pts. Almost all pts switched to ANA from hydroxycarbamide (93%). Most pts discontinued PT before ANA was introduced (66%; Group A). 22% discontinued PT after introduction of ANA (Group B; 17% within the first mo [Subgroup B1] and 5% in the subsequent 5 mos [Subgroup B2]). A further 9% had not discontinued PT by the end of the follow-up (Group C) and 5 pts (3%) were determined to have no PT. At the end of the follow-up, 85% of pts were still continuing on ANA, Groups: A (82%), B1 (93%), B2 (100%), C (81%). 71% of pts achieved platelet responses, Groups: A (67%), B1 (83%), B2 (100%), C (56%); 42% full response (<400x10^9/L) and 29% partial response (400–600x10^9/L or a reduction of ≥200x10^9/L). The median final platelet count was 412x10^9/L and the absolute median change from baseline was -94.5x10^9/L. 75% of pts who received ANA in line with the SPC achieved platelet response vs 54% of those not consistent with the SPC. 46% pts reported adverse drug reactions (ADRs) all described in the SPC. The most frequent were palpitations (13%), headache (11%), diarrhea 6% and asthenia 6%. 17% pts discontinued ANA due to ADRs (mainly palpitations or headache).
Summary / Conclusion: 85% of pts remained on ANA at the end of the 6-mo follow-up. ANA was introduced using the SPC□recommended dosing schedule in 76% of pts and 71% achieved platelet responses. Overall, ANA was well tolerated and the most frequent adverse events were in line with the SPC. Introducing ANA according to the SPC and subsequently withdrawing PT was associated with the highest platelet response rates.
For the France Observatoire Xagrid (FOX) investigators.

B1561

RISK OF LIMPHOPROLIFERATIVE NEOPLASMS IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Background: Over the last years, two large cohorts of patients have reported that chronic myeloproliferative neoplasms (cMPN) patients have a significantly higher risk of developing lymphoproliferative neoplasm (LPN) compared with the general population [1,14% (22/1915) and1,34% (11/820), respectively (Rumi, Haematologica 2011; Vannucchi, Cancer Epidemiol Biomarkers Prev 2009)]. In most cases, diagnosis of LPN was subsequent to the MPN one (91%), and only in 3 cases (9%) the diagnosis of LPN was synchronic or previous to the MPN one. If any genetic susceptibility exists, a random order of onset of the myeloid or lymphoid neoplasm should be expected. Authors of these series attribute the low number of cMPN patients with a previous or concurrent LPN due to a more aggressive behavior of lymphoid neoplasms, so these patients would die before cMPN develops. The molecular mechanisms underline the predisposition of cMPN patients to develop a LPN is not known.
Aims: The **aim** of our study was to evaluate the frequency and time of onset of LPN in patients with cMPN in our health area, and to investigate if specific genetic marker for predisposition to cMPN may also contribute to the risk of developing LPN.
Methods: All consecutive patients with newly diagnosed of cMPN in our unit between 2000 and 2011 were included in this study. Among 155 cMPN cases, 92 (60%) were diagnosed of essential thrombocitemia (ET), 37 (24%) of polycitemia vera (PV), 24 (15.5%) of myelofibrosis and 1 (0,5%) of systemic mastocytosis (SM) by using WHO criteria. In addition to carry out JAK2V617F, c-KIT (D816V) mutation analysis for the SM case, we performed mutational screening of hotspots: CBL (exons 8 and 9), ASXL1 (exon 12), N/K-RAS (exons 1 and 2), IDH1/2 (exon 4), TP53 (exons 4-10) and complete coding regions for RUNX1 and TET2. Mutation analysis was made by conventional Sanger sequencing.
Results: Of the 155 patients included, 4 (2.6%) developed a LPN: in one of them, LPN onset was 2 years before PV whereas in the other 3 patients (2 TE and 1 SM), diagnosis of both neoplasms was simultaneous. All four cases were men over 60 years. Distribution of LPN cases was as following: 2 cases of CLL, both 0-A stage; 1 case of T-LGLL (TCR gamma/delta+), and 1 case of B-cutaneous non-Hodgkin lymphoma. This last patient was the only one who required treatment (CHOP-R and radiotherapy), whereas the rest of the patients remain in therapeutic abstention. In 2 of 3 patients with classic MPN, JAK2V617G mutation was detected, whereas the patient with SM presented the c-KIT mutation (D816V). We were not able to detect any additional mutation genes we had evaluated in patients with both myeloid and lymphoid neoplasms.
Summary / Conclusion: Accordingly to previous studies, the risk of developing a LPN in patients with cMPN is greater in men than women and no correlation with mutational status of JAK2 seems to underlie. By contrast, we did not find a higher incidence of LPN between patients with cMPN over the time, since in our series, both pathologies onset randomly or simultaneously, likely because of the inclusion of LPN patients in early stages of the disease. Despite not described in the two large serious listed above, SM may also be associate rarely to LPN. Although we did not find any additional mutation other than well known JAK2 V617F in classical MPN and c- KIT (D816V) in SM, we cannot exclude that different genes other than *JAK2*V617F might favor the genetic instability predisposing to both LPN and cMPN or that a mutator phenotype exists in these cMPN patients.

B1562

SERIAL ANALYSIS OF GENOMIC ABERRATIONS IN DIFFERENT PHASE OF PATIENTS WITH MYELOPROLIFERATIVE AND MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS

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Background: Myeloproliferative neoplasm (MPN) and myelodysplastic/myeloproliferative neoplasm (MDS/MPN) may transform into secondary myelofibrosis (MF), and evolve into acute myeloid leukemia (AML), possibly preceded by a myelodysplastic phase. Genetic mechanisms underlying disease progression remain to be cleared, while chromosomal aberrations are relatively rare in the chronic phase of MPN.
Aims: The purpose of this study was to identify serial genomic aberrations that are associated with disease progression in MPN and MDS/MPN, using whole genome single nucleotide polymorphism based array (SNP-A), which can detect cryptic aberrations or copy neutral loss of heterozygosity (CN-LOH).
Methods: We investigated serial genomic changes of MPN and MDS/MPN patients in different phases of disease. The study group included 10 MPN and 4 MDS/MPN patients (7, polycythemia vera (PV);2, essential thrombocythemia (ET);1, primary myelofibrosis;2, MDS/MPN-unclassifiable;1, chronic myelomonocytic leukemia;1, atypical CML). Median follow up time was 78 months (range 8-154). Whole genome SNP-A (SNP 6.0, Affymetrix, CA) based karyotyping was performed according to manufacturer's instruction.
Results: Four patients (1 PV, 1 ET, 2 MDS/MPN-U) progressed to secondary MF or AML. All PV patients except one developing to MF showed CN-LOH of chromosome 9p at diagnosis and during follow up. One patient with PV developing to MF showed serial genomic changes by SNP-A; CN-LOH of 9p shown at diagnosis changed to gain (copy number 3) of 9p after 18 months and then additional lesions such as 1q gain and 6p deletion were accompanied at 100 months after diagnosis. Moreover, the copy number of 9p increased to four. One ET patient developing to MF showed CN-LOH of 9p, 6q deletion and CN-LOH of 14q at diagnosis and the same genomic changes showed during 106 months follow up. In the other ET patient without disease progression, any genomic aberrations were not found during 84 months. One MDS/MPN, unclassifiable patient demonstrated chromosome 8 gain, 13q deletion and CN-LOH of 20q at diagnosis and throughout follow-up and he progressed to AML at 14 months after diagnosis. During blastic phase, the same previous aberrations were detected and additional lesions such as 5q and 17p deletions were observed. The other MDS/MPN, unclassifiable patient developing to MF showed normal result by SNP-A during 94 months.
Summary / Conclusion: This study suggests that disease progression from chronic phase to secondary MF or AML is associated with genomic changes that can be identified by SNP-A.

B1563

GENETIC ABNORMALITIES ASSOCIATED WITH THROMBOSIS IN PATIENTS WITH PH-NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Background: Given the fact that one of the main causes of death in Ph-negative chronic myeloproliferative neoplasms (CMPN) patients is thrombotic complication, a consistent pattern of genetic abnormalities associated with thrombosis might be useful for polycythaemia vera (PV), thrombocythaemia (ET), and primary myelofibrosis (PMF) patients risk assessment.
Aims: The aim of this study was to check whether *JAK2* V617F, factor V gene *G1691A* (factor V Lieden, FVL) and *G20210A* prothrombin gene (PT) mutation has the predictive value for thrombosis in patients with Ph-negative CMPN.
Methods: We examined clinical and molecular genetic parameters of 103 PV, 43 ET, 36 PMF patients, and 156 persons with transient myeloproliferative reactions (TMR).
Results: Seven PMF patients (19%), 6 ET patients (14%), 32 PV patients (32%) and 30 TMR individuals (19%) had at least one confirmed arterial and/or venous thrombosis. In *JAK2* V617F positive PMF patients thrombotic complication occurred more often than in PMF patients without mutation (6 of 18 vs. 1 of 18 patients, P=0.043). Relative risk of thrombosis in *JAK2* V617F positive PMF was 6.0 (95% CI=1.01-44.7; p<0.05). Episodes of atypical thrombotic complications manifested in 13 (28%) unselected Ph-negative CMPN patients with thrombotic complication, 2 of them (4%) had both typical and atypical thrombosis, all of these patients were *JAK2* V617F positive. The FVL allele has been detected in 3.3% of Ph-negative CMPN patients (6 of 182 cases) and in 7.7% (12 of 156) of TMR persons. The difference between groups was on the border of statistical significance (P=0.07). The FVL mutation was detected in 3 of 38 Ph-negative CMPN patients with thrombotic event (7.9%) and in 3 patients without thrombosis (2.9%, P=0.2). The separate analysis shown that the prevalence of FVL mutation was higher in PMF patients with thrombosis compared to those without thrombotic episode (2 of 7 vs. 0 of 29 patients, P=0.03). The prevalence of FVL mutation in PV patients did not differ between groups of patients with and without thrombosis. The PT mutation has been detected in 3 (2.1%) of 142 Ph-negative CMPN patients. The prevalence of PT mutation was higher in ET patients with thrombosis compared to patients without this complication (2 of 6 vs. 0 of 37, P=0.02). Transient myeloproliferative reactions patients with thrombotic episodes demonstrated a higher prevalence of FVL mutation than those without thrombosis (6 of 30 vs. 6 of 126, p<0.05). The FVL mutation in TMR patients was associated with a 4.2-fold (95%, CI=1.46-12.06; p<0.05) increase of probability of having any thrombosis. Relative risk of venous thrombosis was 5.6 (95% CI =1.79-17.28; p<0.05) in carriers of FVL allele. The PT mutation has been identified in 8 (5.1%) individuals with TMR. The prevalence of mutant allele was higher in TMR patients with thrombosis compared to those without the thrombotic event (4 of 30 vs. 4 of 126, P=0.027). It was found that individuals with PT mutation demonstrated a 4.1-fold (95% CI=1.09-15.33, P<0.05) greater probability of having thrombosis. All FVL and PT mutations detected in our study were heterozygous.
Summary / Conclusion: Our data confirm that *JAK2* V617F mutation is the predictor of thrombotic complication in Ph-negative CMPN and support the assumption that FVL and PT heterozygous mutation might further increase the risk of thrombosis in PMF and ET patients respectively.

B1564

HEMOGLOBINOPATHIA YPSILANTI – A RARE, BUT IMPORTANT DIFFERENTIAL DIAGNOSIS TO POLYCYTHEMIA VERA

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Background: Hemoglobin Ypsilanti is a rare high oxygen affinity hemoglobin variant involving an amino acid substitution and is inherited autosomally dominant. Due to the high oxygen affinity of hemoglobin Ypsilanti, hypoxia arises in the tissues, and secondary erythrocytosis develops. Before the inclusion of JAK2/exon 12 mutations in the diagnostic criteria, a patient with erythrocytosis could be difficult to diagnose properly with polycythemia vera (PV). The JAK2 mutation is present in more than 95% of patients with PV, and in the rest, a mutation in exon 12 is almost always found. The number of patients with non-clonal PV is therefore very low, but it is very important to obtain a correct diagnosis due to correct patient information and treatment strategy.
Aims: To highlight the importance of proper differential diagnostics in PV.
Methods: We present a case report of a mother and daughter who were initially diagnosed with PV.
Results: In 2003, the mother (age 51 years) was referred to the hematological department due to a high hemoglobin (B-Hgb) level diagnosed during admission for a suspected transient cerebral ischemic attack. In 2007, her daughter (age 24 years) was also referred due to a high B-Hgb level and a three week history of headache. Neither had leukocytosis, thrombocytosis or hepatospleno-megaly. Chest X-rays and peripheral blood smears were normal. Bone marrow biopsies showed hypercellularity and in the case of the mother, the bone marrow examinations were interpreted in concordance with a myeloproliferative disorder. S-EPO was within normal range for both. EPO-stimulated growth of erythroid colonies were normal in both patients. The JAK2/ and exon 12 analyses showed wild-type in both mother and daughter when these analyses became available. The mother later developed pulmonary embolism after immobilization following surgery. Both were treated with venesection regularly according to guidelines for PV to a hct < 45 %, and being comfortable with this. The mother also received anticoagulant therapy, first as acetylsalicylate and later warfarin after the pulmonary embolism. In 2012, a hemoglobin analysis by β-globin gene sequencing was performed and both were heterozygous for the mutation CD 99 G>T (Asp99Tyr), and therefore a carrier of the Hgb-variant Ypsilanti. The regular venesection was stopped and B-Hgb allowed to increase within normal. The daughter has given birth twice to apparently normal offsprings.
Summary / Conclusion: The pursuit of a correct diagnosis is important - especially today where molecular biology offers the possibility of accurate diagnosis. Erythrocytosis due to high oxygen affinity hemoglobin is usually well tolerated in younger patients, but in elderly patients the risk of thrombosis is increased. Standard venesection treatment with high oxygen affinity hemoglobinopathies may not be rational, since the high oxygen affinity causes tissue hypoxia, if there is no compensatory erythrocytosis. Contrary to PV, the risk of transformation to myelofibrosis or leukemia is not present, which has a great impact psychologically. Furthermore, cytoreductive therapy with e.g. hydroxyurea or interferon-α is not indicated and may cause side effects, affecting quality of life negatively and may imply long-term complications. It is important to suspect high oxygen affinity hemoglobinopathy when there is a family history of erythrocytosis and/or when young persons are affected; especially when no apparent cause or clonal marker is identified. The finding of high oxygen affinity hemoglobin is important, for patient information and optimal treatment strategy.

B1565

THE PREVALENCE OF LEG ULCERS IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS TREATED WITH HYDROXYUREA

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Background: Leg ulcers (LU) represent a common adverse side effect of long-term hydroxyurea (HU) therapy, which is often neglected.
Aims: To describe the prevalence of LU in patients with myeloproliferative disorders (MPD) treated with HU and association of LU with chronic venous insufficiency (CVI) and presence of JAK-2 (V617F) mutation.
Methods: 70 patients aged 39 to 89 years, 33 (47%) women and 37 (53%) men treated with HU for MPD (polycytemia vera 34 (49%), essential thrombocythemia 14 (20%), primary myelofibrosis 20 (29%), MPD unspecified 2 (2%). The average duration of HU therapy was 6.66 years with the average HU dose of 2325.8g per patient. JAK-2 (V617F) mutation was examined in 45 (64%) patients; positive results were found in 31 (69%) cases. All patients were assessed using the CEAP classification of CVI, including ultrasonography of lower limb veins.
Results: CVI symptoms were found in 51 (73%) patients, 35 (50%) of them had both lower limbs affected. LU developed in 16 (23%) patients during the period of HU treatment. In 11(69%) of them LU was associated with CVI, while in 6(38%) cases the ulcer affected the leg without CVI. (1 patient with ulcers of both legs had only one limb with CVI). 19 patients without CVI were followed for 19-870 (median 367) weeks and LU developed in 3 (15.8%) of them. 51 patients with CVI were followed for 20-933 (median 318) weeks and LU developed in 12 (23.6%) of them; the difference was not significant (P=0.4828). The time to LU development was 10 to 547 (mean 233) weeks and 196 to 845 (mean 409) weeks in patients with CVI and without CVI, respectively; the difference was not significant (P=0.1495). The mean cumulative dose of HU administered before LU development was 1848 (55 – 4817) g and 3831 (1635-8648) g in patients with CVI and in patients without CVI, respectively; the difference was not significant (P=0.1931). The mean duration of HU therapy till LU development was 257 (10-846) weeks in 7 patients with JAK-2 mutation and 313 (197-535) weeks in 3 patients without JAK-2 mutation. The mean cumulative dose of HU administered was 2238 (57-8648) g and 3243 (1083-7011) g in patients with JAK-2 mutation and in patients without mutation, respectively. 2 (18%) ulcers healed within 18 and 33 weeks during HU therapy. In 15 cases HU therapy was stopped due to non-healing ulcers and an alternative therapy was administered. 14 (93%) ulcers disappeared after discontinuation of HU therapy, 1 patient died shortly after HU suspension. The average time from HU discontinuation to healing of LU were 30 (8-73) weeks and 44 (5-121) weeks in ulcers associated and non-associated with CVI, respectively; the difference was not significant (P=0.5035). The average time of healing was 47 (8-121) weeks and 9 (5-13) weeks in patients with JAK-2 mutation and in patients without JAK-2 mutation, respectively.
Summary / Conclusion: LU is a common complication associated with HU

therapy; its negative impact on quality of life is significant. The ulcers occur more frequently in patients with CVI and JAK-2 mutation; however their prevalence in patients without CVI is not negligible. The role of HU in development of LU is substantial and the discontinuation of HU therapy is required in most patients.

B1566

THE ROLE OF HISTOLOGICAL FEATURES IN PATIENTS WITH POLICYTHAEMIA VERA

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Background: According to WHO revised classification, bone marrow biopsy is no more needed in the diagnostic process of Policythaemia Vera (PV). However, it was widely used in the past years and is still performed in many PV patients at baseline.
Aims: At present, very few data exist on the correlation between histological features and patient follow-up.
Methods: To address this issue, we revised retrospectively the histological features at baseline of 195 patients with PV [M/F 100/95, median age 58.4 yrs (IQR 48.1 – 67.2), median Hb 18.4 g/dl (IQR 17.1 – 20.1), median Ht 56.6% (IQR 53.0 – 61.8), median WBC 10.4 x 10^9/l (IQR 8.4 – 14.0), median PLTs 505 x 10^9/l (IQR 346 – 717)] observed at our Institution from 1/1982 to 12/2010.
Results: The following histological features were considered: bone marrow fibrosis [grade 0 in 128 patients (65.6%) and grade > 0 in 67 patients (34.4%)], number of megakaryocytes [normal in 34 patients (17.4%) and increased with or without clusters in 161 patients (82.6%)], marrow cellularity [normal or increased with erythroid hyperplasia in 152 patients (77.9%) and increased with granulo-megakaryocytic hyperplasia in 43 patients (22.1%)], lymphoid reactive infiltration [absent in 165 patients (84.6%) and present in 30 patients (15.4%)]. The different histological features at baseline were compared with thrombotic episodes during follow-up [reported in 45/195 patients (23.1%)], evolution in myelofibrotic phase (MP) [21/195 patients (10.8%)], evolution in blastic phase [15/195 patients (7.7%)] and overall survival. Marrow cellularity, number of megakaryocytes and lymphoid infiltration did not show any significant prognostic role: on the contrary, the presence of marrow fibrosis > grade 0 was associated with an increased occurrence of thrombotic episodes during follow-up (P=0.020) and an increased rate of evolution in MP (P=0.040).
Summary / Conclusion: In conclusion, while the histological marrow evaluation is not required for the diagnosis of PV, the recognition of fibrosis seems to have a role in the prognostication of adverse events during follow-up and should be evaluated at baseline.

B1567

COMPARISON OF THE EXPRESSION CANCER-TESTES (CT) ANTIGEN PROFILES IN CML AND POLYCYTHEMIA VERA (PV).

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Background: Since 1951 when W. Dameshek supposed closed interrelation within the group of myeloproliferative syndromes many things had happened and it turned out that CML differs from other similar diseases by presence of Ph-chromosome and BCR-ABL gene and that Jak2 V617F and a few other mutations are the molecular hallmarks of PV, ET and IMF. Nevertheless, all these diseases in some features are still quite similar notwithstanding our ignorance about exact causes of this similarity. Cancer-testis (CT) antigens almost universally express in different malignancies varying in frequency and intensity of expression of particular members of CT antigen family. We have supposed that CT antigen expression profiles may explain or at least further highlight molecular similarity and/or difference between CML and Ph-negative Jak2 V617F-positive chronic myelogenouse disorder (CMD).
Aims: To perform gene expression profiling of a number of CT antigens in CML and Jak2 V617F-positive polycythemia vera (PV).
Methods: In this study we used RQ PCR to analyze and compare mRNA expression of CT antigens SP17, GAGE1, HAGE, NY-ESO1, MAGE1, PASD1, SCP, SEMG, SLLP1, SPANXA, SSX1 and PRAME in blood cells. Quantitative analysis of expression level cancer-testis antigens was carried out relatively using the expression of a housekeeping gene ABL as endogenous control to compensate for irregular cell numbers.
Results: In blood of CML chronic phase (CP) primary pts (N=36) we observed HAGE1, SLLP1, SPANXA and PRAME gene expression with frequencies of 61,1% (22/36), 36,1% (13/36),2,8% (1/36) and8,3% (3/36), respectively. In case of Jak2 V617F-positive PV pts (N=33) we observed only HAGE1 (63,6%, 21/33), SLLP1 (84,8%, 28/33) and PRAME (12,1%, 4/33) gene expression. In contrast to CP of CML, expression frequency of SLLP1 and PRAME in PV samples was higher (84,8% and 12,1% against 36,1% and8,3%). Expression rate of HAGE gene in both diseases was generally similar (61,1% and 63,6%). Expression level of genes found in CP of CML did not differ significantly to compare with PV. We did not find mRNA expression both in CP of CML and PV: SP17, GAGE1, NY-ESO1, MAGE1, PASD1, SCP1, SEMG and SSX1. In AP&BC of CML we have observed additional expression of CT22 (14,4%, 1/7), MAGE1 (14,4%, 1/7) and GAGE1 (14,4%, 1/7) (Fig.1).
Summary / Conclusion: Our data suggest obvious similarity of CT expression profiles of CP of CML and Jak2 V617F-positive PV. This finding reflects clinical relation being observed among these two leukemias. Both CP of CML and PV differ in CT expression profile from CML in AP&BC.

Gene	CML (CP) %	CML (AP+BC) %	PV (JAK2-V617F) %
SSX	0	0	0
SCP1	0	0	0
SEMG1	0	0	0
NY-ESO-1	0	0	0
PASD1	0	0	0
CT22	0	14,4 (1/7)	0
GAGE1	0	14,4 (1/7)	0
MAGE1	0	14,4 (1/7)	0
SPANXA	2,8 (1/36)	14,4 (1/7)	0
PRAME	8,3 (3/36)	28,6 (2/7)	12 (4/33)
SLLP1	36 (13/36)	14,4 (1/7)	84,8 (28/33)
HAGE	61 (22/36)	28,6 (2/7)	63,6 (21/33)

Fig.1. CT antigen expression in CML and PV.

B1568

EFFICACY OF PEGYLATED INTERFERON IN MYELO-PROLIFERATIVE DISORDERS

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Background: Pegylated Interferon (Pegasus) is an alternative treatment for patients with myelo-proliferative disorders (MPD), including polycythaemia vera (PV) and essential thrombocytosis (ET).
Aims: Review efficacy of Pegylated Interferon in Myeloproliferative Disorders.
Methods: We reviewed the use of Pegasus in 13 patients with MPDs (ET=10, PV=3) at a single institution over a 13 month period; 7/10 ET and 3/3 PV patients were JAK 2 V617F positive.
Results: 10/13 switched from existing therapies including Hydroxcarbamide, Anagrelide and 3x/week Interferon.We recorded: reasons for switching to Pegasus, starting dose, dose alterations, and any significant side-effects (including derangement in LFTs). Time to response was determined from sequential platelet/haematocrit readings. 5/13 patients (3 ET and 2 PV) commenced weekly Pegasus as a single therapy, of which 3 (all ET) were treatment naïve. The remaining 8 patients all had existing therapies withdrawn following initiation of Pegasus, completing the bridging period over 1 to 7 weeks. 1 ET patient remains on Hydroxycarbamide alongside Pegasus. Reasons for switching to Pegasus included: ease of weekly administration (n=2), planning to or being pregnant (n=4), side-effects of existing therapy (n=3), lower limb ulceration (n=1), non-response to current therapies (n=3). Prior to commencement of Pegasus, baseline median platelet count was 387 x10□/L (range of 173 – 1065x10□/L). Median platelet counts were 413 (124-889x10^9/L) at 1 month and 355 (109-740 x10^9/L) at 3 months after starting therapy. Doses ranged from 45µg every 2 weeks to 135µg/week. Within the PV group, there was no change in median haematocrit between baseline (0.50), 1 month (0.48) and 3 months (0.49). No reported side effects were noted, although 2/13 patients had a mean ALT rise of >90IU/L from baseline, following initiation of therapy.
Summary / Conclusion: Pegylated IFN appears a safe alternative approach in MPD therapy with prompt and sustained control of laboratory parameters.

B1569

HYPEREOSINOPHILIC SYNDROME: DIAGNOSTICS AND THERAPY. SINGLE CENTRE EXPERIENCE

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Background: Hypereosinophilic syndrome (HES) is a group of rare diseases with persistent eosinophilia >1.5x10/L and organ imparement. It may be of non-neoplastic or neoplastic origin. If the cause of eosinophilia is not found the

HES is considered as idiopathic (IHES).
Aims: to represent our experience in diagnostic and therapy of myeloproliferative diseases (MPD) with eosinophilia and IHES with signs of MPD (myeloproliferative variant of IHES).
Methods: from 1985 to 2012 years there were 96 patients with HES followed up in our centre. The ratio of male and female was 72:24 and the median age was 38 (17 – 70). Diagnostic steps were directed to verify diseases with primary and secondary eosinophilia.
For the proof of the clonal MPD existence standard cytogenetic study and, from the 2003 year, the polymerase chain reaction (PCR) with FIP1L1-PDGFRA and ETV6-PDGFRB primers were applied.
Results: reactive eosinophilia was proved in 16% (14/96) cases: adrenal hemangiopericytoma – 1; multifocal liver damage unknown origin disappeared without cure –1, allergic reaction – 1; autoimmune diseases –8, T-cell lymphomas – 3. It is important to point that the first three cases of eosinophilia could be established postfactum only. The standard cytogenetic study was done in 80 and PCR was done in 61 rest cases of HES. Different chromosomal aberrations were found by G-banding only in 8% of cases (6/80) whereas PCR could prove clonality in 50% (30/61) of cases with HES. In most cases the FIP1L1-PDGFRA – gene expression was found (28/30). All the rest cases of HES with unestablished cause of eosinophilia were considered as idiopathic. In 19/46 cases there were signs of myeloproliferation and the syndrome was interpreted as myeloproliferative variant of HES. The therapy of clonal MPDs and myeloproliferative variant of HES included schemes with different cytostatics, alpha-interferon, hydroxyurea. From the 2003 we began to use imatinib at a dose 100-400 mg o.d. Complete hematologic response (CHR) was received only on imatinib and alpha-interferon. The effectiveness of imatinib was 87% (20/23) in cases PDGFRA- and PDGFRB-positive and 36% (4/11) in cases where PCR was negative or not done. In 14 of 15 cases the complete molecular response was received too. At a therapy with alpha-interferon CHR was received in 3 of 7 cases (43%). Neither chemotherapy no hydroxyurea allowed to get any sustained and prolonged result.
Summary / Conclusion: About 1/3 of HES cases are represented by clonal MPD to verify which a special methods such as PCR or FISH are required. In a small percent of HES the association eosinophilia with some diseases can be confirmed only after resolution of symptoms. If the target therapy is nonsufficient the possibilities of conservative therapy are limited. For the young patients is reasonable to conduct bone marrow transplantation.

B1570
USING MYELOPROLIFERATIVE NEOPLASM SYMPTOM ASSESSMENT FORM (MPN-SAF) TO EVALUATE QUALITY OF LIFE FOR PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA IN QATAR
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Background: Myeloproliferative neoplasms (MPNs), that is, essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF) can lead to significant morbidity and mortality among affected patients. Specifically can be predisposed to thrombohemorrhagic events and vascular complications.progressive cytopenias, constitutional symptoms, cachexia , splenomegaly, and risk of blastic transformation
Aims: Symptomatic burden in (MPNs) is present in most of MPN patients we sought to use broadly applicable instrument (MPN-SAF) to assess symptoms in (ET) among populations of Qatar.
Methods: Using the MF-SAF as a base instrument, we added several key additional symptoms previously identified as present in all subtypes of MPNs including headaches, loss of concentration, dizziness, extremity tingling, insomnia,sexual problems and mood changes on a 0 (absent) to 10 (worst-imaginable) scale. The MPN-SAF was administered jointly with the EORTC-QLQ-C30 as the co-validation instrument using prospective cohorts in Qatar (Patients referred to National Centre for Cancer Care and Research).
Results: 123 MPN-SAF surveys were administered (English (45%), Arabic (55%) , 78 ET patients were JAK 2 V617F positive and 45patients were JAK2 V617F negative) an average of 3.6 years from their MPN diagnosis. Participants were of, age range (22 – 58 years) and gender (52% female) characteristic of disease. Prior hemorrhage (10%) and thrombosis (25%) 78% of patients currently received cytoreductive therapy. 19 items assessed in the MPN-SAF demonstrated consistently that the most common symptoms were decreased quality of life (93%), fatigue (84%), insomnia (65%), sad mood (65%), and sexuality problems (62%). The least common symptoms (<50% prevalence) were fevers (15%), weight loss (10%), abdominal pain (23%), cough (34%), headache (50%), and bone pain (48%) Interestingly, night sweats (present in 58%) The majority found the MPN-SAF easy to understand (90%) and "addressed most of my MPN symptoms" (93%). Comparison to EORTC-QLQ-C30: Strong correlations existed between individual items represented on both the MPN-SAF and the EORTC-QLQC30 including pain, fatigue, appetite and insomnia (all P<0. 001). Additionally key symptomatic elements were highly correlated with the EORTC QLQ-C30 functional subscales. Comparison to Physician Perceptions: Comparison of the results of the MPN-SAF to enrolling physicians' blinded opinion of patients symptoms (7 assessed - night sweats, fevers, fatigue, weight loss,and bone pain) showed excellent correlation with corresponding patients' responses (all P<0. 001). Serial MPN-SAF: Pearson correlations indicate that most MPN-SAF items are well correlated (r >0.5, P<. 001) upon repeat survey administration. Items characteristic of advanced disease, including weight loss, fever, and cough displayed lower Pearson correlations (r=0. 46, –0. 08, and 0. 38 respectively)
Summary / Conclusion: MPN-SAF is comprehensive and reliable instrument which is available in multiple languages (including Arabic and English) to evaluate MPN-associated symptoms. The MPN-SAF is recommended as a uniform symptom assessment tool for MPN patient

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B1571
PREGNANCY MANAGEMENT AND OUTCOMES IN WOMEN WITH MYELOPROLIFERATIVE DISEASES
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Background: Myeloproliferative diseases (MPD) rarely occur in women of reproductive age. But in recent years it becomes more often that young women suffer from these diseases. The appearance of new drugs and therapeutic strategies provides good results in survival rate and life prognosis for patients. All this requires special options for management of pregnancy in women with MPD.
Aims: To develop the protocol of preconception planning and pregnancy management and to evaluate pregnancy outcomes and complications in women with MPD.
Methods: Retrospectively we have analyzed 41pregnancies in 21 women (group 1) who did not receive a special treatment of MPD. The prospective group (group 2) included 46 women who were treated according to our algorithm. Our trial included women with main MPD: essential thrombocythemia, polycythemia vera, primary myelofibrosis. Pregnancy management included examination of blood cell count and hemostasis system twice a month, besides this the inherited trombophylia testing, lupus anticoagulant, homocystein level, antiphospholipid syndrome diagnostics and hematologic examination including trepanobiopsy and JAK2V617F mutation. Besides thorough laboratory examination our protocol of pregnancy planning and management included cytoreductive therapy, antiaggregants, low-molecular-weight heparin, plasmapheresis, vitamins of group B. For the cytoreductive therapy we prescribed Interferon alfa which is the safest option in preconception planning and pregnancy management for women with MPD.
Results: Medical abortion was made in 3 (7,3 %) women, spontaneous miscarriages occurred in 65,8% in group 1 without special treatment. First and second trimester spontaneous abortions prevailed among all the miscarriages – 15 (36,6%) cases, stillbirth occurred in 10 (24,4%) cases. Preterm labor were in 14,6 % pregnancies, full-term delivery occurred in 17,1% of cases. Medical abortion was made in 2 (4,3%) women of group 2. Spontaneous miscarriages occurred only in4,5% in the group of women who were treated according to our protocol (2 pregnancies). Five (10,9%) pregnancies ended preterm, while 37 (80,4%) labor were fill-term (*P*=0,000001, OR – 0,07; 95% C.I.: 0,018;0,232). Pregnancy was uncomplicated in 2 (15,4%) and 14 (33,3%) cases in 1 and 2 groups respectively. The most often pregnancy complications were threatening miscarriage - 10 (76,9%) and 22 (52,4%) cases, anemia – 4 (30,8%) and 13 (31%) in 1 and 2 groups respectively. Placental insufficiency complicated 30,8% pregnancies including intrauterine growth retardation (IUGR) in 2 cases in group 1. Although all pregnancies in group 2 were carefully observed and treated 11,2% of them were complicated by placental isufficiency with IUGR in 3 (10,3%) cases.
Summary / Conclusion: Thus pregnancy losses in women suffering from MPD occur in 65,8% without special treatment and complications of pregnancy – in 84,6% cases. The development of protocol for preconception planning and pregnancy management resulted in considerable decrease in miscarriages to4,5% (*P*=0,000005, OR – 24,2; 95% C.I.:4,8;225,9) and a tendency of lowering of pregnancy complications to 66,7% (P=0,5).

B1573
COMMON COMMUNITY INFECTIONS AND THEIR ASSOCIATION WITH MYELOID MALIGNANCIES
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Background: Antigenic stimulation precedes many haematological malignancies. Limited evidence suggests that community acquired infections may increase risk of acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS). However, associations between community infections and chronic

myeloid leukaemia (CML) and myeloproliferative neoplasms (MPNs) have not been investigated.
Aims: Using the SEER-Medicare database, fourteen common community acquired infections were compared between myeloid malignancy patients AML (n=8,489) and CML (n=3,626) diagnosed 1991-2005; MDS (n=3,072) and MPN (n=2,001) diagnosed 2001-2005) and controls (200,000 for AML/CML and 97,681 for MDS/MPN patients).
Methods: Odds ratios (ORs) and 95% confidence intervals were obtained using polytomous logistical regression with adjustment for gender, age and year of diagnosis/selection. Multiple comparisons were considered using Bonferroni correction and significant associations analysed over select time periods.
Results: Respiratory tract infections, including bronchitis (ORs 1.20, 1.25), influenza (ORs 1.16, 1.41), pharyngitis (ORs 1.13, 1.22), pneumonia (ORs 1.28, 1.52), sinusitis (ORs 1.23, 1.25) were associated with AML and MDS, respectively. Cystitis (ORs 1.13, 1.26), cellulitis (ORs 1.31, 1.51) and herpes zoster (ORs 1.18, 1.31) were associated with both AML and MDS respectively while gastroenteritis (OR 1.38) was only associated with MDS. Bronchitis (OR 1.21), pneumonia (ORs 1.49), cellulitis (ORs 1.43) and sinusitis (OR 1.19) were associated with CML while cellulitis (OR 1.34) was the only infection associated with MPNs. Claims for most infections occurred 31-72 months preceding diagnosis although several remained significant >72 months preceding diagnosis.
Summary / Conclusion: Stronger associations between community acquired infections and AML/MDS were observed compared to CML/MPNs indicating that chronic antigenic stimulation may be important in the malignant transformation of immature blood cells of myeloid lineage.

B1574
INTERLEUKIN-8 LEVELS IN ESSENTIAL THROMBOCYTHEMIA: CLONAL OR INFLAMMATORY TRIGGER?
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Background: The patients with essential thrombocythemia (ET) overproduce proinflammatory cytokines known to promote the hematopoiesis, such as interleukin-8 (IL-8). The JAK2V617F mutation expressing cells are featured by an inherited hypersensitivity to cytokine stimulation.
Aims: Therefore, we evaluated platelets, red blood cell (RBC), haemoglobin (Hb) concentration, hematocrit (HCT) and white blood cell (WBC), as myeloproliferative markers, fibrinogen (Fg) and C-reactive protein (CRP), as inflammatory indicators, JAK2V617F mutation and IL-8.
Methods: We recruited 50 patients with ET who fulfilled WHO criteria. Their mean duration of disease was 10 years (range, 5-20 years). Of 50 ET patients, 25 were JAK2V17F mutated (11 males and 14 females, mean age 59 years) and 25 were JAK2V617F WT (9 males and 16 females, mean age 58 years). All patients were on aspirin.
Results: The JAK2 mutated patients had higher platelets, RBC, Hb, HCT and WBC (909±253x109/L, 5.42±7x106/L, 15.2±1.7 g/dl, 46±4%, 11±2.7x109/L) than JAK2 WT patients (753±129x109/L, 5±7x106/L, 12±2 g/dl, 39±3%, 7± 1.5x109/L) (P=0.009, P=0.001, p <.0001, p <.0001, p <.0001), whereas the JAK2 WT patients had higher Fg and CRP (414±3 mg/dl and 6±1 mg/L) than JAK2 mutated patients (286±37 mg/dl and 0.8±1 mg/L) (p <. 0001 and p <.0001, respectively). The JAK2 mutated patients and JAK2 WT patients had elevated IL-8 (274±158 pg/ml and 222±90 pg/ml, respectively).
Summary / Conclusion: On basis of these results it is conceivable that IL-8 may represent the clonal or inflammatory counterpart in mutated and WT patients, respectively. Hence, IL-8 may be an unfavourable prognostic index in mutated ET patients.r

B1575
PRIMARY MYELOFIBROSIS – A SURVEY BASED ON THE 20-YEARS' EXPERIENCE OF A SINGLE CENTER
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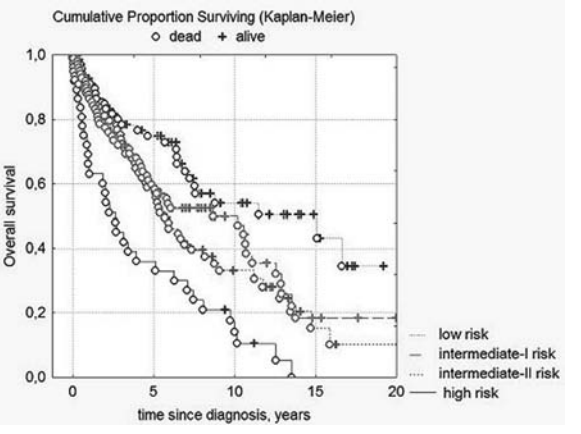
Background: As a result of advances in decryption of molecular mechanisms of pathogenesis and invention of target drugs, the chronic myeloproliferative diseases including primary myelofibrosis (PMF) undergo the renaissance of interest nowadays. The information about historical control is needed to assess potential effect and additional costs of new diagnostic and therapeutic technologies.
Aims: The objective of our study was to review the experience of PMF diagnostic and treatment in our center for the past twenty years.
Methods: Our institution has a primary hematological outpatient department which works for about a half of Saint-Petersburg city inhabitants – that is about 2.4 million people.We reviewed patients' charts to obtain information about incidence, symptoms, diagnostic test results, treatment options and relationship to prognostic factors. Statistical methods included descriptive and Kaplan-Meyer method with log-rank test for survival comparisons in Statistica 7.0 package.



Results: Since 1993 to 2012 there were 296 newly diagnosed PMF patients in our center. This yields stable incidence varied from 0.76 to 1.56 with mean of 1.06 new patient per 100 000 inhabitants per year. The age interval was between 16 to 83 years with median of 62 years. The gender ratio was nearly 2:1 as female:male (192 females and 104 males).The most prevalent symptoms of disease were: splenomegaly (71.4%), constitutional symptoms (fever, night sweats, weight loss) (33.3%), thrombosis 25.4%, portal hypertension (5.1%) with esophageal variceal bleeding (1.6%). The most common lab abnormalities were leukocytosis (80%), thrombocytosis (69.5%), anemia (39.3%), and thrombocytopenia (10.2%). Bone marrow fibrosis as histological findings as grade 0 (prefibrotic stage) were noted in 26.7%, grade 1 in 20%, grade 2 in 29.2% and grade 3 in 24.2% of patients. Cytogenetic banding analyses were performed in 76 cases. Normal karyotype was revealed in 72.4% cases, no mytoses had been obtained in 7.9%. Cytogenetic abnormalities were in 19.7% patients with trisomy 8 and complex karyotype as most frequent (13.3% of whole clonal abnormalities for each). JAK2V617F was detected in 50 of 101 (49.7%) examined patients. In addition MPLW515L mutation was revealed in 1 patient. The results of stratification by IPSS/DIPSS+ risk score were the following: low-risk 27.0%/25.7%, intermediate-I - 38.4%/35.9%, intermediate-II – 14.3%/26.0%, high-risk – 20.3%/12.4%. Patients were treated mainly with hydroxycarbamide monotherapy or in combination with other drugs in 81.9% cases, interferon was used in 21.0%, glucocorticoids were used in 11.1% patients. Overall 10-years survival of patients was 44.4% with median of 7.6 years. Blastic transformation occurred in 5.7% patients with median time to blast crisis as of 5.1 years. Overall survival highly significantly influenced by risk stratification as IPSS and DIPSS+. Survival curves according DIPSS+ groups are presented in fig.1.
Summary / Conclusion: PMF is one of the most common hematological malignancies with reduced life duration. Risk stratification systems had high predictive value. Introduction of innovative drugs should be evaluated in comparison with historical control.

B1576
100 MG WEEKLY IMATINIB MAINTENANCE THERAPY IS SUFFICIENT TO SUSTAIN THE MOLECULAR RESPONSE IN PATIENTS WITH CHRONIC EOSINOPHILIC LEUKEMIA
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Background: CEL is a rare myeloproliferative neoplasm with prominent eosinophilia and presence of a FIP1L1- PDGFRA gene. This fusion gene is much more sensitive to imatinib than BCR-ABL gene, and usually a dose of 100 mg daily is sufficient to achieve response to therapy and molecular remission in most of CEL patient. This imatinib dose is used in induction and maintenance therapy.
Aims: Is the imatinib dose of 100mg weekly sufficient to maintain molecular remission in patinets with CEL?
Methods: 5 patients were diagnosed at the Department of Hematology and Transplantology Medical University of Gdansk. All patients are male and diagnosed was confirm by the presence of FIP1L1-PDGFRA gene detected by the PCR. The median age at diagnosis was 47 (range 39-64) . At the diagnosis median number of WBC was 16 G/L (10,3-29 G/L) and median eosinophils count was 9,2 G/l (5,36-12,0 G/l). Spleen was enlarged (palpable below costal margin) in 3 of 5 patients.

Results: The normalization of eosinophils was achieved at the 25 days (mean value) after starting of induction imatinib therapy. Duration of induction therapy was at least 3 months Confirmation of complete molecular response was performed 3-12 months after starting the imatinib therapy. After normalization of eosinophils count and achieving of molecular response maintance therapy was started (imatinib 100 mg weekly). Confirmatory blood count was performed every 3 months and molecular analysis was performed every 6-12 months. All performed blood count showed normal eosinophils count and nested PCR was negative for the FIP1L1/ PDGFRA gene during maintenance therapy in all patients.
Summary / Conclusion: As the maintenance therapy, imatinib 100mg weekly is effective to sustain the molecular remission in patients with diagnosed CEL.

B1577

A RETROSPECTIVE ANALYSIS ON THE IMPACT OF PROGNSOTIC FACTORS IN 362 PATIENTS WITH PRIMARY MYELOFIBROSIS FOLLOWED IN THE LAZIO REGION DURING A 20 YEARS FOLLOW-UP PERIOD

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Background: Primary myelofibrosis (PMF) is a rare chronic myeloproliferative disorders characterized by a heterogeneous clinical presentation, a relatively shortened survival and a propensity to develop acute myeloid leukemia (i.e. blast phase).
Aims: To correlate the clinic-biologic features at presentation with risk of blast phase evolution, we retrospectively collected the clinical records of 362 patients followed in 12 hematologic units of the Latium region and diagnosed between 1981 and 2010.
Methods: Diagnosis was made according to the criteria accepted at the time when the patient was diagnosed. Two hundred-nine (65%) patients were males, 153 females (38%) (M/F= 1.4). Patients mean age was 67 years. Hb < 10 g/dl was present in 85/274 (31%) of patients. Eigtheen/270 (6.6%) patients presented a WBC count >25 x 10^9/L. The presence of Jak2V617F mutation was checked in 143 patients. However, only 83 patients had JAK2 V617 mutation assessed within 1 year from diagnosis. Among these 83 patients 51 (61%) were JA2V617F-positive. 224 (62%) patients received conventional chemotherapies.
Results: At time of analysis, 180 patients were alive, 137 dead and 45 lost to follow-up. With a median follow-up of 48.4 months (range: 1-252) the actuarial survival rates at5, 10 and 20 years were 70%, 35 % and 22%, respectively. Forty-two of 362 patient (11.6%) progressed to a blast phase after a median time from diagnosis of 92 months (range: 2-250 months) for a cumulative incidence rate of 13%, 28% and 28% at5, 10 and 20 years, respectively. At univariate and multivariate analyses only Hb < 10 gr/dl and presence of blasts (>1%) resulted the only independent prognostic factors significantly affecting the blast phase occurrence. JAK2 V617F did not impact on progression to blast phase. A score system combining these two factors (score 0= none; score 1= 1 of the 2 factors; score 2 = both factors) significantly discriminate patients at high, intermediate and low risk of evolution to blast phase (Figure 1).
Summary / Conclusion: In conclusion, our retrospective analysis on a large series of patients demonstrates that progression to blast phase of PMF patients may be predicted by a score system that includes anemia and presence of blasts. These latter are easily and worldwide detectable parameters able to help risk stratification and decision making for PMF patients, an issue that has became extremely relevant in light of the forthcoming availability of the new targeted drugs.

B1578

THE ASSOCIATION BETWEEN JAK2 46/1 HAPLOTYPE AND SUSCEPTIBILITY TO MYELOPROLIFERATIVE NEOPLASMS

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Aims: To investigate wether JAK2 46/1 haplotype was the genetic susceptibility gene for MPN patients and the relationship between JAK2V617F and 46/1 haplotype.
Methods: MALDI-TOF MS was used to analyze two tag SNPs (rs10974944, rs12343867) of JAK2 46/1 haplotype in 77 MPN patients with known JAK2V617F status and 92 healthy persons as control.
Results: The 46/1 haplotype in genotype distribution between the two groups had a statistically significant difference (P <0.05); Individuals carrying at least one variant allele, compared with the wild-type individuals, had an significantly increased risk of MPN by 2.31 times[the OR (95% CI) were 2.31 (1.21-4.39) and 2.31 (1.2-4.47)];While the MPN risk of people carrying the variant homozygotes was increased by 7-9 times[the OR (95% CI) were 7.54 (2.27-24.99) and 8.9 (2.69-29.42)]. Genotype distribution difference between the JAK2 V617F-negative group and positive group was statistically significant (P <0.05). The V617F-negative group and control group did not exist a statistically significant difference (P> 0.05),while the distribution between the JAK2 V617F-positive group and control group had a significant difference (P <0.05). The test of peripheral blood cell levels by single factor analysis of variance showed that the average blood cell levels of patients with 46/1 haplotype mutation compared with that of wild-type patients, white blood cells increased significantly (11.5608/11.5008 vs. 15.3108/16.3779, P <0.05) while neither hemoglobin nor platelet levels between two groups had a significant difference (P> 0.05).
Summary / Conclusion: Our results indicate that the 46/1 haplotype mutation contributes significantly to the occurrence of MPN in populations in Nanjing region of China.

B1579

PRIMARY AUTOIMMUNE MYELOFIBROSIS: REPORT OF TWO CASES

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Background: Primary Autoimmune Myelofibrosis (PAIMF) is a rare disease characterized by pancytopenia, mild leukoerythroblastosis, isolated serological immune abnormalities and no splenomegaly. Differential diagnosis include idiopathic myelofibrosis, immune myelofibrosis associated with other disorders, particularly sistemic lupus erythematous (SLE) and secondary myelofibrosis.
Aims: To describe clinical features, diagnosis and outcome of two patients with PAIMF at our institution.
Results: Case 1. A 53 year old female with no relevant medical history was admitted because of several weeks of progressive asthenia and pancytopenia. Isolated bruising in the legs were present and no splenomegaly was found. The haemogram showed leukocytes 3.3 x10^9/L (ANC 1.2x10^9/L), Hb 10.5g/dL, Hct 29%, Platelets 42x10^9/L. The immune panel revealed positive anti-nuclear antibodies 1/40 in a mottled pattern and polyclonal hipergammaglobulinemia. JAK-2(V617F) was negative. Paroxysmal nocturnal hemoglobinuria (PNH) was ruled out by flow cytometry. Bone marrow trephine biopsy showed normal morphology and distribution of haematological lineages with megakaryocytes precursors surrounded by reticulinic fibrosis. Methylprednisolone at 1 mg/kg/d was initiated with a rapid improvement of clinical symptoms, reaching normal haematological values within 4 weeks of treatment. Prednisone was discontinued after 4 months and the patient remained asymptomatic, with normal haemogram until last follow up, 14 months after treatment discontinuation.
Case2. A 76 year old female with a history of hypertension and diabetes mellitus was admitted because of constitutional symptoms and pancytopenia. At physical examination she was pale with no splenomegaly. The haemogram showed leukocytes 2.61x10^9/L (ANC 1x10^9/L with Dhöle bodies and mild leukoerythroblastosis), Hb 6g/dL, Hct 19%, Platelets 94x10^9/L. The immune panel revealed positive anti-nuclear antibodies 1/40 in a mottled pattern. JAK-2(V617F) was negative and PNH was ruled out by flow cytometry. Bone marrow aspiration was dry tap and trephine biopsy showed hypercelularity with isolated groups of small megakaryocytes and areas of reticulinic fibrosis. Methylprednisolone at 1 mg/kg/d was initiated with rapid improvement of clinical symptoms, reaching normal haematological values within 3 weeks. After 2 months treatment was discontinued and the patient remained asymptomatic with normal haemogram until last follow up 5 months after treatment discontinuation.
Summary / Conclusion: PAIMF is an infrequent and benign disease that should be distinguished from other disorders having myelofibrosis. The main difference with idiopathic myelofibrosis is mild leukoeritroblastosis without marked teardrop poikilocytosis in peripheral blood, lack of clustered or atypical megakaryocites in bone marrow and absence of splenomegaly. PAIMF should also be distinguished from myelofibrosis associated with immune disorders, mainly SLE. In cases of co-existing SLE or other immune disorders, combined immunosuppressive agents are usually necessary. Due to the excellent response to corticosteroid therapy, empirical treatment should be considered to patients with *atypical* myelofibrosis.

B1580

CLINICAL-BIOLOGICAL CHARACTERISTICS AND RISK FACTORS ASSOCIATED TO PROGRESSION IN A SERIES OF MYELOFIBROSIS PATIENTS IN A SINGLE CENTER: VALIDATION OF DIPPS-PLUS PROGNOSIS MODEL

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Background: Identification of myelofibrosis (MF) patients with expected shorter survival is important for appropriate decision making. For risk stratification, it is recommended to use International Prognostic Scoring System (IPSS) at diagnosis and Dynamic-IPSS (DIPSS) anytime during the disease course. Marked inter-patient variability within IPSS and DIPSS risk groups has been

observed, suggesting a potential role of other risk factors. Incorporation of 3 other factors into the DIPSS-plus scoring system was based on single institution data, which warrant further validation.
Aims: The aim of this study was to analyze the factors associated to disease progression and evaluate whether the new DIPSS-plus prognosis model can be validated in our independent dataset.

Table 1	N
Median age (range)	67 (37-84)
Male/female	14/12 (54/46%)
Symtoms and signs:	16 (61%)
• asymptomatic	10 (38%)
• anemia	10 (38%)
• constitutional syndrome	6 (23%)
• symtomatic splenomegaly	5 (19%)
• palpable splenomegaly	16 (61%)
Hb, g/dL*	11.15 ± 2.47
WBC, x 10^9/L*	13.35 ± 10.49
Platelets, x 10^9/L*	445 ± 284
High LDH levels	23 (88%)
Circulating CD34+,mcL*	102 ± 105
JAK2V617F available	22 (84%)
JAK2V617F/JAK2wt	12/10 (55%/45%)

*Mean ± SD

Methods: All patients consecutively diagnosed in our center of MF according to WHO criteria since 2000 were eligible. Clinical-biological characteristics at presentation and complications during evolution were registered. Based on EUMNET criteria, progression was defined as appearance of constitutional symptoms; or, decrease of Hb ≥ 20 g/L, transfusion requirement for non-transfusion-dependent patients, or increase ≥ 50% of transfusion requirement; or, substantial increase of spleen size; or leukemic transformation. Association between progression and factors at presentation such as grading of bone marrow fibrosis, splenomegaly, LDH, Jak2V617F, circulating CD34+ cells in peripheral blood (PB), unfavorable cytogenetic (CG), anemia, constitutional symptoms, DIPPS and DIPPS-plus risk scale were evaluated by X2 test. Progression free survival (PFS) and overall survival (OS) analysis was carried out with the Kaplan-Meier method. The log-rank test and Cox regression were applied for univariate and multivariate analysis, respectively.
Results: 26 patients were included. Patients´ characteristics at presentation are shown in Table 1. CG analysis were available in 23 cases: standard risk in 87% of the evaluable cases (normal: n=15; del 20q: n=3; del 13q: n=2) and unfavorable risk in the other 13% (+8: n= 2; -5: n=1). Almost 80% of the patients were treated with hydroxyurea and 69% (18/26) did not have any complication. The other 8 patients had different complications including new thrombotic (n=2) or hemorrhagic (n=5) events, infections (n=1), other neoplasms (n=1) or progression (n=5, 2 because of leukemic transformation and the other 3 because of marrow failure). Two factors associated to disease progression: constitutional symptoms (P=0.01) and CD34+ cells in PB (174±100/uL vs 79±90/uL, P=0.08). PFS was 88 months (range 72-103). Due to sample size, we grouped patients in two groups for DIPPS-Plus: Low+Intermediate (Int)-1 vs Int-2+High Risk. Applied at the time of diagnosis, DIPPS-Plus was predictive of PFS (105±4 in low/Int-1 vs 69±13 months in Int-2/high risk, P=0.01). Univariate analysis of the weight of each variable in DIPPS-Plus showed that constitutional symptoms, leukocytosis >25x10^9/L and transfusion requirement are predictive of shorter PFS. In the multivariate analysis, however, only the presence of constitutional symptoms and leukocytosis >25x10^9/L bordered statistical significance. OS was 76 months (range 57-96). An association between risk increases in the DIPPS-Plus model and decrease in OS (91, 86, 74 y 34 months for low, int-1, int-2 and high risk respectively) was observed.
Summary / Conclusion: Our data suggest that DIPSS-plus prognosis model can classify patients into categories with a different PFS, when applied either at diagnosis or throughout disease evolution.

B1581
TWO CASES OF JAK2 V617F-NEGATIVE PV WITH THE N542-Z543DEL MUTATION IN JAK2 12TH EXON.
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Background: The discovery of V617F mutation in Janus kinase gene (JAK2) revolutionized the classification and diagnosis of BCR/ABL-negative chronic myeloproliferative disorders (BCR/ABL- CMPDs), which includes polycythemia vera (PV), essential thrombocytosis (ET) and primary myelofibrosis (MF). Nevertheless, in a great number of cases, these pathologies exist in absence of this mutation, so it must be other alterations related with their origin and development. It has been demonstrated that mutations in 12th exon of JAK2 are involved in the PV development. Until date, 37 different mutations has been found in the adjacent region to pseudokinase domain of JAK2 (JH2), all of them curses with a PV phenotype similar to that caused by JAK2 V617F mutation.
Aims: Description of two cases of JAK2 V617F-negative PV with a mutation (n542-z543del) in JAK2 12th exon.
Methods: We studied 29 patients diagnosed as PV according to the WHO criteria (2008). Hematological data were obtained with a hematologic counter (Coulter LH750).

PATIENT	SEX	Age at diagnosis	Risk factors	Hb (g/dL)	Ht (%)	RBC (x10^6/L)	Platelets (x109/L)	WBC (x109/L)	EPO (mU/mL)	JAK2 V617F	JAK2 exon12
26	♀	46	Smoker	17,00*	55,70*	8,10**	370,00	8,60	1,7*	-	n542-z543 del
Others (10 cases)	♀	65± 19	Specific of patient	16,26* ± 2,38	49,56* ± 6,31	5,51* ±1,56	482,30* ±198,63	8,89 ±3,93	n/d	+	Normal
66	♂	35	Smoker	20,00*	63,70*	7,60**	164,00	10,00	1,54*	-	n542-z543 del
Others (13 cases)	♂	67 ± 13	Specific of patient	17,92* ± 1,54	54,69* ± 5,50	6,25* ± 0,91	487,31* ± 283,47	11,53 ± 3,90	n/d	+	Normal

Table 1: Clinical data of patients. Where Hb= Hemoglobin, Ht= Hematocrit, RBC= Red blood cells, WBC= White blood cells, EPO= Erythropoietin, JAK2= Janus Kinase2, n/d= not determinated *=Out of normal range. [Normal ranges for Female (12.0 - 16.0 g/dL; 36 – 46%; 3.5 - 5.5 x10^6/L, 150-400 x109/L, 4-12 x109/L, 5-30 mU/mL) and Male (13.5 - 17.5 g/dL; 41– 53%; 4.3 - 5.9 x10^6/L; 150-400 x109/L; 4-12 x109/L, 5-30 mU/mL)]. In "Others" the values shown are the average of individual parameters ± their standard deviations.

To determinate the presence of the JAK2 V627F mutation a PCR-ARMS was performed with DNA from whole blood obtained by manual methods (salting out). In some cases in which the mutation was not found, due to clinical evidences (like low erythropoietin levels), we sequenced JAK2 12th exon, using the same biological source, but employing an automatic method for the extraction (Biorobot EZ1). Automatic sequencing was performed following the supplier's instructions in an ABI PRISM® 3100 Genetic Analizer sistem.
Results: From all cases studied, 23 of them (79.3%) showed the JAK2 V627F mutation, 2 (6.9%) carries alterations in the 12th exon of the same gene (in both cases, the n542-z543del deletion in heterocygotic state, previously described by Scott et al. 2007) and in 4 cases (13.8%) any of the mutations studied were found. Clinical features of these patients are summarized in the attached table; they shown middle age at diagnosis (35-50 years), low erythropoietin (EPO) levels, hemoglobin (Hb), hematocrit (Ht) and erythrocyte count (RBC) values over the normal range but no splenomegaly neither thrombotic events.
Summary / Conclusion: The 86,2% of patients diagnosed as PV carried mutations in JAK2 (23 V617F + 2 n542-z543del). Both mutations affect the function of JH2 domain, which loses their regulatory function on the catalytic domain, being constitutively active and over activate their related molecular pathways. This explains, at least in part, the features of the disease.
So, is not strange that the phenotype caused by both mutations was similar but, in cases with exon12 mutations, the age at diagnosis was lower and the hematological parameters a little higher (with predominant erythrocytosis). These data are consistent with those founded in the literature.
Furthermore, there are four cases where mutations studied were not found, but showing a clear PV phenotype (so, it must be caused also by other molecular alterations in JAK2 or other genes). These facts show that other mutations in adition to JAK2 V617F, even if minorities should have a great importance in CMPDs. That might explain phenotypic differences between patients and forms of the disease (PV, ET, MF) and their implications in the clinical, diagnosis and treatment of the CMPDs must be studied more deeply.

B1582
PHILADELPHIA CHROMOSOME - NEGATIVE MYELOPROLIFERATIVE DISEASES AND GASTROINTESTINAL BLEEDINGS
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Background: Philadelphia chromosome-negative chronic myeloproliferative diseases (CMPD) are a group of malignant clonal diseases of hematopoietic stem cells, which are characterized by an overgrowth of one or more blood lines with normal or nearly normal maturation of those cells in the bone marrow and extramedullary hematopoietic organs. In dependence on the dominating type of cells, morphological and clinical characteristics, we include in this group the following diseases: 1) Polycythemia vera (PV); 2) Agnogenic myeloid metaplasia with myelofibrosis or idiopathic myelofibrosis (IMF); 3) Essential thrombocythaemia (ET); 4) Myeloproliferative diseases that cannot be classified (MPS).
Aims: The aim of the paper is to determine what is the incidence of gastrointestinal bleedings in patients with chronic myeloproliferative disorders.
Methods: The investigation included 155 patients of both sexes aged between 17 and 83 years with a diagnosis of CMPD. Patients with a diagnosis of CMPD are divided into four groups. We used methods of clinical examination, labora-

tory tests, ultrasound examination of the abdominal organs and esophagogastroduodenoscopy and rectosigmoidoscopy.
Results: Gastrointestinal symptoms (nausea, urge to vomit, abdominal pain, feeling of satiety) were found in 36.99% of patients with CMPD. The highest percentage of gastrointestinal symptoms was observed in patients with IMF (46.15%), while in the other group recorded percentage was significantly less, but not statistically significant. The presence of various forms of gastrointestinal bleeding (melena, hematochezia, hematemesis) were observed in less than one fifth of patients with CMPD (19.18%). The highest percentage of bleeding was originating from the proximal gastrointestinal tract (80.2%), as a result of gastric and duodenal ulcers. Only 18.8% of bleedings were from the distal part of the gastrointestinal tract. In groups with PV, ET, between 30 and 40% of patients were with ulcer disease that was significantly higher in comparison to those with IMF and MPS ($p < 0.001$). The highest percentage was in patients with PV, and immediately after them are those with ET. Patients with PV had an increased incidence of peptic ulcer disease, caused by the thrombosis of small arteries in the mucous membrane of the stomach and intestines, the effects of gastric juice, which is almost always hyperacid in those patients, basophilia and hyperhistaminemia.
Summary / Conclusion: Our investigations have shown an increased incidence of gastrointestinal bleedings in patients with true polycythemia, but also a significantly higher incidence of those complications in patients with essential thrombocythemia.

B1583

USE OF RADIO-ACTIVE PHOSPHORUS (P 32) IN THE MANAGEMENT OF POLYCYTHAEMIA RUBRA VERA (PRV) AND PRIMARY THROMBOCYTHAEMIA (ET) IN EAST KENT UNIVERSITY HOSPITALS NHS TRUST (EKUFHT) UK (01/01/2008 – 31/12/

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Background: : As per the British Committee for Standardisation of Haematology (BCSH) guidelines for ET and PRV published in 2010 and 2005 respectively, P32 therapy is effective in controlling blood counts with few acute side effects. The usual dose is 150-300MBq, and can be repeated after 3 months. The use of P32 is recommended as second line therapy in those who are older than 70 years who fail to respond to or are intolerant of the first line therapy (hydroxycarbamide). P32, the radioactive isotope of Phosphorous, had long been successfully used in treatment of myeloproliferative disease, namely PRV and ET. It is a pure beta emitting radionuclide with a physical half-life of 14.3 days, given intravenously or orally in aqueous solution. It is actively incorporated into the nucleic acids of rapidly proliferating cells, thus suppressing hyperproliferative cell lines rather than eradicating them. Early side-effects of transient leucopoenia and thrombocytopenia are observed. The late potential for leukaemogenicity is a risk but its incidence is comparable to that associated with the chemotherapeutic agents commonly used in management of these conditions. (EANM procedure guideline for ^{32}P phosphate treatment of myeloproliferative diseases; Eur J Nucl Med Mol Imaging (2007)34:1324-27)
Aims: To audit the efficacy of radio-active phosphorus(P32) in the management of PRV and ET and the impact on the haematology out-patient clinic case-load in EKUFHT.
Methods: Data regarding the total number of episodes, Sex and Age group was collected via the Patient Administration System (PAS) of EKUFH NHS Trust. Data regarding the original diagnosis and outcome was collected from the APEX system used in the pathology department of EKHUFHT and from outpatient clinic letters. Response was assessed using the following criteria: Complete Response (CR) = HCT < 0.450 + Platelet count <600X10^9/L at 3-6 months post P32 injection + Not on any cytotoxic drugs OR venesection OR Interferon-alpha OR Anagrelide + being considered for discharge from clinic; Partial Response (PR) = reduced dose of cytotoxic therapy OR reduced frequency of venesections OR on Anagrelide/Interferon alpha (alone/in combination).No Response (NR) = No change in management plan.
Results: Between 01/01/2008 – 31/12/2012 there were 43 episodes (21 male/22 female) of usage of radio-active phosphorus (P32) in EKHUFT (23 (53.5%) episodes for treatment of PRV and 20 (46.5%) episodes for treatment of ET). 25 (58.1%) episodes were CR, 8 (18.6%) were PR and 10 (23.3%) were NR. This led to 18 episodes of discharge from the haematology out-patient clinics (41.9% of the total episodes).
Summary / Conclusion: Radio-active phosphorus (P32) is an effective modality of treatment for those over 70 years of age with a diagnosis of PRV or ET, which reduces/abolishes outpatient clinic attendance; thus contributing to improved quality of life.

B1584

2 CASES OF IDIOPATHIC HYPEREOSINOPHILIC SYNDROME: A RARE DISORDER WITH SEVERE THROMBOHAEMORRHAGIC COMPLICATIONS

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Background: Idiopathic hypereosinophilic syndrome (HES) is a rare diagnosis with a poor prognosis. Management of HES is challenging due to heterogeneous clinical presentation. Complications include haemorrhage, thrombosis, multi-organ failure, rapid clinical deterioration and death. Thromboses can vary from ischemic colitis, sinus thrombosis to DIC and TTP (Ohguchi ,2009).There is an urgent need to control the eosinophil count and prevent thrombosis and haemorrhage. Conventional treatment includes steroids, hydroxycarbamide and interferon-alpha. Several case reports document the utility of cytotoxic agents such as AraC either alone or in combination.
Aims: We describe two cases of HES with eosinophil counts > 100x10^9/L highlighting diagnostic and management challenges.
Methods: CASE 1: 60 year old female on rivaroxaban thromboprophylaxis post knee replacement presented with swelling and discolouration of her right leg. Ultrasound Doppler was negative for a deep vein thrombosis. DVT was excluded and intramuscular bleed was diagnosed, rivaroxaban was stopped. FBC was Hb 7.6g/dl, WCC 7.5x10^9/L, eosinophils 0.3x10^9/L and platelets 265x10^9/L. She was readmitted the following week with exertional dyspnoea.FBC now Hb 10.5g/dl, WCC 100x109/L (Eo 83x10^9/L). Prednisolone 100mg and heparin anticoagulation commenced for suspected drug- related hypereosinophilic syndrome. BM: mature eosinophilic hyperplasia with normal maturation. Cytogenetics and FIPILI-PDGFRA status negative. Within 24 hours she developed acute onset right hand weakness and CT/MRI imaging suggested transverse sinus thrombosis. Hydroxycarbamide was started, eosinophils remained high. 4 days later, acute confusion occurred. WCC was 134 x10^9/L (Eo 118x10^9/L). AraC 20mg bid normalised eosinophil counts within a week. However falling GCS and hypoxia developed. Repeat CT confirmed multiple cerebral infarcts with small haemorrhages, and anticoagulation discontinued. 7 days later a myocardial infarction occurred and neutropenic sepsis with acute eosinophilic lung disease. Multi-organ failure progressed with further acute cerebral events leading to signs of brainstem infarction/bleed and the patient died a few days later. CASE 2: 55 year old male admitted with headache, flu-like symptoms and bilateral purpuric rash. WCC 16.4x109/L,mild neutrophilia and anaemia. CT revealed hepatosplenomegaly, mediastinal lymphadenopathy and adrenal mass. Investigations for vasculitis and infective causes were excluded. The rash progressed WCC 69x10^9/L, Eo 39x10^9/L, BM: reactive eosinophilia, no evidence of CML/MPN. Commenced prednisolone for suspected vasculitis. Extension of his rash, ulcers and pneumonia occurred with WCC 184x10^9/L, Eo69.9x10^9/L. Methylprednisolone and broad spectrum antibiotics were initiated. He developed ascites and coagulopathy. Repeat BM consistent with HES, commenced Hydroxycarbamide, but continued to deteriorate with confusion, progressive MRSA pneumonia needing ventilation. Interferon alpha was not effective. Neurological progression with unilateral weakness; CT: multiple cerebral infarctions, ground glass shadowing in lungs and bronchial lavage revealed eosinophilic infiltrate. AraC 10mg bid controlled the eosinophil count. Ventilation was required for 7 weeks and on-going Hydroxycarbamide and interferon alpha. He was discharged for intensive rehabilitation and continues to improve.
Results: As above
Summary / Conclusion: Our cases illustrate diagnostic and management challenges; rapid disease course reinforces the need to urgently control the counts to minimise the thrombo-haemorrhagic complications. Early use of AraC seems an effective cytoreductive agent in HES with extreme eosinophilia.

B1585

SINGLE-AGENT LENALIDOMIDE IS EFFECTIVE FOR TRANSFUSION INDIPENDENCE IN A PATIENT WITH REFRACTORY ANEMIA WITH RING SIDEROBLASTS, THROMBOCYTOSIS AND JAK2 (V617F)

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Background: Refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T) is a rare myelodysplastic/myeloproliferative disorder, that has been proposed as a provisonal entity in the 2001 and 2008 WHO classification. JAK2-V617F has been shown in the majority of these patients. The clinical course of RARS-T could be better than that of RARS and worse than that of essential thrombocythemia; however, the clinical and therapeutic experience with such cases is limited, due to the rarity of this disease. Lenalidomide is effective in patients with myelodysplastic syndromes with or without the del(5q) cytogenetic abnormality to reach transfusion independence, while no efficacy data of Lenalidomide are available in essential thrombocythemia to reduce platelet count. The efficacy of single-agent lenalidomide in RARS-T has been recently reported in one report (two patients described).
Aims: We report here the clinical outcome of Lenalidomide in the treatment of one patient with RARS-T and JAK2 V617F.
Methods: A 58-year-old caucasian man was admitted at our hospital in September 2006 for anemia (Hb 9.8 g/dL) and thrombocytosis (platelet count: 1163 x10^9/L). The bone marrow showed approximately 80% cellularity, increased atypical megacaryocytes with often lobulated nuclei and erythroid dysplasia with 30% ring sideroblasts. Cytogenetic analysis showed a normal karyotype, FISH examination was negative, while PCR revealed the presence of JAK2 V617F; a diagnosis of RARS-T associated to JAK2 mutation was made. Due to increase platelets count (1340x10^9/L), hydroxyurea was started after 11 months from diagnosis, with worsening of anemia (Hb 9.6 g/dL pre-therapy to 8.3 g/dL after three months of cytoreduction). Mild steroid therapy (prednisone 10 mg/day for 16 months) showed a transient efficacy while treatment with recombinant erytropoietin (30.000 units/weekly) was no successful. Thus, blood red cells transfusion treatment was started, after 40 months from diagnosis. Because of a high transfusion needs (2 units every months), Lenalidomide 10 mg daily for 21 days consecutive was started after 48 months from diagnosis and Hydroxyurea was concurrently interrupted.
Results: After 3 cycles of lenalidomide, anemia improved (Hb 9 g/dL) and the platelet count decreased to 700 x 10^9/L.
After 6 cycles, the hemoglobin level reached a stable value at > 9 g/dL without need of blood red cell transfusion and the platelet count reached the stable value of 500-600 x 10^9 /L.
Currently, the patient successfully continues 21 days-cycles of 10 mg lenalidomide and he maintains the transfusion independence, after a total of 28 cycle in 2 years of follow-up. No adverse event have been recorded until now.
Summary / Conclusion: Therapeutic experiences with RARS-T cases are limited. We confirm that Lenalidomide as single agent is efficacy in the treatment of our patient with RARS-T and JAK2 V617F, to reach transfusion independence and to maintain the platelet count within the target threshold after a long follow-up. Biological and histological analysis to demonstrate the morphological and molecular response are being carried out.

Non-Hodgkin lymphoma - Biology

B1586

CANINE DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) AS A SPONTANEOUS ANIMAL MODEL OF NON-GERMINAL CENTER DLBCL FOR PRECLINICAL STUDIES

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Background: Relevant preclinical animal models of human cancers are highly warranted to accelerate transfer of innovative therapies into human clinical practice. Spontaneous tumors in dog share important clinical, pathological, and diagnostic similarities with human tumors. Non-Hodgkin lymphoma is highly frequent in dog (33/100,000) and diffuse large B-cell lymphoma (DLBCL) is the most common subtype of lymphoma in dog. However, similarities between human and canine DLBCLs have been poorly investigated so far.
Aims: In order to evaluate the interest of spontaneous DLBCL in dog as a valid animal model for preclinical studies in human, we applied Hans' algorithm to canine DLBCL samples and evaluated the prognostic value of a canine-adapted immunohistochemical double-hit score.
Methods: Between 2005 and 2011, 100 dogs have been diagnosed with DLBCL in the College of Veterinary Medicine of Nantes (France). All DLBCL samples were evaluated for CD10, BCL-6, MUM-1, BCL-2 and c-MYC (clone Y69) by immunohistochemistry using an automated stainer. Thresholds for positivity were 30% for CD10, BCL-6, MUM-1; 10% for BCL-2 and 80% for c-MYC. Dogs' outcomes were obtained from referring veterinarians and owners.
Results: To date, 49 samples have been evaluated. Thirty-three dogs (67%) presented with stage III disease according to the World Health Organization (WHO) staging system of canine lymphomas. Six (12%) samples were classified as immunoblastic, while 43 (88%) were centroblastic. Seven cases (25%) were classified as germinal center-DLBCLs according to Hans' algorithm and 42 cases (75%) were non-germinal center-DLBCLs. 26 cases (53%) carried overexpression of c-MYC. A high immunohistochemical double-hit score (DHS) (c-MYC and BCL-2 positive) was observed in 6 cases (12%). Twelve dogs (24%) received no treatment, 26 (53%) received prednisolone alone, and 11 (22%) received CHOP-like chemotherapy regimen. Median overall survival according to treatment options was 19 days, 45 days and 220 days, respectively. In multivariate analysis, 4 parameters (absence of treatment, high WHO clinical stage, non-germinal center phenotype and a high DHS) were associated with shorter OS (P=0.01).
Summary / Conclusion: The present analysis shows that Hans' algorithm could be applied in dog and that non-GC DLBCL is more frequent than GC DLBCL. As observed in human DLBCL, a high DHS is associated with worse outcome. Our preliminary results suggest that spontaneous DLBCL in canine may be used as a model for non-germinal-center DLBCL (and not GC) for preclinical investigations.

B1588

SHRNA AGAINST CD44 EFFECT DIFFUSE LARGE B CELL LYMPHOMA CELLS

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Background: CD44, expressed predominantly in activated B-cell-like diffuse large B-cell lymphoma(DLBCL), has been reported to predict inferior survival in DLBCL patients.We have previously substantiated that CD44 expression still remain its prognostic significance even in the R-CHOP era.
Aims: To study the effect of CD44 down-regulation with lentivirus-mediated small hairpin RNA (shRNA) interference on the biological features of activated B-cell-like diffuse large B-cell lymphoma cell line SUDHL-2.
Methods: A lentiviral vector for RNAi of CD44 which contained green fluorescent protein(GFP) was constructed Package cells phoenix293 was used to produce virus stocks Then SUDHL-2 cells were infected with the recombinant lentivirus and the cells with CD44 knock-down were selected by FACS for GFP expression CD44 expression in the cells was determined by RT-PCR and FACS The proliferation,apoptosis and invasion were evaluated by MTT methods, FACS and transwell migration assay, respectively.
Results: DNA sequencing demonstrated that the lentivirus RNAi vector was constructed successfully Clones of SUDHL-2 cells infected with the recombinant lentivirus were selected and exhibited substantial knock-down of CD44 mRNA and protein expression compared with the control cells The proliferation ability of cloned SUDHL-2 cells were inhibited The apoptosis rates at early and late phase were 6.26%, 36.40% respectively in cloned SUDHL-2 cells and in control cells were 2.9%, 2.56% at early phase and 6.1%, 6.58% at late phase. The migrating number of cloned SUDHL-2 cells(34.53±8.05)% was also significantly decreased compared with the control cells(78.67±2.64 %, (78.00±6.13% (*P*=0.290).
Summary / Conclusion: The lentivirus-mediated shRNA of CD44 is efficient in down-regulating CD44 expression and inducing apoptosis, inhibiting proliferation and invasiveness of SUDHL-2 cells, suggesting that CD44 might have an oncogene role in the tumorigenesis and progression of ABC-DLBCL.

B1589

SEVEN MICRORNAS DIFFERENTIATE PATIENTS WITH EBV-POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA OF THE ELDERLY FROM DIFFUSE LARGE B-CELL LYMPHOMA NOT OTHERWISE SPECIFIED: A MICRORNA SIGNATURE PROFILE PROPOSAL

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Background: EBV-positive diffuse large B-cell lymphoma of the elderly (EBV+DLBCLe) is considered a provisional entity in the latest World Health Organization classification. It affects individuals older than 50 years without prior documented immunodeficiency. This disorder has unfavorable clinical course even after the advent of immunotherapy associated to anthracycline-based chemotherapy. It is linked to Epstein-Barr virus and the physiopathology is related to the presence of the virus itself, senescence and immunological deterioration. Currently there is not a characteristic pattern of expression of microRNAs in EBV+DLBCLe.
Aims: To characterize a signature profile for this new entity and to explore its peculiar characteristics as biomarkers and potential alternative therapeutic targets for EBV+DLBCLe.
Methods: One hundred and twenty four cases of patients of DLBCL treated at Hospital São Paulo UNIFESP/ EPM between 2000 to 2010, had paraffin blocks available for immunohistochemical and molecular analyses. Seventy of 124 patients have more than 50 years and are potential candidates to be considered EBV+DLBCLe. *In situ* hybridization was used for EBV detection (EBER-1, Invitrogen) in a tissue microarray (TMA) slide. Total RNA was obtained from tumor slides using the kit Recover All Total Nucleic Acid Isolation (Applied Biosystems). From the total RNA we obtained cDNAs using Megaplex Pools for microRNA Expression (Applied Biosystems). The cDNA was inserted into two platforms containing 384 human microRNA each (Taqman Low Density Arrays) on 7900 Real Time PCR Systems (Applied Biosystems). Data analyses were made in mathematical-statistical environment "R". The normalization method dct-2 was performed using the endogenous RNU48, and it was identified as the most stable among samples by software Normfinder. It was also used RNU6 recommended by the manufacturer, in a comparative way. MicroRNAs differentially expressed in EBV-positive group compared to EBV-negative were identified by means of nonparametric tests rank products (package RankProd) and Wilcoxon rank-sum (R-Stats). We considered differentially expressed microRNAs which average fold change above or below 1.5.
Results: 8.5% of cases of DLBCL were considered EBV+DLBCLe after *in situ* hybridization for EBV. We selected four samples of EBV-positive cases and four of EBV-negative matched by age, gender, stage and IPI to be analyzed in the PCR platforms. We found 10 deregulated microRNAs among the two groups. However, only seven microRNAs achieved statistically significant differences and will be the start point of a microRNA signature profile proposal to be validated in a larger multicentric cohort (total of 20 EBV+DLBCLe versus 40 DLBCL, paired by age, gender, stage, IPI and cellular origin according to Hans et al. algorithm). Among them 6 were overexpressed in EBV+DLBCLe comparing to EBV-negative DLBCL whereas 1 was underexpressed.
Summary / Conclusion: All microRNAs found are already described and involved in cancer. Five are reported in previous studies in non-Hodgkin lymphomas and one of them is correlated with clinical outcome and poor prognosis. The main routes involved are reduction of NF-KappaB DNA-binding activity and deregulation of the PI3K-AKT pathway. The merit of the present study is to put all the microRNAs together to create a specific expression profile signature for EBV+DLBCLe with the aim to define potential therapeutic targets as alternatives for R-CHOP. (Supported by FAPESP 2010/17668-6).

B1590

SYNERGISTIC CYTOTOXICITY OF EDELFOSINE, GEMCITABINE AND CLOFARABINE IN T-CELL LYMPHOMA

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Background: Edelfosine (1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine, ET-18-O-CH3) is an antitumor alkylphospholipid, a new type of anticancer drug, with immunomodulatory activity. It has shown clinical activity in lung and brain cancers, and pre-clinical activity in non-Hodgkin lymphoma, leukemia, breast cancer and pancreatic carcinoma. Edelfosine (Ed) induces apoptosis by recruitment of Fas/CD95 and subsequent DISC formation in lipid rafts. On the other hand, gemcitabine (Gem) and clofarabine (Clo) are nucleoside analogues which are used for treatment of various solid tumors and hematological malignancies. These are antimetabolites which inhibit DNA synthesis and repair, and induce apoptosis.
Aims: To investigate Ed for a potential synergistic cytotoxicity with Gem and Clo in lymphoma cells.
Methods: Cells were exposed to drugs individually, or in combinations, for 48 hours and analyzed by MTT assay, flow cytometry and Western blot.
Results: Exposure of J45.01 cells to IC_{10} – IC_{20} of these drugs for 48 hrs resulted in strong synergistic cytotoxicity. While the individual drugs inhibited cell proliferation by 5-20%, their combination inhibited survival by ~85%. Apoptosis with triple-drug combination increased to ~70%. The observed [Ed+Gem+Clo]-mediated cell death correlated with activation of DNA damage response as shown by increased phosphorylation of histone 2AX, SMC1 and KAP1. Furthermore, changes in the mitochondrial integrity are indicated by a dramatic decrease in the mitochondrial transmembrane potential, increase in the production of reactive oxygen species and release of pro-apoptotic factors from the mitochondria to the cytoplasm. The activation of apoptosis is also shown by cleavage of PARP-1, caspase8, caspase3, MCL1 and ANP32B. Exposure of the Jurkat-derived cell line I9.2, which is null for caspase8, to [Ed+Gem+Clo] resulted in only 40% inhibition of cell proliferation (as compared with 85% inhibition in caspase 8-positive J45.01 cells), suggesting the relevance of caspases in the antitumor activity of the three-drug combination. These observations are consistent with decreased phosphorylation of the prosurvival proteins p38, MAPK and AKT in cells exposed to [Ed+Gem+Clo]. The three-drug combination also caused translocation of nucleolar proteins to nucleoplasm, suggesting induction of nucleolar stress, which has been shown to activate p53-dependent cell growth inhibition.
Summary / Conclusion: Combination of the alkylphospholidid Ed with the nucleoside analogues Gem and Clo provides synergistic cytotoxicity in lymphoma cells by activating DNA damage response, induction of apoptosis via increase in reactive oxygen species and leakage of mitochondrial membrane, inhibition of the p38, MAPK and AKT signal transduction pathways, and induction of nucleolar stress. The observed synergism can be used as a mechanistic impetus for evaluating this drug combination as a part of cytoreductive treatment programs in patients with lymphomas.

B1591

MOLECULAR AND IMMUNOHISTOCHEMICAL ANALYSIS OF CELL CYCLE REGULATORY GENES IN DIFFUSE LARGE B-CELL LYMPHOMAS

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Background: Diffuse large B-cell lymphoma (DLBCL) is an aggressive malignancy of mature B cells with heterogeneous prognosis. Biological markers have been used in an attempt to improve the discriminative capacity of the International Prognostic Index (IPI). Abnormalities of cell cycle constitute a marker of clonal expansion and may contribute to disease progression; thus the investigation of cell cycle regulatory genes´ expression may be of prognostic relevance.
Aims: This study focuses on verifying the findings of our previous analysis regarding the expression levels of cell cycle regulatory genes in DLBCL. The first cohort of patients was treated with CHOP chemotherapy and the analysis was performed at the mRNA level. The second group included patients treated with R-CHOP and the analysis was performed by immunohistochemistry.
Methods: Our first analysis included 45 newly diagnosed cases of DLBCL and 15 cases of non-neoplastic lymphadenopathies used as controls. RNA was extracted from fresh frozen tissues and analyzed by RNase Protection Assay. Two sets of probes were used that included cyclins, cyclin dependent kinases and their inhibitors. This study included 99 newly diagnosed cases of DLBCL. Immunohistochemistry was performed on paraffin-embedded tissue sections. A semiquantitative analysis was performed and the intensity of immunostaining was assessed as follows: 0 (<1% positive cells), 1-weak staining (1-10%); 2-moderate staining (>10-50%) and 3-intense staining (>50%). 20,2% of the examined cases scored 0, 22,2% scored1, 30,3% scored 2 and 27,3% scored 3. Clinical characteristics were recorded. There were 50 males and 49 females. The median age was 66 years. The median freedom from progression (FFP) was 3 months, the median relapse-free survival (RFS) 18 months, the median progression-free survival (PFS) 16 months and the median overall survival (OS) 23 months. The revised IPI score was estimated as “very good” for the6,8% of patients, “good” for the 33,9% and “poor” for the rest of the patients (59,3%).
Results: Regarding our first cohort of patients the expression at the mRNA level of the cell cycle regulatory genes did not correlate with any of the clinical parameters nor the prognostic markers. 92% of the CHOP-treated patients with low IPI (0,1) expressed cyclin-dependent kinase (cdk10), while only 56% of the patients with high IPI (2,3,4,5) expressed this gene. The expression of cdk10 was also associated with significantly better overall survival (P=0,0329). Regarding the second cohort of patients treated with R-CHOP immunohistochemichal analysis was performed focusing on cdk10 expression with the following results. 40,3% of the examined lymphomas belonged to the GBC phenotype and 59,7% of the cases to the non-GBC phenotype, according to Hans *et al* algorithm. The association between cdk10 expression and clinicopathological variables revealed that a significantly higher number of cases within the non-GBC group was cdk10 positive, compared to the GBC group (P=0.03). Cdk10 expression was not associated with survival or disease free survival. However, cases with cdk10 positive expression were associated with significantly shorter freedom from progression time (P=0,006).
Summary / Conclusion: Our study has shown that putative markers of prognostic significance already published at the era of CHOP chemotherapy in DLBCL, lose their discriminative capacity in the era of R-CHOP. Although the expression of cdk10 at the mRNA level was associated with low risk disease and higher overall survival, these results were not confirmed at the protein level in the era of R-CHOP chemoimmunotherapy.

B1592

CYTOGENETIC CHANGES AND HISTOLOGIC ASSESSMENT OF BONE MARROW INVOLVEMENT OF NK/T CELL LYMPHOMA IN KOREAN

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Background: NK/T cell lymphoma is derived from a proliferations of NK cell and is characterised by an immunophenotype of CD2+, surface CD3-, cytoplasmic CD3e+, CD56+ and T cell receptor (TCR)-, lack of TCR gene rearrangement, and strong association with EBV. No specific chromosomal translocation has been identified by conventional cytogenetics, so far, though del(6q), i(1q), +8, i(17q), and 11q23 rearrangement are reported.
Aims: To investigate the patterns of bone marrow involvement and recurrent cytogenetic changes in NK/T lymphoma,we retrospectively studied bon marrow biopsy specimens, and fluorescent in situ hibridization (FISH) for del(6q), i(1q), +8, i(17q), and 11q23 rearrangement.
Methods: We retrospectively studied trephine biopsy specimens by CD56 immunohistochemistry and Epstein-Barr virus-–encoded RNA in situ hybridization (EBER ISH) from 68 consecutive patients. FISH for del(6q), 1q, +8, i(17q), and MLL 11q23 rearrangement were performed on available bone marrow mononuclear cells in 11 patients with microscopic BM involvement, using 19 probes (MYB(6q23), CEP XY, Tri-1q, cep8(+8), Rb(del13q), 7q31, p53(del17q), N-myc, BCL6, PDGFR-a, EGR, MLL, TEL-AML, inv16, 19q, del20q, BCR-ABL, PML-RARA, IGH-MYC). Results of conventional cytogenetics are reviewed.
Results: The incidence of BM involvement was 30.9% (21 of 68 patients) and in patients with BMI, CD56 were positive in infiltrated cells in 80.0% (12/15), and granzyme in 80.0% (4/5) and EBER in 66.7% (6/9). 1 of 12 patient showed EBER positive cells without evidence of infiltration of malignant cells. By G-banding, 8 of 43 patients (18.6%) showed cytogenetic aberrations. Cytgenetic aberrations were present in 27.8% (5 of 18 patients) among patients with BMI, while among in patiens without BMI, 12.5% (3/24) showed aberrations. FISH analysis revealed sex chromosome loss (45.5%), MLL rearrangement (9.1%), monosomy 11 (9.1%) and p53 deletion (9.1%). trisomy8, 6q deletion, iso17q and 1q gain were not detected.
Summary / Conclusion: Frequency of BM involvement in NK/T lymphoma in Korean is higher, compared to reported low frequencies of BMI. For determination of BM involvement which is pivotal in directing treatment strategy, IHC and EBER are essential. G-banding in conjunction with FISH analysis could give addtional complementary information for BMI.

B1593

FREQUENT LOSS OF THE TUMOR SUPPRESSOR TNFAIP3 IN SÉZARY SYNDROME

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Background: TNFAIP3 (A20) is known as one of the negative regulators of the NF-κB pathway. It`s inhibitory effect on NF-kB is based on the co-operative activity of two ubiquitin-editing domains. This function of A20 has been recognized for a number of substrates involved in transmission of signals from cell surface receptors to NF-κB, for example the receptor-interacting protein 1 (RIP1), the TNF receptor associated factor 6 (TRAF6) and the I-kB kinase (IKK). Particularly interesting is the NF-κB signaling pathway, since its constitutive activation has been identified as a key feature in CTCL (Cutaneous T Cell Lymphoma), including Sézary syndrome. Despite recent therapeutic improvements, the prognosis for patients suffering from Sézary syndrome is still poor.
Aims: To establish more specific and targeted therapies, a better understanding of the underlying molecular mechanisms driving the aberrant proliferation is required.
Methods: Using high resolution comparative genomic array hybridization (array CGH) we found bi- and monoallelic deletions of the TNFAIP3 gene in a high proportion of SS patients as well as a biallelic deletion in the SS-derived cell line SeAx.
Results: We demonstrated that inhibition of A20 activates the NF-κB pathway thereby increasing the proliferation of normal T lymphocytes. On the other hand, the reconstitution of A20 expression slowed down the cell cycle in SeAx cells. In a current study we extended the number of Sézary syndrome patient samples and determined gene copy number of A20 using an optimized competitive PCR assay. In this independent group of SS patients deletions were found in nine out of 17 (53%) Sézary syndrome samples.
Summary / Conclusion: In this study we showed that A20 is also a putative tumor suppressor in the T-cell malignancy - Sézary syndrome and underscore the relevance of A20 deletion in this tumor entity.

B1594

GENETIC POLYMORPHISM OF GLUTATION-S-TRANSFERASES M1 AND T1 (GSTM1 AND GSTT1) AND ENZYMES OF FOLATE METABOLISM IN THE PATIENTS WITH NON-HODGKIN MALIGNANT LYMPHOMAS

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Background: Non-Hodgkin's lymphomas (NHL) is a heterogeneous group of malignant lymphoproliferative disorders that have different rates of tumor progression, which may be the result of not only mutations in a phase of initiation, promotion and progression of lymphoma, but the initial genetic characteristics of thecells, such as polymorphisms of genes. NHL are most commonly diagnosed at advanced stages (III и IV). It is important to research new factors of the etiology of this disease. Genetically determined differences in the activity of enzymes of the second phase of xenobiotic biotransformation and folate metabolism can cause unequal predisposition to cancer, including NHL.
Aims: of this study was to investigate the role of deletions in glutathione-S-transferases M1 and T1 and some SNPs in folate genes (the C677T and A1298C SNPs in the *MTHFR* gene, A2756G in *MTR*, A66G in *SHMT1*, G1958A in *MTHFD1* and 844ins68 in *CBS)* in genetic susceptibility to non-Hodgkin's malignant lymphoma in the west-Siberian region.
Methods: 146 unrelated patients from the Novosibirsk City Haematological Center with various types of NHL were investigated. Genomic DNA was isolated from leukocytes in venous blood and also from buccal epithelium, using the standard methods of DNA separation. A PCR-restriction fragment length polymorphism (RFLP) assay was used to detect the *MTHFD* G1958A and *CBS* 844ins68 SNPs. Genotyping of the *MTHFR*, *MTR*, *MTRR* and *SHMT1* gene SNPs was carried out by real-time PCR allelic discrimination with TaqMan probes, *GSTM1* and *GSTT1* genes were analyzed by using real-time PCR with SYBRE Green and the following registration of melting curves. The alleles and genotypes distribution of SNPs in patients were compared with their distribution in healthy white Russian subjects from Novosibirsk.
Results: We determined the allele and genotype frequencies for *GSTM1* and *GSTT1* genes and seven SNPs in folate metabolism in NHL and control groups. For all these SNPs, the genotype frequencies were in Hardy-Weinberg equilibrium in the control group. For *GSTM1* gene it was noticed a higher rate of deletion genotype in patients with indolent lymphoma compared with the control group and aggressive NHL. This fact allowed us to estimate the contribution of *GSTM1* gene deletion in a predisposition to development of indolent NHL. It was revealed, the carriers of mutant *GSTM1* genotypes had 2-fold increased risk of indolent NHL in comparison with the normal genotype carriers (OR =1,96, CI [1.072-3.581], p <0,02). But the polymorphisms G1958A *MTHFD1* and C1420T SHMT1 showed significant association with aggressive NHL. Allele *1958A MTHFD1* was associated with decreased risk of diffuse large B-cell lymphoma (OR=0,429; C.I. [0.279-0.659], P<0,00008). Allele 1420T *SHMT1* was associated with increased risk in the group of another types of large B-cell non-Hodgkin's lymphoma (OR =1,862; C. I. [1,073 –3,231], p < 0,026). Any association of folate genes with indolent non-Hodgkin's lymphoma was not revealed.
Summary / Conclusion: Thus, the diverse impact of single nucleotide substitutions in the genes of the second phase of xenobiotic biotransformation (*GSTM1*) and folate metabolism (*MTHFD1, SHMT1*) on development of indolent and aggressive NHL can show the different molecular mechanisms of neoplastic transformation and it requires the investigation of the gene complex in order to determine predisposition to the development of non-Hodgkin's lymphoma.

B1595

GERMINAL CENTER LYMPHOMA CELL LINES: TOOLS FOR THE STUDY OF CLONAL EVOLUTION?

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Background: The evolution of tumor clones and the clonal architecture of tumors can be followed by the analysis of clone-specific mutations. These studies are done best at the single cell level. However, the necessary techniques are very challenging. Immortalized cell lines providing unlimited supplies of clonal cells would facilitate this work substantially. Yet, according to a widely accepted concept cell lines originate from one cell of a tumor carrying a set of mutations that allow continuous growth *in vitro.* If more than one cell happens to divide in the culture vessel, one clone should eventually outgrow the other. The data presented here contradict this common view.
Aims: We set out to find out whether molecularly-defined subclones can be detected in immortalized cell lines.
Methods: Immunoglobulin hypermutation and cytogenetic analyses were performed to detect subclones in B-cell lines. Gene expression of subclones was performed by microarray analysis and qRT-PCR analysis. Methylation specific PCR and bisulfite sequencing were conducted to determine the methylation status of individual genes.
Results: In several germinal center lymphoma-derived cell lines, we detected subclones with unique immunoglobulin hypermutation patterns. Clone-specific expression of various B-cell markers in the diffuse large B-cell line U-2932 allowed separation of the individual clones. According to the combined results of immunoglobulin hypermutation and cytogenetic analysis, both U-2932 clones derived from one mother clone with genomic *BCL2* amplification. The subclones acquired secondary lesions leading to the overexpression of *BCL6* in one, and *MYC* in the other subclone. Some 200 genes were differentially expressed in the two clones including transcriptional targets of *BCL6* and *MYC*. Other genes were epigenetically regulated as evidenced by DNA methylation analysis. Ectopic regulation of *BCL6* variously modulated new candidate target genes, confirming dual activating and silencing functions.
Summary / Conclusion: Genetically distinct subclones of cell lines may prove to model tumor heterogeneity *in vitro* allowing for functional analysis of oncogenes against a syngenic background.

B1596

CHARACTERIZATION OF CD19+CD5+ NON-CLL LYMPHOPROLIFERATIVE DISORDERS WITH AND WITHOUT T(11;14)

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Background: Mantle cell lymphoma (MCL) is characterized by the presence of t(11;14)(q13;q32), and shows a typical immunophenotype CD19+CD5+ that can be identified by flow cytometry (FCM). However, cases with typical MCL immunophenotype without t(11;14) are frequently detected in the clinical routine flow cytometry laboratory.
Aims: To characterize cases CD19+CD5+ (no CLL) lymphoproliferative disorders, analyzed in the clinical routine laboratory of flow cytometry, trying to identify profiles that could predict the presence of t(11;14).
Methods: We retrospectively analysed a total of 119 patients with CD19+CD5+ (non-CLL) lymphoproliferative disorders detected in the clinical laboratory of the University Hospital of Salamanca (Spain), in which information of immunophenotype, cytogenetics, and molecular biology (bcl-1) was available. The clonal B cell population was detected in peripheral blood (n=63), bone marrow (n=35) and lymph node (n=21). Erythrocyte-lysed samples were stained using selected panels of monoclonal antibodies, using four-color direct immunofluorescence technique and according to previously well described methods, aimed to identify and characterize B neoplastic cells; the complete diagnosis was performed with ancillary technics as molecular biology (bcl-1), karyotype and fluorescent in situ hybridization (FISH) for t(11;14).
Results: From the 119 samples with CD19+CD5+ immunophenotype (no CLL), 49% (59/119) of cases were t(11/14) positive. (8% by the three techniques; 8% by karyotype and FISH; 19% by FISH and MB; 46% only by FISH; and 19% only by MB). The 83% of t(11;14) positive cases showed a typical CD22+/CD23- immunophenotype, in addition to bright expression of CD20 and FCM7+ positivity. CD38 expression was negative in 53% of cases. Negative cases for t(11;14) showed CD22+/CD23- and CD38 in a slight lower frequency (60% and 32%, respectively), with similar expression of CD20. In t(11;14) positive cases the CD22+/CD23- was more homogeneous. The percentage (mean) of clonal B cells infiltrating PB, BM and lymph nodes was of 46%; 28% and 68% in cases t(11;14) positive vs 34%, 32%, and 57% in t(11;14) negative cases. Therefore, in general the percentage of infiltration was slightly lower in t(11;14)cases. Concerning clinical behaviour, as expected, the percentage of infiltration was higher in cases with a clinical behaviour of CLPD than in cases with a clinical behaviour of NHL, in cases both with and without t(11;14) (mean 39% and 44% vs 29% and 21%). Of the 23 cases with lymph node study, 2 cases were classified as splenic marginal zone NHL, 1 as DLBCL and 20 cases were classified as MCL. From the total, 73% of cases received therapy until now, and 83% of them had a NHL clinical behaviour. The rest 17% treated patients had a CLPD clinical behaviour, and the time to treatment was longer (mean 395 days) than in cases with NHL clinical behaviour (mean 95 days) (P=0.02).
Summary / Conclusion: Although cases with typical MCL immunophenotype are frequently detected in the routine clinical laboratory, the t(11;14) is detected only in half of the cases, and both t(11;14) positive and necative cases can have a indolent or agresive behaviour.

B1597

GLUTATHIONE S-TRANSFERASE GENOTYPES INFLUENCE PROGNOSIS OF DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma, accounting for approximately 30% of all newly diagnosed cases and more than 80% of aggressive lymphomas. The aim of this study was to investigate whether the genetic polymorphism of glutathione S-transferase P1 (GSTP1), which is a phase II detoxifying enzyme known to be a resistance factor for anticancer drugs, could be a prognostic factor of DLBCL.

Aims: patients with DLBCL

Methods: The case group comprised 82 patients with DLBCL (median age: 48 years, range: 18–76; males: 37, females: 45) treated in the National Cancer Institute, Ukraine. The patients were treated with R-CHOP-like regimens. Genomic DNA from peripheral blood of all individuals was analyzed for identification of *GSTP*1 polymorphism (c. 313 *A* > *G*, p. Ile105Val) using Allelic Discrimination Real-Time PCR.

Results: Of the 82 patients with DLBCL, 44 (53.7 %) were homozygous for the A313A GSTP1 genotype, 27 (32.9 %) were heterozygous (A313G), and 11 (13.4 %) were homozygous for the G313G genotype. The GSTP1 genotype distribution was conformed to Hardy-Weinberg equilibrium ($\chi 2$=1.88; P=0.17). Observed distribution was in agreement with previously reported findings in a healthy Ukrainian. We did not fined associations between demographic characteristics of the patients and GSTP1 genotype. The frequency of the homozygous wild genotype of the GSTP1 was higher in patients with advanced disease (stages III-IV) than in patients with stages I-II (59.5 versus 49.5 %, P=0.12). The overall response rate was 78.1 % (64/82) with a complete response of 72 % (59/82) and a partial response – 6 % (5/82). A complete response rate after the first-line therapy was better for the patients with the *A*313*G* or *G*313*G* genotype in comparison with the patients with homozygous wild genotype (79 % (30/38) versus 66 % (29/44), P=0.05). The highest incidence of primary drug resistance was observed for patients with A313A genotype of GSTP1 (62 %, 13/21) compared to patients with *A313G* (28.5 %, 6/21) and *G313G* genotypes (9.5 %, 2/21). Among the patients with complete response 17 (28.8 %) had relapse or progression and 7 patients dead (11.9 %) during the follow-up (median – 18 months; range 6–36 months). There was a trend for a lower risk for early relapse for patients with increasing number of mutant G allele (P=0.09) in our cases, but it should be confirmed by further studies with a larger cohorts of patients.

Summary / Conclusion: The obtained results suggest that the homozygous wild genotype of GSTP1 gene (*A*313*A*) is associated with a worse clinical response to the therapy and a higher risk of the early relapse for the DLBCL patients. Hence the further investigations of GSTP1 polymorphism is very promising, since it might provide a possible application of this genetic marker as an independent prognostic factor of DLBCL.

B1598

PRIMARY CENTRAL NERVOUS SYSTEM DIFFUSE LARGE B-CELL LYMPHOMA (PCNSDLBCL) HARBOR FREQUENT BCL6 BUT NOT MYC OR BCL2 TRANSLOCATIONS, AND BELONG TO THE NON-GERMINAL CENTER SUBTYPE (NON-GC)

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Background: Translocations involving *MYC* gene can occur in up to 10-15 % of diffuse large B-cell lymphoma (DLBCL), and have been associated with a bad prognosis, especially if present concomitantly with *BCL2* or *BCL6* translocations ("double-hit" and "triple-hit" lymphomas). Translocations involving those genes have not been widely evaluated in PCNSDLBCL.

Aims: The objective of the study was to evaluate the immunophenotypic and molecular profile of PCNSDLBCL, focusing on the presence or absence of *MYC*, BCL6 and BCL2 translocations.

Methods: Cases of PCNSDLBCL were collected from the Department of Pathology of two institutions (Hospital Germans Trias i Pujol, Badalona and Hospital de Cruces, Bilbao, Spain). Immunohistochemical stains regarding CD20 (clone L26, Dako), CD3 (clone PS1, Novocastra), CD10 (clone 56C6, Novocastra), BCL6 (clone PG-B6p, Dako), and multiple myeloma oncogene 1 (MUM1) (clone MUM1p, Dako) were re-evaluated and cases were classified as GC-like or non-GC according to Hans algorithm. The proliferating index was evaluated using the Ki67 antibody (clone MIB1, Dako). The status of *MYC*, *BCL2* and *BCL6* genes was evaluated by fluorescent in situ hybridization (FISH) using dual-colour break-apart commercial probes (LSI MYC DC BA, LSI BCL6 DC BA and LSI BCL2 DC BA; Abbot Molecular, Abbot Park, IL, USA) in two tissue microarray constructed with representative cores.

Results: A total of 49 cases were included in the study. The median age was 59 years (range 13-80) and 23 were female (27%). In 9 cases there was a subjacent immunodeficiency (8 cases with HIV infection, 1 case was a renal transplant recipient). Immunohistochemical staining was positive for CD10 in 3/49 evaluable cases (6%), BLC6 in 34/48 (71%), and MUM1 in 48/49 cases (98%). According to this phenotype, 4 cases were classified as GC-like. The mean proliferative index (Ki67) was 72 % (range 40-95%). *MYC* translocation was demonstrated in 1 of 48 evaluable cases (2%) and *BCL6* translocation in 19 of 47 (40%). *BCL2* translocation was not demonstrated in any case. Moreover, copy gain or losses without translocations involving *MYC*, *BCL2* and *BCL6* genes were observed in 17%, 36% and 11% of the cases respectively.

Summary / Conclusion: PCNSDLBCL belong to the non-GC subtype and undergo frequent translocations of *BCL6*, but not *BCL2* or *MYC* genes, indicating a different molecular profile from its nodal counterpart.

B1599

GENETIC POLYMORPHISMS ARG399GLN IN DNA REPAIR GENE XRCC1 AND RISK OF HIGH-GRADE NHL ENTITIES

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Background: The pathogenesis of non-Hodgkins lymphomas (NHLs) as and other tumors remains unknown. The results of epidemiologic studies suggest that single nucleotide polymorphisms in DNA repair genes may contribute to individual susceptibility for development of cancers different localization. Gene *XRCC1* (X-ray repair cross-complementing group I) participate in control of cell cycle and genome stability. Protein XRCC1 enrolled in organization of enzymes complex, which are repairing DNA. Polymorphism Arg399Gln gene *XRCC1* results in restitution of arginine to glycine in protein XRCC1 structure and depression of its activity. A number of molecular epidemiologic studies investigated association polymorphisms Arg399Gln in the DNA repair gene XRCC1 with different neoplasm's including Hodgkin's disease and acute lymphoblastic and myeloblastic leukemias, but insufficient evidence is available for NHL.

Aims: The purpose of the present study was study genotype distribution of single nucleotide polymorphism Arg399Gln gene *XRCC1* in patients with different histological NHL entities and estimate the association of this polymorphism with NHL risk.

Methods: We studied the single nucleotide polymorphism Arg399Gln gene *XRCC1* in 98 unrelated patients with NHL. To estimate the association of polymorphism with NHL risk the genotype distribution of Arg399Gln single nucleotide polymorphism in patients were compared with the distribution 180 in healthy, white Caucasians Russian subjects. Genotyping was carried out with use of PCR-RFLP.

Results: We evaluated a significant elevation of mutant Gln/Gln genotype *XRCC1* frequency in patients with NHL compared with normal controls. Analysis indicated that mutant gomozygosity is a significant risk factor of NHL (OR=2.2, 95% CI, 1.19-4.12). A positive association between genotype Gln/Gln gene *XRCC1* and risk of high-grade lymphomas (OR=2.6, 95% CI, 1.20-5.73), in particularly Diffuse large B-cells lymphoma (DBLCL) (OR=4.88, 95% CI, 1.62- 14.77), was observed. In contrast, Arg/Arg genotype was significantly lower in the cases, especially in patients with high-grade lymphomas and DLBCL than the controls and persons with Arg/Arggenotype *XRCC1* showed a more significant decrease in risk of high-grade lymphomas (OR=0.42; 95% CI, 0.18-0.98). Non-statistically changes genotypes distributions were observed for patients with low-grade NHLs and the controls. There was no statistical low-grade NHLrisk change in persons with the genotype Arg/Arg (OR=0.74; 95% CI, 0.34-1.44) or the genotype Gln/Gln (OR=1.79; 95% CI, 0.84-3.83) of the *XRCC1* Arg399Gln polymorphism.

Summary / Conclusion: These data suggest that genetic polymorphisms Arg399Gln in DNA repair gene *XRCC1* may modify the risk of high-grade NHL entities.

B1600

CASTLEMAN'S DISEASE: IMMUNOHISTOCHEMICAL ASSESSMENT OF NEOANGIOGENESIS IN POEMS-SYNDROME

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Background: Castleman`s Disease (CD) is an IL-6-dependent angiofollicular hyperplasia of the lymph nodes. The lesion occurs in two histological patterns (hyaline vascular and plasma cell) and may be local or multicentric. POEMS-syndrome is a rare complication of multicentric CD. The features of POEMS are polyneuropathy (P), organ enlargement-organomegaly (O), endocrinopathies (E), M-protein in the serum (M), and skin (S) changes. Most often the syndrome manifests with edema of the skin, subcutaneous fat and effusions in the serous cavities of the body. Overproduction of VEGF is currently discussed among probable pathogenetic mechanisms of POEMS.
Aims: The aim of the study was to check whether neoangiogenesis in the affected lymph node correlates with the development of POEMS in multicentric CD patients.
Methods: 15 patients with multicentric CD were included in the study. The diagnosis of CD was based on histopathological and laboratory findings. The spread of the CD as well as presence of the fluid in the serous cavities were estimated with CT scans. Polyneuropathy was proven by neuro-muscular conductometry. Presence of M-protein in the serum was detected by electrophoresis. Immunohistochemistry for CD31 was used to detect vascular endothelium in the lymph nodes. Perimeters of the vessels and vessel fraction area were calculated using image analysis VideoTest® software. Vessel fraction area index was calculated as mean value in 10 standard fields of view per case. The statistical significance was considered with p≤0,05 (Student criterion).
Results: Among 15 patients with multicentric CD included in the study 11 patients had hyaline vascular histological pattern and 4 patients had plasma cell or mixed pattern. All of 4 patients with plasma cell or mixed pattern had manifestations of POEMS. Vessel fraction area index in patients without POEMS was 11,5±0,83 % (M±m), and in patients with POEMS raised up to 29,4±8,4 % (p≤0,05). For comparison, single patient with tuberculosis infection and reactive lymphadenopathy had vessel fraction area index of only6,5%.
Summary / Conclusion: In multicentric CD substantial increase in vessel fraction area index is observed in patients with POEMS-syndrome as compared to those without POEMS. This finding may be related to the expected role of VEGF in the pathogenesis of POEMS.

Non-Hodgkin lymphoma - Clinical

B1601

DIAGNOSTIC SIGNIFICANCE OF PRETREATMENT SERUM LEVEL OF OSTEOPONTIN AND MACROPHAGE CHEMOTACTIC PROTEIN-1 IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

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Background: Diffuse large B cell lymphomas (DLBCL) are heterogeneous diseases which vary in biological expression and clinical course. While standard clinical prognostic factors predict outcome in DLBCL, predicting the outcome of patients might be further refined using biological factors. Some biological factors play a role in stimulation of malignant growth, metastasis and angiogenesis; however, their clinical relevance has not yet been well established for most of them.
Aims: The focus of this study was to determine pretreatment serum level of osteopontin (OPN) and macrophage chemotactic protein-1 (MCP-1) in patients with diffuse large B cell lymphoma and to investigate whether these factors provide prognostic information.
Methods: We measured pretreatment serum levels of OPN and MCP-1 by Enzyme-Linked Immunosorbent Assay (ELISA) in 67 patients newly diagnosed as diffuse large B-cell lymphoma and in 30 healthy controls. All patients were treated with rituximab-CHOP chemotherapy.
Results: The serum OPN levels were found elevated in untreated DLBCL patients compared to controls: in patients ranged from 25 to 238 pg/ml; median 94.2 pg/ml while OPN levels of the healthy controls ranged from 13 to 46.5 pg/ml; median 30.0 pg/ml (P=0.00008). There were significant differences in the serum MCP-1 levels between DLBCL patients and controls (median 1395.14 pg/ml vs. 779.3 pg/ml, P=0.035). Serum OPN levels higher than the median level was related to advanced Ann Arbor stage (P=0.026), International Prognostic Index of 2 or higher (P=0.005), ECOG ≥2 (P=0.004). The complete remission rate after treatment was higher in patients with low OPN serum levels than in those with high OPN serum levels (67.5% versus 32.4%, P=0.002). Elevated serum levels of OPN were strongly associated with shorter overall survival (P=0.007) and event-free survival (P=0.04). In multivariate analysis with International Prognostic Index criteria, OPN remained a significant predictor for overall survival (P=0.043). MCP-1 level was significantly correlated with age (P=0.005) and serum lactate dehydrogenase level (P=0.046), bat were not strongly correlated with other potential prognostic factors and it failed to show prognostic significance. A more advanced disease and/or poor prognostic factors were seen in patients who had both serum OPN and MCP-1 levels higher than the median level of the patients.
Summary / Conclusion: Our results showed that pretreatment serum level of OPN is significantly related to outcome in DLBCL patients.

B1602

THE USE OF PET-CT IN THE DIAGNOSIS OF ADULT T CELL LEUKAEMIA – LYMPHOMA SUBTYPES

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Background: The treatment of adult T-cell leukemia lymphoma (ATLL) is poor with median survival rarely exceeding 10 months in the aggressive subtypes. The diagnostic sub categorisation of the disease is important prognostically according to the original classification proposed by Shimoyama, which comprises acute (leukaemic), lymphomatous, smouldering and chronic subtypes. Acute and lymphomatous ATLL constitute the aggressive types and convey a worse prognosis. The role of PET-CT has been extensively investigated in the context of both Hodgkin's and Non-Hodgkin's lymphoma. However, to date no publications have characterised the appearances of the discrete ATLL subtypes on PET-CT.
Aims: Our aim was to characterise the appearances of ATLL subtypes on PET-CT, with particular emphasis on distinguishing aggressive disease from the indolent forms. In addition, we wished to investigate whether a correlation existed between the degree of marrow involvement and 18F-fluoro-deoxyglucose (FDG) uptake on PET-CT.
Methods: We retrospectively analysed 21 cases of ATLL treated at our institutions diagnosed between 2001–2012. 17 of these patients had PET-CT scanning performed as part of their initial diagnostic investigations and 4 after treatment or at progression. Bone marrow samples were described according to degree of disease involvement and correlated to intensity of FDG uptake on PET-CT. Clinical outcomes were correlated to PET-CT and bone marrow findings.

Results: The median age of patients was 49 years (male =9, female =12). 3/21 (14.3%) were categorized as aggressive acute subtype, 12/21 (57.1%) lymphomatous, 3/21 (14.3%) smouldering and 3/21 (14.3%) chronic. The median overall survival for all subtypes was 20.9 months (range 1.3-73.4 months). 16/21 (76.2%) of the patients have died. Patients with intense uptake on PET-CT at baseline (11/17, mean SUVmax 29.6, range SUVmax 9.6-85.2) displayed a tendency to markedly shortened median survival of 10.5 months compared to those with minimal/no uptake (5/17, mean SUV max 2.2, range SUV max 0-3.06) of 24.6 months regardless of subtype, although this was not statistically significant with our number of patients (P=0.28). All lymphomatous cases (n=10) demonstrated evidence of intense FDG avidity at baseline (mean SUVmax 24.5, range SUVmax 9.6-41.0) except 1 patient with no FDG avid disease who had a solitary lesion excised. All 3 cases of smouldering disease had either low or no FDG uptake (mean SUV max 2.0, range SUV max 0-2.88), no evidence of bone marrow involvement and remain alive and well. In 2 cases with excision of a solitary lesion, no uptake was demonstrated elsewhere. Of 19 assessable patients, 7 had documented marrow involvement: 3 had <5% disease bulk of which 1 had FDG uptake in bone. The 2 patients with high bone marrow involvement of 40% and 85-90% respectively both demonstrated intense skeletal FDG avid uptake (mean SUV 15.9).
Summary / Conclusion: In this series, patients with lymphomatous aggressive ATLL all demonstrated intense FDG avid uptake. All cases of smouldering subtype demonstrated minimal FDG uptake. In patients with resected localised skin disease and no evidence of FDG uptake, observation with serial HTLV-1 viral loads may identify those patients at high risk of progression to a more aggressive phenotype. The number of patients with marrow involvement was small, and intense FDG uptake appears to have been associated with greater disease bulk and more aggressive subtypes. Overall this retrospective analysis suggests PET-CT is useful in the diagnostic subclassification, and potentially prognosis of ATLL.

B1603

THE IMPACT OF FCΓ RECEPTOR POLYMORPHISMS AND GLUTATHIONE-S- TRANSFERASES POLYMORPHISMS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH R-CHOP

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Background: FcγRIIIa polymorphisms have been associated with response to single-agent rituximab in patients with follicular lymphoma and Wandelstrom macroglobulinemia but data regarding DLBCL and follicular lymphoma patients treated with R-CHOP are conflicting. Glutathione transferases (GSTs) polymorphisms have been associated both with favorable and unfavorable outcomes in a variety of cancer types. In Korean DLBCL patients they have been associated with chemotherapy related toxicities.
Aims: In the present study, we evaluated the prognostic impact of polymorphisms of the FcγRIIIa, GSTT1, GSTM1 and GSTP1 genes on the outcome of patients with DLBCL treated with R-CHOP.
Methods: DNA was isolated from peripheral blood or bone marrow samples of 109 patients. The 158V/F polymorphism of the FcγRIIIa gene and the deletions of GSTT1 and GSTM1 were analyzed using multiplex PCR techniques. The Ile^{105}Val polymorphism of the GSTP1 gene was analyzed using a PCR-RFLP technique. PCR products were evaluated on ethidium bromide-stained agarose gels.
Results: Of the 109 patients tested for the FcγRIIIa-158V/F polymorphism, 28 (26%) were carriers of FcγRIIIa-158V/V, 23 (21%) of F/F and 58 (53%) of V/F. With respect to GST polymorphisms, 45 patients (52%) were GSTM1-null, 22 (25%) were GSTT1-null, 10 patients (11%) were GSTP1-105V/V, 42 (47%) were Ile/Ile and 38 (42%) were heterozygous. Eleven patients (13%) were GSTM1/GSTT1-double null, while 45 (52%) had only one deleted gene and 31 (36%) had no deletions. There were no significant associations between FcγRIIIa or GST genotypes and patients' characteristics. Presence of GSTM1-null genotype was not associated with concomitant presence of GSTT1-null genotype (P=0.85), neither was presence of GSTP1-105V/V associated with concomitant presence of GSTM1/GSTT1-double null genotype (P=0.77). The 5-year EFS was 76% for FcγRIIIa-158V/V, 70% for V/F and 64% for F/F (P=0.68). For the GSTP1-105I/V polymorphism, 5-year EFS was 86% for V/V, 67% for V/I and 74% for I/I (P=0.58). The 5-year EFS was 72% for GSTM1-null patients versus 69% for GSTM1+ (P=0.66) and 77% for GSTT1-null versus 69% for GSTT1+ (P=0.47). Finally, 5-year EFS was 90% for GSTM1/GSTT1-double null patients versus 68% for patients with one deleted gene and 71% for patients with no deletions (P=0.59). Given that patients with GSTP1-105V/V genotype and patients with GSTM1/GSTT1-double null genotype had the highest EFS rates (86±13% and 90±9% respectively), further evaluation showed that presence of either GSTM1/GSTT1-double null genotype or GSTP1-105V/V genotype (19 patients in total), was associated with a marginal improvement in EFS compared to any other genotype (P=0.13).
Summary / Conclusion: Our data indicate that there is no individual effect of GSTs polymorphisms on EFS of DLBCL patients treated with R-CHOP. However, carriage of either GSTP1-105V/V polymorphism or GSTM1/GSTT1-double null genotype is present in 20% of patients and is slightly associated with improved EFS. This finding needs confirmation in a larger group of patients.

B1604

MANTLE CELL LYMPHOMA WITH AN INDOLENT BEHAVIORS

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Background: Mantle cell lymphoma makes up from 3 to 10% of non Hodgkins lymphomas. Median age of presentation is 60 years with male predominance. Morfologically limphocytes are small with a slightly indented nucleus and have a characteristic chromosomal translocation (11;14) (q13;q32). The survival of patients varies from 3 to five years, prognostic factors associated with a poor outcome comprise an increased mitotic index, blastic variant, 12 trisomy, aberrant karyotype, p53 gen mutations and peripheral blood involvement.
Aims: Evaluate Mantle cell lymphoma with indolent behavior in our institution
Methods: We have reviewed all mantle cell lymphomas diagnosed during the last 6 years in our Hospital. We studied 20 patients, 16 of them showed an indolent behaviour similar to B cell chronic lymphoid leukemia in A stage of Binet. We showed the results of these patients
Results: The male/female ratio of the patients was 2 to 4. The median age of our subjects was 72 years (range: 62-84 years) At diagnosis most of the patients had associated comorbidities, the most frecuent was HTA. Two patients suffered autoimmune hemolytic anemia before diagnosis of MCL was made and both of them showed good response to corticotherapy. Perypheral blood involvement was present in all cases , no anemia, and no thrombocytopenia appeared, morfologically limphocytes were small with a slightly indented nucleus coarse chromatine, without nucleoli, in one patient blastic cells were present in blood. Cells expressed strong inmunoglobulin , more often lambda light chain, CD5 and FMC7 while they were negative for CD10 and CD23 antigens. Only 3 patients had supra and infradiafragmatic lymphadenopathies inferior to 2 cms. 5 cases showed moderate splenomegaly . A FISH study in peripherical blood was performed in all patients which confirmed positivity for the translocation (11;14), without any others added abnormalities. None of the patients received treatment because of they were asymtomatic, the median follow up was 3 years, range (2-7 years)
Summary / Conclusion: There is a variant of indolent MCL similar to B cell chronic lymphoid leukemia in that affects peripheral blood without adenopathies. This asymptomatic patients without adenopathies should be treated by therapeutical abstention.
All of them were diagnosed following a inmunophenotype in peripheral blood on finding a lymphocytosis. It is important study prognosis factor of an index type of proliferation and added genetic anomalies to t (11;14) so as take therapeutical decisions

B1605

SIMPLE PROGNOSTIC PARAMETERS IN DLBCL: PRETREATMENT SERUM ALBUMIN LEVEL AS AN INDEPENDENT PREDICTOR FOR EFS AND OS

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Background: The International Prognostic Index (IPI) is a widely used and accepted prognostic model for diffuse large B cell lymphoma (DLBCL). In the rituximab era, R-IPI is used as well. In the last years, several groups looked for additional simple prognostic parameters, not included in the original IPI, i.e. absolute lymphocyte count (ALC), absolute monocyte count (AMC) and absolute neutrophil count (ANC), with diverse results.
Aims: The aim of the present study is to evaluate the prognostic value of these parameters such as, ALC, AMC, ALC/AMC, ANC/ALC, hemoglobin and albumin level at diagnosis in patients with DLBCL and compare with known prognostic models as IPI and R-IPI.
Methods: We retrospectively reviewed data of 166 adult patients with DLBCL who were diagnosed at Rabin Medical Center, Israel, between the years 2004-2008. The mean age was 63.4 years, 43% were male, 62% with stage III/IV, 28% with ECOG performance status 0-2, 59% with elevated LDH level and 85% were initially treated with R-CHOP. The median follow up was 6.6 years (range 3.6-9 year).
Results: The 5 years overall survival (OS) for the entire group was 67.5%; for patients with lower IPI (0-2) – 76% (n=83); and for patients with higher IPI (3-5) – 48.8% (n=80). In univariate analysis, pretreatment hemoglobin and albumin levels had statistically significant effect on EFS and OS. Five years OS was 77.3% in patients with pretreatment albumin >3.5 g/dl, compared with 47.9% in patients with albumin level ≤3.5g/dl (P<0.001). Five years EFS was 68.9% *vs.* 45.8%, respectively (P<0.001). Five years OS was 81.7% in patients with pretreatment hemoglobin >12g/dl, compared with 56.5% in patients with hemoglobin level ≤12g/dl (P=0.003). Five years EFS was 74.6% *vs.* 51.1%, respec-

tively (P=0.002). However, univariate analysis did not identify pretreatment ALC, AMC, ALC/AMC and ANC/ALC as predictors for EFS and OS. Multivariate analysis using Cox regression, included the above parameters as well as IPI/R-IPI, showed that pretreatment albumin level was independent prognostic factor (P=0.001) for EFS and OS and its effect was significant as IPI or R-IPI effect.
Summary / Conclusion: Our data showed that albumin level is a strong prognostic factor for EFS and OS, and its effect is as good as IPI or R-IPI effect.

B1606
MANAGEMENT OF THE HBV REACTIVATION IN ISOLATED HBCAB POSITIVE PATIENTS AFFECTED WITH NON HODGKIN LYMPHOMA
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Background: Occult HBV infection (OBI) is defined by the persistence of HBV in the liver without serum HBsAg and HBVDNA. It represents a life threatening risk if the carrier experiences immunosuppression. An OBI can be present in about 18% of HBcAb+ patients. International guidelines suggest a strict surveillance for ALT and HBV markers in patients undergoing immunosuppressive therapies, in particular monoclonal antibodies. In Non-Hodgkin Lymphoma (NHL), OBI reactivation can occur in 3 to 25%. The real prevalence remains to be established.
Aims: To determine the prevalence of occult HBV reactivation in a large cohort of patients undergone immunosuppressive treatments for NHL and to confirm the association with monoclonal antibodies.
Methods: We analysed 498 NHL patients in a single centre of Southern Italy from 2005 to 2011. We evaluated HBV markers, type and NHL localization, treatment type and HBV reactivation.
Results: Fourty percent was treated with monoclonal antibodies and 60.3% without. Ninety six patients were HBcAb+ and HBsAg-. HBV reactivation occurred exclusively in ten subjects of this subgroup, 5 treated with Rituximab and 5 without. Every patient was treated with Lamivudine. No one experienced liver-related death.
Summary / Conclusion: Our data report a prevalence of OBI reactivation of 10.42% in HBcAb positive patients. This event occurred in 50% of cases in patients treated with no monoclonal antibodies. Each reactivation was treated with Lamivudine. This report enlightens the importance and the cost-effectiveness of a strict surveillance in HBcAb+ HBsAg- patients, in order to detect an occult HBV reactivation, also in NHL patients treated with monoclonal antibodies-free protocols.

B1607
EPIGENETIC MARKERS AS PREDICTORS OF RESPONSE IN YOUNG POOR RISK DIFFUSE LARGE B CELL NON HODGKIN'S LYMPHOMA (DLBCL)
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Background: Young patients with poor risk DLBCL are characterized by truly refractory disease. Dose intensification of the standard combination chemotherapy is one of the methods to improve treatment results. DNA methylation is an important regulator of gene transcription; alteration in DNA methylation is common in development and progression of malignancy
Aims: To evaluate the promoter methylation (PM) of 4 genes: p16, p53, MGMT and GST as effective tools in predicting the outcome and prognosis of young poor risk DLBCL patients treated with CHOEP – 14 regimen
Methods: The study included 51 patients with high and high intermediate risk aaIPI DLBCL patients who were treated with CHOEP – 14 regimen. Promotor methylation of the tested genes was done by specific methylation PCR.
Results: Complete remission (CR) was achieved in 38 patients (74.5%). The CR rate was significantly affected by B-symptoms (P=0.03), extra nodal sites (P=0.03) and dose intensity of myelosuppressive drugs (P=0.001). PM of the studied genes was associated with significantly poor response (P=0.001) for the four tested genes. There was also significant relation between the methylation index and the response rate. The relapse rate was 23.7%. The 3 years DFS was 68.8% and 4 years OS was 96% with only one treatment related mortality. There was significant relation between the DFS and p53 PM as well as with p16 PM (P=0.02 and 0.05 respectively) while this relation was not significant with the other two tested genes. The main reported toxicity was myelosuppression, while non hematologic toxicities were infrequent and regressive
Summary / Conclusion: Hypermethylation of the studied genes correlated with poor response; however more studies are required to validate these findings.

B1608
NEITHER CONSOLIDATION RADIOTHERAPY NOR MAINTENANCE WITH RITUXIMAB OFFER ADVANTAGE TO PATIENTS WITH LARGE B CELL LYMPHOMA TREATED WITH STANDARD THERAPY
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Background: Most patients (pts) with diffuse large B cell Lymphoma (DLBCL) treated with R CHOP achieve complete remission (CR). However, a significant percentage relapses. Different strategies try to decrease relapse. Consolidation with radiotherapy (RT) has played a role in the management of DLBCL, but its utility in the Rituximab era is less known. Although a modest benefit of Maintenance with Rituximab (MR) has been suggested, guidelines do not support its use in DLBCL. We present the results of 105 pts with DLBCL who achieved CR after treatment with R CHOP(Epirubicin 75 mg/m^2 substituted Doxorubicin= R CEOP).
Aims: Assess the potential benefit of consolidation RT or MR to decrease relapse. Four groups of pts were defined: first, pts with no further treatment after R CEOP courses, consolidation with RT comprised the second group, MR formed the third group and a fourth group included pts who received RT and MR.
Methods: Between January 2006 and December 2011 all pts ≥ 18 years in CR were included. Risk factors reviewed: age <60 versus ≥60 years, Eastern Cooperative Oncology Group performance status (ECOG 0-2vs >2), Ann Arbor Stage (I-II vs III-IV), Extranodal Disease (ED), Bulky disease(BD) (≥10 cm), International Prognostic Index (IPI low vs high risk),6 versus 8 courses R CEOP, RT and MR. After CR and R CEOP courses, physicians decided if adjuvant treatment was necessary based on their clinical judgment. Involved field RT was delivered at a total dose of 36 Gy. MR was started 3 to 6 months after the last course of R CEOP and consisted of 2 different schedules: Rituximab 375 mg/m^2 one dose weekly for 2 consecutive weeks every 6 months or Rituximab one single dose every 3 months; both schedules until completing 2 years. Time to relapse was measured from the application of the sixth course of R CEOP to relapse.
Results: Median age was 61 years (18-86). Median follow up of all pts was 24 months (1-72). Fifty seven (54%) pts were ≥60 years (69% of them did not receive adjuvant therapy). Seven (7%) subjects had ECOG >2. Advanced Disease was present in 38 (36%)pts. Fifty one (48.5%) pts showed ED. BD was observed in 50 (48%) pts (54% of them received RT). High risk IPI in 22 (14.6%)pts. Seventy six (72%) pts received 6 courses of R CEOP. Sixty six pts (62.8%) received adjuvant therapy: RT 21 (20%) pts, MR 31 (29.5%)pts and 14 (13.3%)pts received RT plus MT. The differences between groups were an older age for the no adjuvant therapy group (p< 0.05) and BD for the RT group (p< 0.04). In all, 17pts (16.1%) have relapsed at a median time of 9 months (3-54).The group with no therapy had 8 relapses, RT 3 relapses, MR 5 relapses and one relapse occurred in the group with both therapies.The 3-year relapse free survival rates were 79.4%, 85.7%, 83.8% and 92.8% for the no adjuvant therapy, RT, MT, and both therapies groups (p <0.4), respectively. For those relapsing, the risk factors were advanced stage (p < 0.009) and high risk disease (p < 0.008).
Summary / Conclusion: The majority of pts are in CR. Nevertheless, most cases had low risk disease. We did not observe difference between groups. BD is usually an indication for RT. However, near half of the pts with BD did not receive RT, and did not have an increase incidence of relapse. MR did not offer benefit. Advanced and High risk diseases were the only risk factors associated to relapse. Therefore, it seems reasonable not to use any further treatment in low risk disease after standard therapy. Prospective trials with larger number of pts are needed to evaluate RT in the rituximab era.

B1609
PROGNOSTIC RELEVANCE OF BASELINE NEUTROPHILE-TO-LYMPHOCYTE RATIO IN DIFFUSE LARGE B-CELL LYMPHOMA : RESULTS FROM A PROSPECTIVE COHORT STUDY
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Background: The systemic inflammatory response and host immunity has been associated with prognosis of various human cancers. The neutrophile-to-lymphocyte ratio (NLR), representing both inflammatory response and imuune status, has been proposed as a reliable marker to predict clinical outcomes in cancer patients.
Aims: The aim of this study is to determine prognostic significance of baseline NLR in patients with diffuse large B-cell lymphoma (DLBCL).
Methods: Subjects were selected from the Samsung Medical Center Lymphoma Cohort Study (a prospective cohort study registered at www.clinicaltrials.gov as NCT00822731). Survival analysis were performed by Kaplan-Meier methods and the log-rank test. To evaluate the independent prognostic significance of NLR, multivariate Cox regression models were applied. The optimal

cutoff value of NLR was decided by using ROC curve analysis.
Results: A total of 334 DLBCL patients receiving R-CHOP chemotherapy were included in this study. A NLR value of 3.1 corresponded to the maximum combined sensitivity and specificity on the ROC curve and the AUC (area under the curve) was 0.635 (95% CI 0.581-0.687, P=0.0009). With a median follow-up of 31.1 months (range, 0.3-55.5), patients with baseline NLR>3.1 (n=125) showed poorer overall survival (OS) (P<0.001) and progression-free survival (PFS) (P<0.001) compared to patients with baseline NLR≤3.1 (n=209). The expected 3-year OS and PFS rates were 63.0% vs 86.1% (P<0.001) and 58.0% vs 77.7% (P<0.001), respectively. In univariate analysis, the following variables were predictive of OS: NLR>3.1 (P<0.001), age older than 60 (P<0.001), ECOG≥2 (P<0.001), stage≥3 (P<0.001), high-intermediate or high IPI risk group (P<0.001), extranodal involvement≥2 (P<0.001), and bone marrow involvement (P=0.001). In multivariate analysis, NLR>3.1 (HR 2.0; 95%CI 1.2-3.4, P=0.009), age older than 60 (HR 3.0; 95%CI 1.8-4.9, P<0.001), ECOG≥2 (HR 1.9; 95%CI 1.1-3.3, P=0.021) retained its statistical significance as independent poor prognostic factors for OS.
Summary / Conclusion: This study suggests that baseline NLR is a simple and significant independent prognostic factor for OS in DLBCL patients treated with R-CHOP chemotherapy. Further studies are anticipated exploring the mechanisms of associations between NLR and clinical outcomes in DLBCL, such as inflammatory transcription factors and cytokines.

B1610

18-FLUORODEOXYGLUCOSE (FDG) OUTPERFORMS 18F-FLUOROTHYMIDINE (FLT) IN IDENTIFYING TRANSFORMATION OF FOLLICULAR LYMPHOMA, IN PARTICULAR THROUGH HETEROGENEITY IN UPTAKE

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Background: Diagnosing transformation of follicular lymphoma (FL) to diffuse large B-cell lymphoma is important, since therapy regimens for FL are not effective in transformed lymphoma. Currently, transformation is probably underdiagnosed as transformation is known to occur focally whereas in general a biopsy is performed randomly. FDG-Positron Emission Tomography (PET), imaging glucose utilization, is known to correlate with proliferation rate. However, FLT might more specifically image proliferation through visualization of thymidine uptake.
Aims: Therefore we performed a prospective study to identify which PET-tracer, FDG or FLT, can be used to distinguish between FL and transformed lymphoma.
Methods: FDG- and FLT-PET scans were performed in 17 patients with FL and 9 patients with biopsy-proven transformed lymphoma. We measured SUVmax (standardized uptake value) of the lymph node with the highest uptake per patient and measured the range of uptake in involved nodes per patient. To reduce partial volume effects only lymph nodes larger than 3cc (measured as the A50 isocontour on the PET scan) were incorporated in the analysis. Scans were made on the Philips Gemini TF PET-CT camera, 1 hour after injection of 185 MBq of FDG or FLT.
Results: The SUVmax was significantly higher in transformed lymphoma as compared to FL for both FDG (median 22.0, range 14.6-42.4 in transformed and 10.9 (5.2-20.4) in FL, P<0.0001) and FLT (median 11.5, range 5.5-16.3 in transformed and 8.0 (3.6-16.6) in FL, P=0.03), however, with considerable overlap. Additionally we determined the range of FDG and FLT uptake in each individual patient. The FDG range was significantly higher in patients with transformed lymphoma versus patients with FL (6.0-37.5 versus 0.03-7.9, P<0.001) allowing discrimination between transformed lymphoma and FL. In contrast, FLT did not discriminate (P=0.07, ROC curve: AUC for FDG 0.967 vs FLT 0.716).
Using ROC curve analysis cut off values could be determined. Using a cut off of 14.5 for FDG SUVmax in the lymph node with the highest uptake, transformation was diagnosed with a sensitivity of 100% and a specificity of 82%. With a cut off of 6 for FDGrange, sensitivity was 100% and specificity 71%. For FLT no cut off values could be determined due to overlap of values. For validation, we analyzed FDG PET scans in 4 additional patients with transformed lymphomas and 5 patients with FL using these cut off values. Moreover, we used the same cut off values for 9 transformed lymphomas and 11 FL from literature (Bodet Millin Haematologica 2008). In these validation sets the 100% sensitivity could only be retained using the cut off of 6 for FDG range. The lower specificity found in the validation set (55-80%) is acceptable since it only leads to excess biopsies (when a FL is misclassified as a transformed lymphoma) and not to mistreatment of a patient (when a transformation is missed).
Summary / Conclusion: FDG-PET distinguishes better than FLT-PET between FL and transformed lymphoma. An intraindividual FDGrange of 6 or higher is highly suspicious of transformation and should guide diagnostic procedures.

B1611

FIRST-LINE THERAPY COMBINATION WITH RITUXIMAB AND FLUDARABINE IN PATIENTS WITH EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA OF THE MUCOSA-ASSOCIATED LYMPHOID TISSUE TYPE.

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Background: There are no consensus guidelines regarding the best therapeutic option for patients for patients with extranodal marginal zone lymphomas of the mucosa-associated lymphoid tissue (MALT) type.
Aims: To evaluate the efficacy and safety of combination first-line therapy with Rituximab plus Fludarabine in extranodal MALT lymphoma patients.
Methods: Patients with untreated or de novo extranodal MALT lymphoma received a combination of rituximab (375 mg/m2 iv) on Day 1 and fludarabine (25 mg/m2 iv) on days 1 to 5 (patients aged > 70 years days 1-3), every 4 weeks. After 3 cycles a work-up was done: those patients who achieved complete remission (CR) received an additional cycle and those in partial remission (PR) received three more cycles.
Results: Twenty-seven patients were included. The median age was 60 years (32-83 y) and 15 (56%) were women. Twelve had gastric MALT lymphoma and 15 extragastric MALT lymphoma. Nine patients (33%) had stage IV disease. After 3 cycles, 16 (59%) of the patients were in RC and 10 (37%) were in PR (1 patient was not evaluated). Thirteen (48%) patients received a total of 4 cycles, 11 (41%) 6 cycles and 3 (11%) 3 cycles. At the end of treatment, 23 (89%) of the patients were in CR and 3 (11%) in PR. With a median follow-up of 66 months, 20 patients (74%) are in CR and 4 (15%) in PR. One patient died due to another malignancy and in two patients we lost follow-up. Toxicities were mild and mostly hematological.
Summary / Conclusion: First-line therapy with Rituximab and Fludarabine is a very active treatment, with a favorable safety profile and long lasting responses for patients with extranodal MALT lymphomas.

B1612

RITUXIMAB, CYTARABINE AND PLATINUM-BASED SALVAGE REGIMEN FOR RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMAS (DLBCL): A SINGLE RETROSPECTIVE SURVEY OF 66 PATIENTS

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Background: Addition of Rituximab to CHOP-like first-line chemotherapy regimen has dramatically improved the outcome of DLBCL. Approximately 33% of patients (pts) experience relapse or refractory disease and underneed salvage therapy including high-dose therapy and autologous stem-cell transplantation (ASCT). However the best pre-transplant salvage regimen still remains to be determined (*Gisselbrecht C., CORAL study, J.C.O, 2010*).
Aims: The aim of this retrospective study is to evaluate the response rate after Rituximab, Cytarabine and Platinum-based salvage therapy in relapsed or refractory DLBCL before ASCT.
Methods: Sixty-six pts with refractory or relapsed de-novo DLBCL (n=52) or transformed indolent lymphoma (n=14) are reviewed between 12/2006 and 11/2012. All patients except 1 receive Rituximab in first or subsequent line treatments and all receive salvage regimen based on Rituximab 375 mg/m² d1 i.v., Dexamethasone 40mg/d d1-4 p.o., Cytarabine 2g/m² d2-3 i.v., with either Cisplatinum 100mg/m² d1 i.v.(n=22; 33%) or Carboplatinum AUC 5 d1 i.v. (n=44; 67%). Tumor response is assessed after 3 or 4 cycles by CT-scan (n=4; 6%), PETscan (n=60; 91%) or clinical assessment (n=2; 3%). When it is feasible, pts with CR or PR undergo BEAM conditioning regimen followed by ASCT.
Results: Relapse after first-line therapy occures in 16 pts with a median time of 18 months (7-76). In 50 pts, disease is considered as refractory based on positive PETscan (Deauville criteria) at intermediate assessment after 4 cycles of RCHOP-like regimen (n=43; 86%) or after the end of the front-line therapy (n=7; 14%). Median age is 57 years (23-73). Salvage therapy is administered for 2 (n=11; 17%), 3 (n=48; 73%) or 4 cycles (n=7; 11%) before assessment. The ORR is 74% with 41% CR, 33% PR,6,1% SD and 19,7% PD. Among the responding pts (n=49; 74%), 43 pts (88%) receive BEAM conditioning followed by ASCT. Six pts (12%) are not transplanted because of age (n=4), comorbidity (n=1), active infection (n=1). After a median FU of 13mo (3-65), over the 66 pts, 49 pts (74%) are alive, in CR (n=40; 61%) or in PR (n=3; 5%), 13 pts (20%) died from progressive disease. Among the whole cohort, 5y-OS is 67% without median reached. Among pts in ORR, 1y- and 3y-DFS are 86% and 78% respectively.
Summary / Conclusion: Rituximab-Cytarabine and Platinum-based salvage therapy shows an impressive response rate in relapsed or refractory DLBCL. Although retrospective date, our results compare favourably with published results.

B1613

CELL PROLIFERATION (KI-67) AND BCL2 AS PROGNOSTIC MARKERS IN PERIPHERAL T-CELL LYMPHOMA

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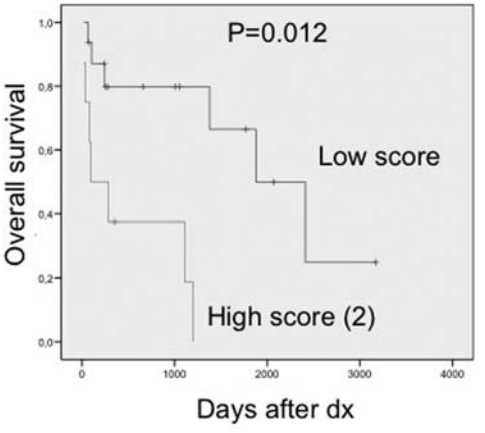
Background: Peripheral T cell lymphomas (PTCL) comprise a heterogeneous group of rare malignancies, characterized by an aggresive clinical course and a poor survival. Although the International Prognostic Index (IPI) and the PTCL prognostic index (PIT) are used for prognostic stratification, their predictive utility is in need of improvement. Proliferation index and chemokine expression may have prognostic significance.
Aims: To investigate the prognostic value of proliferation index (as determined by Ki-67) and BCL2 protein expression (assessed by immunohistochemical methods) in a retrospective cohort of pts with PTCL.



Methods: 37 PTCL cases (28 male, 9 female; median age, 58 year [range, 28-81]) diagnosed between the years 2002 and 2012 were identified. Histology subtypes were as follows: PTCL-not otherwise specified (18, 49%), anaplastic large cell lymphoma-primary systemic type (9, 24%), angioimmunoblastic T-cell lymphoma (7, 19%), T/NK nasal type (2, 5%), intestinal T-cell lymphoma (1, 3%). Proliferation index and bcl-2 expression were reviewed by local hematopathologist. Clinical data was obtained thorough chart review.
Results: Most patients had advanced stage disease, 92% were stage III-IV (Ann-Arbor). 69% had elevated IPI score (3-4-5), and 84% elevated PIT score (2-3-4). The majority of patients (35) received initial treatment with CHOP-like regimens. For the whole group with a median follow-up of 43 months (range 2-139), the median OS was 12.7 months (95% CI, 0-42.1). The median profliferation rate (Ki-67) for the entire group was 80% (range, 10 - 91%; interquantile range, 66 - 80%). Pts with high Ki-67 (>= 80%) at diagnosis (n=21) had a worse OS (median 9.5 months) compared to those with Ki-67 <80% (80.3 months). This difference showed statistical significance (P=0.04) and the Hazard ratio (HR) for Ki-67 >= 80% was 2.7. We found no significant correlation between IPI or PIT scores and Ki-67. By histologic type, angioimmunoblastic T-cell lymphoma showed lower Ki-67 than all other types (55% vs 80%; P=0.013) and this subgroup had better outcome. BCL-2 expression was detected in 76% of the cohort, and showed negative impact on survival (P=0.015; HR=2.347).
We incorporated these variables into a scoring system: 1) a Ki67 index >= 80%, 2) BCL-2 expression. Patients were stratified into two risk groups: low (0 or 1 risk factor) and high risk (2 risk factors). Patients with a high score (2) had: less OS, when compared to the low-risk population (P=0.012) (fig. 1).
Summary / Conclusion: Ki-67>=80% and BCL-2 expression have a negative impact in survival in pts with PTCL, and should be applied in clinical practice.

B1614

AGE DISTRIBUTION OF LYMPHOMA PATHOLOGY SUBTYPES AS PART OF HAEMATOLOGICAL MALIGNANCIES IN JORDAN: A RETROSPECTIVE ANALYSIS OF 2653 CASES IN A TERTIARY CANCER CENTRE.

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Background: Lymphoma is the fourth most common newly diagnosed cancer in Jordan, a small country with a population of 5.85 million.
The age distribution of lymphoma pathology subtypes as a part of the overall haematological malignancies burden in Jordan has not been hitherto well characterized.
Aims: To characterize the clinico-pathological features of lymphoma, and their age distribution, in patients referred to King Hussein Cancer Center (KHCC)(aka Al Amal Cancer centre in the period 1997 to 2003), the major cancer tertiary referral centre in Jordan.
Methods: A retrospective analysis was conducted of all lymphoma patients referred to KHCC/Al Amal Centre, between 1/6/1997 and 31/1/2012. Clinical features and histological subtypes were retrospectively collected for all patients with a diagnosis of lymphoma, and incorporated in the Lymphoma Service Database
Results: Over the 15 year period of 1997-2012, 5153 patients with haematological malignancies were referred to KHCC/Al Amal Centre . Of those, 2653(51%) had a diagnosis of lymphoma, and thus were registered in the Lymphoma Service Database, of whom 160 (3%) were diagnosed with SLL/CLL and were included in the lymphoma cohort for the purpose of this analysis. 3219 of the cases above(62%) were aged 18 or older. 1692 patients (38%)younger than 18 years of age were classified as pediatric cases. 1150 of the latter group had a diagnosis of leukemia(53% of leukemia cases). On the other hand, 542 pediatric patients were diagnosed with lymphoma, constituting 20% of the lymphoma cases. There were 1074 cases (21% if all cases) of Hodgkin Lymphoma. B-cell lymphomas formed 1310 (87.3%) of the NHLs, whereas T-cell lymphomas formed (104)12.7% of the NHL total. Diffuse large B-cell lymphoma was the most common subtype, with 510 cases (50.7% of all NHLs). Follicular centre-cell lymphomas, B-cell small lymphocytic lymphoma, mantle-cell lymphoma, marginal zone B-cell lymphomas (including MALT lymphomas), and Burkitt lymphoma amounted to 90 (6.4%), 160(11.3%), 14(1.0%), and 43 (3.0%) and12(0.9%) respectively. Among the T-cell lymphomas, mycosis fungoides and anaplastic large-cell lymphomas of T/null-cell type accounted for 31(2.1%) and 27(1.9%) of all NHL cases, respectively.
Summary / Conclusion: To our knowledge, this is the biggest lymphoma series to be reported in Jordan to date. Non-Hodgkin lymphoma appears to constitute a smaller share of the lymphoma burden in Jordan in contrast to Hodgkin Lymphoma, as opposed to Europe and the US, with nearly one third of the cases being classified as Hodgkin Lymphoma. T cell lymphomas constitute a smaller proportion of NHL as opposed to other reports from Eastern Asia, Lymphoma constituted the vast majority of haemtological malignancies in adults, with HD (21%of all cases) and DLBCL (9.9%of all cases) accounting for roughly two thirds of all adult cases. ALL/LL, as expected, accounted for the majority of the haematological malignancies in the pediatric age group The lymphoma clinicopathological features, however, show important differences from those described in the rest of the world. Follicular lymphoma and mantle-cell lymphoma are less common in Jordan compared to Europe and the USA. Peripheral T-cell lymphomas and T/NK-cell lymphomas of nodal and extranodal nasal types, which are common in other Asian countries, are also less prevalent. DLBCL, as a result, formed a bigger proportion of NHL in Jordan. As described above, HD formed a bigger proportion of haematological malignancies in general, and lymphomas in particular, in Jordan.

B1615

MUM1/IRF4 /BCL6 EXPRESSION FAILS TO PREDICT SURVIVAL IN PATIENTS WITH GERMINAL CENTER B LYMPHOMA TREATED WITH IMUNOCHEMOTHEARPY-SINGLE CENTAR EXPERIENCE

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Background: Diffuse large B-cell lymphoma is subclassifed into molecular subgroups that correspond to different stages of lymphocyte developmentnamely germinal center B-cell like and activated B-cell like. Myeloma associated oncogene MUM1/ IRF4 is a hallmark of activated B cell lymphoma (ACB). But MUM1 is also expressed in the nucleus or cytoplasm of small percentage of germinal center (GC) B cells located in the “light zone . Unlike normal GC B cells, lymphoma cells in approximately 50% of MUM1 (+) DLCL-B co expressed MUM1 and BCL6.
Aims: In line that MUM1 is predictor of inferior survival the aim of this study was to evaluate MUM1/IRF4/BCL6 immunohistochemical approaches for predicting the survival of patients with GCB lymphoma treated with imunochemotherapy
Methods: Our study enrolled 84 patients diagnosed and treated at the University Clinic of Hematology in the period between January 2002 and January 2012. They were all treated with R-CHOP regimen and the median follow-up of the patient was 36 months. We analyzed biopsy simples immunochemically for markers of germinal center(BCL6), postgerminal center(MUM1), and apoptosis marker (BCL2).
Results: The patients were divided in two groups BCL6+/MUM1+ (39pts;46,4%)and BCL6+/MUM1-(45pts;53,5%). The median overall survival time(OS) were 122,4 months in BCL6+/MUM1-group, and 90 months in BCL6+/MUM1+group respectively. The groups were statistically comparable regarding the prognostic parameters as IPI, performance status.
Summary / Conclusion: Our results did not show any statistical survival advantage and better outcome for the patient classified as GCB DLBL(BCL6/MUM1-) when treated with R-CHOP and indicate that immunohistohemical markers do not really reflect the molecular diversity of the tumor. They also support the studies that suggests that Rituximab eliminates or modulates the significance of some already established prognostic markers for DLBL and indicate that previously recognized markers should be re-evaluated in the context of the

modern therapy and that new prognostic indicators for DLBL has to be identified.

B1616
THE USE OF FLIPI AND FLIPI 2 IN FOLLICULAR LYMPHOMA
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Background: Is has been proposed the use of two different prognostic scores when staging Follicular Lymphoma. FLIPI (Follicular Lymphoma International Prognostic Index) is a prognosis index developed in the pre-rituximab era and divides patients into different overall survival (OS) risk groups. FLIPI 2 was developed in the post-rituximab era and is prognostic for progression free survival (PFS).
Aims: To evaluate FLIPI and FLIPI 2 prognostic value.
Methods: We analysed retrospectively 106 patients with follicular lymphoma diagnosed (1997-2012) in our hospital.
Results: The median age at diagnosis was 56 years-old [33;85], 54.7% were male and 43.4% were over 60 years. The median follow-up was 43 months [1;175]. 65.1% patients were in advanced stage (III-IV-Ann Arbor staging system) and 19.1% had B symptoms at diagnosis with 60.4% of extranodal sites. Lymphoma grade was classified (WHO) 1)31.6%, 2)50.7% and 3A) 17.7%, 3B grade lymphomas were not included in the analysis. 36.6% had liver/spleen enlargement and 7.6% had serous effusions. Bulky mass (>6cm) was present in 10.7% and 9.7% had >= 5 nodal sites involvement. 80% had hemoglobin <=12g/dl, no patients had leucopenia (<1.0x10^9/L) and 3% had thrombocytopenia (<100x10^9/L), 31.4% and 24.4% had LDH (lactate dehydrogenase)/B-2 microglubulin elevated. FLIPI: low risk: 45.9%, intermediate risk: 38.8% and high risk: 15.3% (98 patients) and FLIPI 2: low risk: 53.4%, intermediate risk: 29.3% and high risk: 17.3% (75 patients). The treatment strategy was grouped: "Watch&Wait" (WW) (33%) and chemotherapy/radiotherapy (67%). The majority of WW were patients with early stage disease (I-II:58,8%; P<0.0001) and 68.8% had low FLIPI risk (P<0.02). Patients treated with chemotherapy/radiotherapy were mainly at advanced stages (III-IV:79.7%; P<0.0001) and 50% had intermediate FLIPI (P<0.02). No significance found in FLIPI 2 between these two. Comparing different variables in FLIPI/FLIPI 2 we found 13.5% with both high B-2 microglubulin and LDH and 29.8% with only one (P<0.01). In advanced stages 72.1% had bone marrow involvement (P<0.0001). No significance found between nodal regions >=5 vs bulky mass. The 5-year OS was 93.8% and PFS was 63.5%. Six patients have died. FLIPI showed significance in OS with 100% (low risk), 89.1% (intermediate risk) and 83.6% (high risk) patients alive after 5 years (P<0.046) and in PFS with 72.9% (low risk), 59.6% (intermediate risk) and 25.9% (high risk) without progression/relapse after 5 years (P<0.035). FLIPI 2 showed no significant differences either in OS or PFS. In FLIPI, only age and hemoglobin had significance in OS 98,1% (< 60years;P<0.05) and 97.1% (hemoglobin <=12 g/dl; P<0.02) and this was also true for PFS using FLIPI 2: 74% (P<0.001) and 75.1% (P<0.0001) respectively.
Summary / Conclusion: Only variables common to FLIPI and FLIPI 2 had significant prognostic value. Surprisingly patients with hemoglobin lower than 12 showed better OS and PFS at 5 years. As expected FLIPI had value in OS but FLIPI 2 did not show significance in PFS. Whether FLIPI or FLIPI 2 should be used to decide treatment in not clear. This study showed that 68.8% WW group had low FLIPI, hence this maybe helpful to determine therapeutic strategy. The group chemotherapy/radiotherapy was mainly at advanced stages with 50% intermediate FLIPI.

B1617
BENDAMUSTINE PLUS RITUXIMAB IN PATIENTS WITH RELAPSED OR REFRACTORY WALDENSTRÖM'S MACROGLOBULINEMIA (WM)
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Background: WM is an incurable disease, with an overall medial survival of only 5-6 years. Age, hemoglobin level, platelet count, β(2) microglobulin, and monoclonal IgM concentrations are characteristics required for prognosis. First-line therapy of WM has been based on single-agent or combination therapy with alkylator agents (e.g. chlorambucil or cyclophasphamide), nucleoside analogues (cladribine or fludarabine), and the monoclonal antibody rituximab.
Aims: Novel therapeutic agents that have demonstrated efficacy in WM include thalidomide, lenalidomide, bortezomib, everolimus and bendamustine.
Methods: We report the treatment outcome for 16 (9 male, 7 female; median age: 70y, range: 67-78) relapsed/refractory Waldenström's macroglobulinemia (WM) patients. Treatment consisted of bendamustine (90 mg/m(2) I.V. on days2, 3) and rituximab (375 mg/m(2) I.V. on day 1) for all patients. One rituximab-intolerant patient received bendamustine alone. Each cycle was 4 weeks, and median number of treatment cycles was 4.
Results: The clinical stage (remission, progression or stable disease) was defined with clinical re-evaluation after chemotherapy and re-staging 6 months after end of therapy. At best response, median serum IgM declined from 3500 to 500 mg/dL, and hematocrit rose from 29.9% to 37.8%. Overall response rate (CR + PR) was 81.2%. Overall therapy was well tolerated. Prolonged myelosuppression was more common in patients who received prior nucleoside analogues.
Summary / Conclusion: Bendamustine in combination with Rituximab demonstrates an excellent effectiveness in previously treated WM patients, with an acceptable toxicity profile. These agents, when compared to traditional chemotherapeutic agents, may lead in the future to higher responses, longer remissions and better quality of life for patients with WM.

B1618
ORAL MUCOSA NON-HODGKIN'S LYMPHOMAS: A CLINICOPATHOLOGIC STUDY OF 22 CASES
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Background: Oral non Hodgkin lymphomas (NHL), is a rare subgroup of NHL. Represent less than 5% of the oral cavity malignancies and 2% of all extranodal NHL. The vast majority of them is located in the Waldeyer's ring, mostly in tonsils (70%), but they also may affect the salivary glands, the bone of the jaws and the oral mucosa.
Aims: To report the clinical and laboratory characteristics, as well as the outcome of patients with oral mucosa-NHL (OM-NHL).
Methods: We performed a retrospective analysis of 22 patients records, diagnosed with OM-NHL from 1997 until 2012. Patients with Waldeyer's ring lymphoma (including tonsils), salivary gland lymphoma, and lymphomas that affected the bone of the jaws were excluded from this study. Data, related to histological subtype, clinical stage at diagnosis, international prognostic index (IPI), bulky disease, B-symptoms, treatment administered, response rates, 5-year failure free survival (FFS) and overall survival rate (OS), were recorded.
Results: 12 patients were were male and 10 female, with a median age of 58 years. After staging, (An-Arbor System), oral mucosa was the only site of involvement in 73% (Stage-IE), while 18% were clinical stage II and 9% clinical stage IV. The location of NHL was: tongue 45%, gingival mucosa 23%, palate 14%, lips 14% and the mouth floor 4%. A B-cell phenotype was documented in 64% and a T-cell in 36%. The most common histological subtype was found to be diffuse large B cell lymphoma (DLBCL) in 45%, followed by peripheral T-NHL (PTCL-NOS) in 32%. 9% were diagnosed with mantle cell lymphoma (MCL), 9% with follicular lymphoma (FL) and 5% with anaplastic large cell lymphoma (ALCL). Increased levels of LDH were recorded in one patient, bulky disease in 4/22 patients (2 DLBCL, 1FL, 1 MCL pleiomorphic), B-symptoms also in 4/22 patients (1 DLBCL, 1 FL, 1 MCL, 1 MCL pleiomorphic). Treatment included immunochemotherapy (41%), chemotherapy (31%), immunotherapy (23%) and radiotherapy (RT) followed by chemotherapy (5%). 5/9 patients with NHL of T-cell origin were placed on Interferon-α (INF-α) treatment, while 8/13 patients with NHL of B-cell origin received as first line treatment immunochemotherapy R-CHOP. Among the treatment modalities used in the T-NHL group, INF-α, either as monotherapy or as a rescue therapy in relapsing patients, was highly effective, producing long lasting CRs. After a median follow-up of 64 months, the vast majority of our patients (91%) is alive, while 77% of them are in complete remission (CR). We did not record any significant difference in response and survival rates between the two major histological subtypes (B-NHL versus T-NHL).
Summary / Conclusion: This is one of the largest series focusing on NHL exclusively on the oral mucosa. Our results are contributing in the understanding of the clinical behavior of this NHL subgroup. The percentage of OM-NHL originating from T-cells was significantly higher (36.4%) than that reported for the nodal T-NHL. Response rates are remarkably high, with a long lasting overall survival, independently of the histological subtype.

B1619
RETROSPECTIVE REVIEW OF PERIPHERAL T-CELL LYMPHOMA IN A SINGLE INSTITUTION: OUTCOME AND CENTRAL NERVOUS SYSTEM INVOLVEMENT
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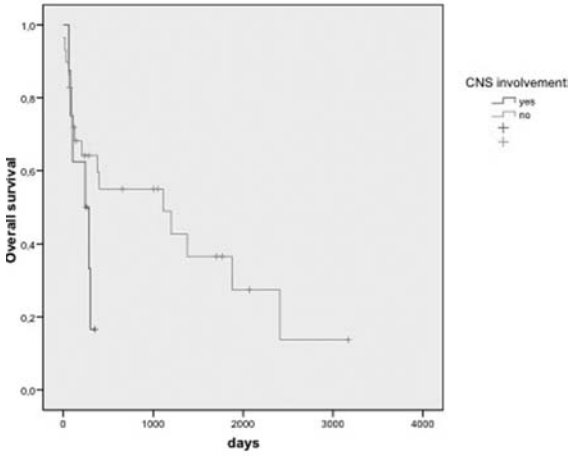
Background: Peripheral T cell lymphomas (PTCL) comprise a heterogeneous group of rare malignancies, characterized by an aggresive clinical course and frequent extranodal involvement. Moreover, little data exist on the risk of central nervous system involvement (CNS) by these lymphomas.

Aims: To describe the clinical characteristics and outcome of PTCL patients, and to estimate the frequency of CNS involvement.
Methods: Retrospective analysis of all PTCL cases diagnosed in Gijon (Asturias, Spain), that covers a population of approximately 450,000 people, between 2002 and 2012. Clinical data were obtained thorough chart review. CNS involvement was defined by positive finding in citology or flow cytometry analysis of CSF.
Results: A total of 37 cases were identified, the histology subtypes were: PTCL-not otherwise specified (18, 49%), anaplastic large cell lymphoma-primary systemic type (9, 24%), angioimmunoblastic T-cell lymphoma (7, 19%), T/NK nasal type (2, 5%), intestinal T-cell lymphoma (1, 3%). 3 patients had received another diagnosis (B-cell lymphoma, Hodgkin's disease) prior to the current diagnosis of PTCL. One case was diagnosed post-mortem. Median age at time of presentation was 58 (range 28-81) years, 35% were ≥65 years old. ECOG was 2-4 in 57%, and 68% had B symptoms. Most patients had advanced stage disease: 92% were stage III-IV (Ann-Arbor), 76% had extranodal involvement, and 38% had documented bone marrow involvement. 69% had elevated IPI score (3-5), and 84% elevated PIT score (2-4). Laboratory data showed elevated LDH level in 73% and elevated Beta-2-microglobulin in 76%. A total of 35 pts received initial treatment, the majority received CHOP-like regimens. 8 pts (22%) received consolidation with autologous stem cell transplant (ASCT) in 1st remission. At the end of first line therapy, overall response was 57% (17 pts CR; 4 pts PR), while 7 cases (19%) had disease progression and 22% experienced early death during treatment. Overall, 19 pts (51%) presented relapse/progression: 15 were treated with platinum-containin regimens, and 5 pts underwent salvage SCT (4 allogeneic, 1 autologous). With a median follow-up of 1298 days (range 79-4173), the median OS for the whole group was 382 days (95% CI, 0-1262). Pts who received transplant in 1st remission had better outcomes than as salvage treatment (P=0.029). CNS disease, as detected by flow cytometry, was found in 8 pts (22%), with no case found in angioimmunoblastic subtype and the highest number of cases in ALCL (4 cases, 50%), and NOS (3 cases, 37.5%). Median time to CNS involvement was 67.5 days (range, 33-296), and univariate analysis only identified elevated LDH (P=0.05) as risk factor for CNS disease, without correlation with BM involvement. Median OS for this group of pts was particularly dismal (244 days, CI 29 - 459 days) (fig. 1).
Summary / Conclusion: This 10-year review of patients treated in a single institution confirms the poor clinical outcomes of PTCL: advanced stage, extranodal involvement, high rate of early death, poor response to front-line treatment, and short OS after relapse. We also find a strikingly high frequency of CNS involvement, as detected by flow cytometry. A CNS surveillance and prophylaxis strategy should be considered for this type of lymphomas.



B1620
IMPACT ON SURVIVAL ACCORDING TO HISTOLOGICAL GRADE (1-2 VS 3A) IN A SERIES OF 128 PATIENTS DIAGNOSED OF FOLLICULAR LYMPHOMA IN THE RITUXIMAB ERA. EXPERIENCE IN ICO-HOSPITAL DURAN I REYNALS.
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Background: Around 15-25% of all follicular lymphoma (FL) are histological grade 3a and comparing with grade 1-2 have different clinicobiological features.
Aims: To analyze clinicobiological characteristics, response to therapy and outcome of a series of 128 patients according to histological grade 1-2 vs 3a in a single institution.
Methods: Between 2004 to 2012 were identified in our data base among 128 patients diagnosed with follicular lymphoma. Histological grades were: grade 1-2, 91 patients (71%); grade 3a, 30 patients (23%) and grade 3b, 7 patients (6%). Histological grades were reviewed according to WHO criteria. We analyzed the clinical and biological features, response rate and prognosis of 121 patients with histological grades 1-2 and 3a.

	FL grade 1-2 (N=91)	FL grade 3a (N=30)	P
Watch and Wait	6/91	0/30	NS
1st line Chemotherapy (N)	91	30	
R-CHOP/R-COP like (%)	82 (90)	30 (100)	NS
R plus Fludarabine analogues (%)	6 (7)	0 (0)	NS
Monotherapy with rituximab(%)	3 (3)	0 (0)	NS
Complementary Radiotherapy	1 (1)	4 (13)	0.04
CR and CRu (%)	59/89* (66)	26/30 (90)	**0.015**
OS at 5 years (%)	81	73	0.39
EFS at 5 years (%)	59	43	0.4
CD10 (%)	93	82	0.12
Bcl2 (%)	96	93	NS
Bcl6 (%)	100	100	NS
Ki-67 > 50% (%)**	17	41	**0.052**

* Two patients not yet evaluated **Performed in 30 and 22 cases, respectively

Results: The clinical characteristics of patients with histologic grade 1-2 vs 3a were: 41M/50F vs 8M/22F; median age, 57 vs 62.5; stage III-IV, 90% vs 77%; B symptoms, 16% vs 10%; high LDH, 31% vs 41% and high β2microglobulina 57% vs 59%, respectively. The percentage of patients with high risk IPI, FLIPI and FLIPI 2 according to grade 1-2 vs 3a was: 6.5% vs 15%, 42% vs 38% and 43.5% vs 45% respectively. Biological data at diagnosis, therapy, response rates, overall survival and event free survival at 5 years are shown in the table. With a not reached median overall survival, 21 (17%) patients died, 14(15,4%) of them with histological grade 1-2 and 7 with histological grade 3a, mostly due to disease progression. Ki-67 index > 50% at diagnosis predict a worse OS at 5 years (54% vs 91%, P=0.072). The probability of transformation to high-grade lymphoma for grade 1-2 vs 3a was 5.5% vs 10% at 5 years (P=0.13), respectively. In multivariate analysis, IPI, FLIPI and FLIPI 2 at diagnosis of all series were the main prognostic factors for survival.
Summary / Conclusion: In our series, histologic grade follicular lymphoma 3a has peculiar biological characteristics, lower expression of CD10 and especially a higher proliferative index. Although patients with histological grade 3a have a significant higher rate of complete remission, OS and EFS are similar in both groups.

B1621
PROGNOSTIC VALUE OF 18 F-FDG TEP IN DLBCL ELDERLY PATIENTS TREATED WITH IMMUNO-CHEMOTHERAPY
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Background: Diffuse large B-cell lymphomas are heterogeneous family of blood diseases which prognosis depends on specific phenotypic, molecular and pathological features. One of the techniques for the evaluation and response is 18 F-FDG PET scanner. If PET scanner imagings at diagnostic (initial extension) and at the end of the treatment (response evaluation) haveal-ready shown their interest through several studies, the impact of the interim evaluation on survival is not yet consensual.
Aims: We conducted a retrospective, monocentric cohort study, to characterize the predictive value of such interim PET scanner (3 courses of treatment) in DLBCL elderly patients treated with R-PMitCEBO.
Methods: We evaluated39 patients aged over 65 years, diagnosed with DLBCL between 2006 and 2011. Patients received immunochemotherapy (R-PMitCEBO regimen) for 6 cycles. Therapeutic response was evaluated according to Cheason 2005 criteria, after 3 and 6 cycles of chemotherapy. An assessment of overall survival (OS) and disease-free survival (DFS) was performed for all patients. Two evaluations were conducted, the first to compare patients in complete remission versus those who are not (CR / nonRC). The second evaluation compares the patients in complete remission versus those in partial remission. (CR / PR).
Results: Among the 39patients, 24 were in complete remission (CR) after 3 courses, 10 in partial remission (PR) and 5 in stable disease (SD). Two year outcome evaluation (CR / notCR at 3 cycles) find PFS at82% for patients with CR and only 29% for patients in nonCR; OS was 78% in formers against 38% among others. More precisely, evaluation of patients with CR compared to PR, provides a two-year PFS was 82% for formers against 50% for laters and a two-year OS of 78% and 50% respectively.
Summary / Conclusion: In elderly patientstreated with R-PMitCEBO, immunochemotherapy for DLBCL, assessment by 18 F-FDG PET scanner is an essential tool for diagnosic and therapeutic monitoring. The interim evaluation after three cycles of chemotherapy has a strong predictive impact on overall survival and disease-free survival for this profile of patients. The use of interim PET scanner allows to rapidly switch the treatment for a another one to maximise the chances to obtain complete remission instead of partial remission or stable disease.

B1622

LANGERHANS CELL HISTIOCYTOSIS IN ADULT PATIENTS: SINGLE INSTITUTION EXPERIENCE

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Background: Objective: Langerhans cell histiocytosis (LCH) is characterized by abnormal proliferation of histiocytes. It is rare disease with an incidance of 1 -2/ million. Although it is more frequent in children at 1-3 years old, it can be diagnosed in all ages. Disease can be presented by multifocal or localized organ infiltrations. Although all systems and organs mıght be infiltrated, main sites for disease is bone, especially skeletal bones. Treatment options differs according to its presentation as local or multifocal. At local disease, only radiotherapy can be effective modality but patients with multifocal disease should be treated with systemic chemothepies or with combination.
Aims: At this study, we aimed to retrospectively analyse our adult LCH patients diagnosed between 1992-2012
Results: Twenty-one patients, 13 male and 8 female, were retrospectively analyzed. Median age was 29(range, 18-53). All of the patients had bone involvement and bone pain has been most prominent complaint according to the involvement site. We documented poliuria and polidipsia in one patient due to hypophysis involvement in addition to bone. 13 (%15 female, %85 male) patients were presented with local disease and 8(%25 male, 75 female) patients had multifocal disease. The characteristics of the patients were given at table 1. The patients with local disease were treated with only radiotherapy and then followed up. The patients with systemic disease were treated with both radiotherapy and chemotherapy. During the treatment period,any grade 3-4 hematological side effects were not documented. The median period of follow-up was 19 (range, 4-120) months. We determined 7 relapses in 4 patients. All of the relapses were detected with bone lesions and they were treated with radiotherapy successfully. Median overall survival was 19 months. 6 patients were lost to follow up. No deaths were recorded during follow up.
Summary / Conclusion: At this retrospective study with relatively limited number of patients, we reported that adult onset LCH patients were mostly presented as a focal disease with bone pain. The radiotherapy was an effective tretatment modality at these patinets. Although, LCH is a rare disease in adult age groups, it should be considered in patients with bone lesions.

B1623

RITUXIMAB MAINTENANCE THERAPY IN DIFFUSE LARGE B-CELL LYMPHOMA: SINGLE CENTER EXPERIENCE

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Background: Diffuse large B cell lymphoma (DLBCL) are curable group of lymphoma with improved outcome mainly due to the incorporation of the anti-CD20 monoclonal antibody, Rituximab (R) to the standard chemotherapy regimens. According to clinical and pharmacokinetic data, prolonged exposure to Rituximab is associated with higher response rates and improved quality of response. Although most of the big clinical studies are not finished, so far, initial findings are not very promising in regard to the overall survival (OS) and progression free survival (PFS) for the patients receiving maintenance treatment in comparison with patients without maintenance treatment after completion of standard therapy with R-CHOP.
Aims: Here, we present our experience with the Rituximab maintenance treatment of DLBCL patients (pts) that were treated at the University Clinic of Hematology in the past 4 years.
Methods: Since 2006, at our Clinic, 42 pts with DLBCL that were not included in a clinical study underwent R maintenance treatment. Pts received Rituximab (375 mg/m^2) every 3 months for 2 years. Our control group consisted of 65 DLBL pts that were treated in same period at our Institution and do not underwent maintenance treatment. Our two groups were comparable regarding the age, gender and IPI score distribution. All evaluated pts initially received 8 cycles of standard R-CHOP regimen. Only pts in complete remission underwent R maintenance treatment. CR was required for entrance in the control group two. We evaluated and compared the progression free survival (PFS), overall survival (OS) between the two groups. Moreover, we evaluated the tolerance of R maintenance treatment and the impact of prolonged R use at pts quality of life (QoL).
Results: After a median follow up of 42 months, PFS was excellent with 32,5% in the treatment group and 27.7 % in the control group. There was no significant statistical difference regarding those two parameters in both groups (P>0.05). Maintenance therapy was generally well tolerated, but we noticed marked and prolonged hypogammaglobulinaemia in the maintenance group. Further investigation of the median initial and follow up serum imunnoglobulin G (IgG) levels in both groups showed : 10,7 g/L and5,07 g/L in the treatment group, respectively, and 13,8 g/L and8,9 g/L in the control group. Statistical correlations of those results showed that maintenance group has statistically significant lower IgG levels (t-test, P<0,05).Furthermore, more frequent hospitalizations and requirement of immunoglobulin therapy were registered in the maintenance group due to the occurrence of recurrent infections, which worsened QoL of those pts.
Summary / Conclusion: In our experience R maintenance therapy did not improve PFS or OS in pts with DLBCL. More over, pts from the maintenance group had a significantly higher risk of developing hypogammaglobulinemia. Our results suggest that risk of developing symptomatic hypogammaglobulinemia should be considered before starting R maintenance. It is in our opinion that evaluation of a larger patient population, together with a longer follow-up is needed before establishing R-maintenance treatment as standard therapeutical approach for DLBL pts..

B1624

CARDIOTOXICITY MONITORING WITH A NOVEL COMBINED APPROACH OF TELEMEDICINE AND BIOMARKERS IN LYMPHOMA PATIENTS TREATED WITH CONVENTIONAL AND LIPOSOMAL ANTHRACYCLINES

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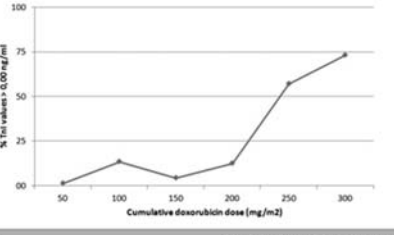
Background: Anthracyclines (AC) are highly effective cytotoxic drugs both in solid and hematological malignancies, whose use, however, is limited by the occurrence of cardiac toxicity (CT). In recent studies intensive cardiac monitoring (with echocardiography *or* biomarkers) has shown to provide an early identification of subclinical cardiac damage: in this case prompt suspension of the AC treatment and aggressive management of the asymptomatic cardiac dysfunction can lead to reversal of the cardiac injury. Liposomal AC formulation has been invoked as a CT-sparing treatment with similar antitumoral activity, especially in old patients or those with cardiac disease.
Aims: Study endpoints are: to measure with the combined monitoring approach the incidence of CT, measured as reduction in the left ventricular ejection fraction (LVEF), rises in TnI levels, significant ECG changes.A telemedicine (TM) system was integrated in this setting to allow for an optimization of health care resources, an increased compliance to intensive monitoring without impacting on the quality of care provided



19 patients	Cumulative doxorubicin dose(mg/m^2)					
	50	100	150	200	250	300
TnI controls performed	71	59	45	32	14	15
TnI values > 0,00 ng/ml*	1	8	2	4	8	11
% TnI values > 0,00 ng/ml	1.4	13.6	4.4	12.5	57.1	73.3

Fig.1: Trend for increasing TnI values with increasing cumulative doxorubicin dose

*Only 2 values above 0,08 ng/ml

Methods: This is a prospective observational trial in lymphoma patients undergoing treatment with conventional or liposomal AC. Informed consent was obtained from each enrolled patient. We used a comprehensive approach to monitor for AC CT, using echocardiography, ECG and biomarkers (Troponin I - TnI). Clinical, echo, ECG and TnI data were acquired in our Hematology Clinic and transferred via TM to be evaluated by the reporting cardiologist.
Results: The study enrolled 20 patients, 13 males and 7 females. The median age at diagnosis was 40.9 years (range 20.1 to 78.2 years). 14 patients had a diagnosis of non-Hodgkin's lymphoma and 6 of Hodgkin's lymphoma. Two patients underwent chemotherapy with liposomal AC, all the others with conventional AC. Three patients had at least one cardiovascular risk factor. In six months we performed 216 TM assessments. Compliance to the protocol was excellent as 97.4% of the planned TM evaluations and 94% of the programmed TnI controls, were actually performed. In all cases, the data were successfully transferred to the cardiologist's client. The average time to perform a complete TM assessment was 19 m and 47s, while less than 6 minutes were used for the echocardiographic examination. It took less than 5 minutes on average by the cardiologist to evaluate data from 1 TM assessment and to produce a report. The primary endpoint (reduction in LVEF), did not occur in any of the 19 evaluable patients. In two patients the monitoring detected asymptomatic signs of cardiac ischemia (in one case diffuse T-waves inversion in anterior leads, in the other posterior wall hypocinesia and moderate TnI rise): both patients underwent percutaneous coronary intervention with optimal myocar-

dial revascularization. In both cases patients were aged >65 years and had a cardiovascular risk factor. These cases were not thought to be related to AC. The patients completed the planned chemotherapy course and they are well. In six patients, at some point during the monitoring, a pericardial effusion was found, in all cases not symptomatic. Except the above mentioned case, none of the patients developed a TnI rise above our laboratory cut-off (0.08 ng/ml). However, with increasing cumulative AC doses, we observed a progressive increase in TnI values > 0.00 ng/ml (Figure 1).
Summary / Conclusion: AC CT monitoring is recommended by most guidelines but seldom practiced. Our innovative approach of integrating different detection methods through a TM system allows for a feasible and accurate monitoring of AC CT, and makes possible the application of a preventive strategy for AC CT.

B1625
LOW DOSE BENDAMUSTINE IN LYMPHOPROLIFERATIVE DISEASE: A SINGLE CENTER EXPERIENCE
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Background: Experience with Bendamustine has grown in various hematologic neoplasms both in relapsed/refractory patients and at diagnosis. Results are very encouraging with clinical and long lasting response. Treatment is usually well tolerated also in older and/or unfit patients and in those with renal impairment.
Aims: We report here our observation of low dose bendamustine in a wide group of hematologic patients treated at our Institution.

Table 1	Patient's features		
Diagnosis		Total number	38
Waldestrom disease	3	Male/Female	19/19
DLBCL	3	Median age	67,4 (20-89)
Multiple myeloma	1		
Mantle cell lymphoma	2	Disease status at treatment	12 (3,5%)
Hodgkin disease	3	Diagnosis	5 (13,3%)
Hairy Cell Leukemia	6 (15,8%)	Refractory Patients	21 (55,2%)
Chronic lymphocytic leukemia	7 (18,4%)	Relapsed	
Follicular lymphoma	10 (26,4%)		

Table 2	ORR	PR	CR	NV	NR	PFS	DFS	OS
All patients	73,6 %	39,5 %	34,2 %	7,9 %	18,4 %	54,5 %	46,3 %	77,3 %
Disease status								
Diagnosis	100 %	41, 7 %	58,3 %	-	-	100 %	100 %	100 %
Refractory	60 %	60 %	-	-	40 %	33 %	50 %	53 %
Relapse > 2	44,4 %	44,4 %	-	11,1 %	44,4 %	23 %	66 %	56 %
Relapse < 2	74,9 %	33,3 %	41, 6 %	16,6 %	8,3 %	75 %	37,5 %	100 %
Diagnosis								
HCL	100 %	-	100 %	-	-	100 %	-	100 %
Follicular Lymphoma	80 %	40 %	40 %	10 %	10 %	88 %	-	67 %
CLL	71,4 %	42,8 %	28,6 %	14,6 %	14,3 %	57 %	-	100 %

Methods: We considered a total of 38 patients with different hematologic diagnosis with a median age of 67.4 years (20-89). 36/38 were treated with low dose (50-60 mg/m^2) Bendamustine (days1,2) in association with rituximab 375 mg/m^2 (day 3) with the exclusion of MM and HL cases. Cycles (4 to 8) were repeated every 28 days. Two patients were treated with lower dose due to comorbities. Patients were treated at diagnosis (31.5%), relapse (55.2%) and progression (13.3). Patients and treatment characteristics are summarized in table 1. Response was defined according to Cheson Criteria, toxicities on the basis of CTC criteria. Statistical analysis we utilized Prism software (MacOS).
Results: 36/38 were evaluable. Treatment was well tolerated in the majority of patients. One patient had to stop treatment due to skin lesions, that occurred in 4 patients (10.5%). Infections were noted in 8/36 patients (21.5%) but not clinically relevant. Hematologic toxicities were mild with 4 case of grade IV neutropenia (10.5%). Among the whole group overall response rate (ORR) was 73.6 % with complete (CR) and partial response (PR) respectively of 34.2 % and 39.5 %. Non responders patients were 3/38 (7.9%). We look for response considering diagnosis (CLL, follicular NHL, HCL) and disease status at treatment (diagnosis, progression and relapse). Patients with HCL (n=6) had impressive response with 100 % CR and no relapses, in those with follicular NHL and CLL had ORR of 80% and 71.4 % respectively Patients at diagnosis have an ORR of 100 % (PR 41,7 %, CR 58.3%), refractory patient responded in 60% cases. We split the group of relapsed patients in those who had less or more than two relapses. Patients in 1st o 2nd relapse had a ORR , PR and CR of, 74.9%, 41.6% and 33.3 %, while in those in subsequent relapse ORR were 44,4% with only PRs and 4/9 patient refractory to treatment. OS, DFS and PFS at 15 months for the whole group were respectively 77,3 %, 46,3 %, 54,8%. But when we split PFS in groups we see how patients at diagnosis had a PFS 100 %, in 1st and 2nd relapse of 77 % , while those treated later or in progression of 33 % and 23 %. Response are summarized in table 2.
Summary / Conclusion: Our experience with low dose bendamustine showed a very good safety and tolerance profile also in elderly and unfit patients with mild infective and hematology toxicities. Responses were satisfactory in the whole group that comprised also multi-treated and refractory patients. Patients at diagnosis and in particular those with hairy cell leukemia had better response with ORR of 100 % and no relapse observed so far. Even those who were treated later in disease history or in progression could be rescued, but often with not long lasting response. In conclusion we confirm the satisfactory data with bendamustine in lymphoproliferative disorders, results that can be obtained also con lower dose and in unfit patients. Results are much better in untreated/low treated patients and with low grade lymphomas, especially in HCL. These observation should be confirmed with longer follow up and more patients.

B1626
HIGHLY ACTION ANTIRETROVIRAL THERAPY (HAART) IMPROVED EFFICIENCY OF CHEMOTHERAPY IN PATIENTS WITH HIV-ASSOCIATED NON-HODGKIN'S LYMPHOMAS (HIV-NHL)
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Background: In total 40 315 patients were diagnosed with HIV infection in Samara region of Russia: 89.8% directly in the town and 11.2% in the surrounding region. 4774 (11.8%) pts died at the time of this analysis. Risk of developing of NHL in these patients was more than 100 times greater than in the general population.
Aims: To analysis of the epidemiology of HIV-NHL, their own experience of their treatment.
Methods:
During2002-2010, 47 patients with NHL-HIV were treated in our center. Three groups of patients were analyzed: (1) 25 received HAART plus CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy (CT) and (2) 15 – only CHOP chemotherapy and 17 only best supportive care. HAART consisted of a combination of three antiretroviral drugs. Response on HAART was defined as increasing the number of CD4+ cells >10x10^6/L and a decrease in viral load <500 copies/ml.
Results: Median of time from diagnosis of HIV infection to NHL development was 3.7 years. Median age pts was 32,5±2,4 years (range, 01-99). Abs (67%) cases were classified as diffuse large B-cell lymphoma (DLBCL), abs (6%) – spleen marginal zone B-cell lymphoma (SMZL), and abs (27%) follicular lymphoma (FL). Abs (47%) patients had a number of CD4+ cells <10x10^6/L at the start of the NHL treatment. The median overall survival (OS) was 18.4 months for the group 1 (HAART plus CT) against 7.5 months (P<0.05) for group 2 (CT) and 4.4 months (P<0.05) for the group 3 (best supportive care). For patients, which therapy was initiated (n=42), unfavorable prognostic factors were age >60 years, AIDS–phase of HIV, Hb <10.0 g/dl, elevated levels of LDH and non-availability of HAART (P<0.05).
Summary / Conclusion: Our data retrospective data suggest that combination of chemotherapy with HAART improved outcome in patients with HIV-NHL. Increasing the number of CD4+ cells and reduction of viral load is essential for the success of chemotherapy.

B1627
THE ROLE OF CONSOLIDATIVE RADIOTHERAPY IN DIFFUSE LARGE B CELL LYMPHOMA PATIENTS WITH BULKY DISEASE.
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Background: Bulky disease, remains to be a significant prognostic factor for non Hodgkin lymphoma patients .The role of consolidative radiotherapy in these patients is still contraversial. Diffuse large B cell lymphoma patients is 30%>40% of all non Hodgkin lymphoma patients.Bulky disease, remains to be a significant prognostic factor for non Hodgkin lymphoma patients .The role of consolidative radiotherapy in these patients is still contraversial.
Aims: So, we aimed to asess the role of radiotherapy in diffuse large B cell lymphoma patients with bulky disease.
Methods: We retrospectively included 92 patients with non hodgkin lymphoma, subtype of diffuse large B cell who had bulky disease in Ankara Oncology Hospital.Bulky disease was defined as the size of lymphadenopathy over 5 cm. Patients characteristics were summarized in Table 1. All patients were treated with six –eight cycles of RCHOP chemotherapy.We excluded patients in whom complete remission with first line chemotherapy could not be achieved and grouped 65 patients in first complete remission in to those treated with radiotherapy(n:22) and not(n:43).We aimed to asess the relapse rates and progression free survival difference between the groups
Results: Median follow up of the patients were 30(6-149) months.Patients in both groups were similar according to age,sex and IPI score. There were statistically significantly higher number of patients with advanced disease status

in non radiotherapy group. The number of patients who relapsed during the follow up were not statistically different between the groups. (5/22 vs 10/43 , p:0.962).Although, 4 years progression free survival was slightly higher in radiotherapy arm(79% ±11% vs 64%±11%) ,it was not statistically significant(p:0,282).
Summary / Conclusion: The use of RT was associated with significant improvements in OS and PFS for all patients with DLBCL in previously reported studies.Although PFS was longer in radiotherapy group, it was not found to be statistically significant in our series.This results has to be assesed in prospective clinical trials.

Age	54(17-88)
Sex F/M	43/49
IPI 0/1/2/3/4/5	1/24/27/22/11/7
Stage 1/2/3/4	23/16/31/22
Bulky disease size 5-7 cm 7-10 cm >10 cm	 23 16 27
Radiotherapy 0/1	62/30
Response to CT CR/PR/Ref/NA	65/5/15/7

	Radiotherapy (n:22)	No Radiotherapy(n:43)	p
age	55(17-77)	44(20-81)	0,072
Sex F/M	11/11	21/22	0,929
Stage 1/2/3/4	10/6/4/2	8/7/18/10	0,031
IPI 0/1/2/3/4/5	0/12/6/2/1/1	1/8/17/12/4/1	0,067

B1628
A 10-YEAR SINGLE-CENTER EXPERIENCE IN PRIMARY GASTRIC DIFFUSE LARGE B CELL LYMPHOMA: FROM PRESENTATION TO TREATMENT AND PROGNOSIS
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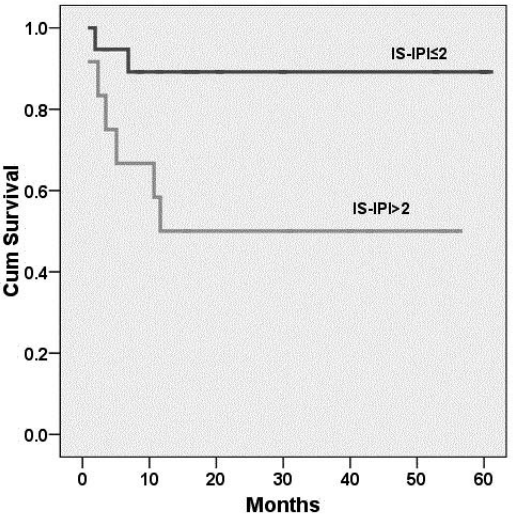
Background: Diffuse large B cell lymphoma (DLBCL) is a common subtype of non Hodgkin lymphomas (NHL), and its extranodal variant can arise from extranodal lymphatic tissue or non-lymphatic tissue. Primary gastric (PG) non Hodgkin lymphoma is a malignancy localized in the stomach with or without abdominal and/or extraabdominal lymph nodal involvement and constitutes 20-30% of all extranodal NHLs. Management of primary gastric diffuse large B cell lymphoma (PG-DLBCL) remains controversial as well as reliable staging system and prognostic factors. Few decades ago surgery has played a central role in the diagnosis, staging, and treatment of PG-DLBCL but adition of chemotherapy significantly improved survival. Since the availability of Rituximab, there is a lack of comparative studies investigating clinical effectiveness between surgery with immunochemotherapy and immunochemotherapy alone.
Aims: The aim of this study is to compare two treatments (immunochemotherapy alone and surgery plus immunochemotherapy) as well as to define most important prognostic factors.
Methods: Records of all-stages patients with a diagnosis of PG-DLBCL which were treated in the Clinic for Hematology Clinical Center of Serbia, between 2002 and 2012, were reviewed. Patients fulfilling the following criteria were included in this study: patients with histologically proven large-cell B lymphoma of the stomach who received Rituximab plus CHOP (R-CHOP) regimen as first-line immunochemotherapy with or without additional surgical resection.
Results: From 73 patients who were fulfilled inclusion criteria 44 received R-CHOP and 29 underwent surgical resection followed by R-CHOP. All clinical and pathological features were similar between the two groups. 45 patients (61,5%) had complete response to treatment, 11 (15,1%) had partial response to the treatment, 2 (2,7%) had stable disease and 15 (20,5%) had progressive disease. Tumor resection did not improve 5-years OS (75,9% and 65,9%, for surgery plus immunochemotherapy and immunochemotherapy alone, respectively, P=0.293). Ann Arbor clinical stage ≥II (P=0.047), ECOG≥2 (P=0.008), IPI≥2 (P=0.038), stage-modified IPI (for II2 grade of the Lugano staging system) (P=0,036), trombocytosis > 450x10^9/l (P=0.001), level of CRP ≥5mg/l (P=0.028) and albumins level low than 28g/l (P=0.047) were predictors of OS in patients with PG- DLBCL. A new *inflammatory stage* IPI (IS-IPI) risk score (smIPI plus level of CRP) was recognized as the best prognostic tool (P=0,045) in multivariate analysis. There were significant differences among patients with low-risk (score 0,1,2) and intermediate/high-risk groups (score >2) in 5-years OS (89.5% vs 50.0%, P=0.021).
Summary / Conclusion: IPI staging system modified for high level inflammation has shown to be the best prognostic tool for overall survival of PG-DLBCL patients. Adition of tumor resection to immunochemotherapy did not improve survival. In preventing morbidity arising from early or late complications from surgery, immunochemotherapy should be a primary option for DLBCL of the stomach. To confirm clinical effectiveness of Rituximab beyond 5 years studies with longer follow up are needed.



B1629
DIFFUSE LARGE B CELL LYMPHOMA PRESENTING WITH OSSEOUS INVOLVEMENT, CLINICOPATHOLOGICAL CHARACTERIZATION AND SURVIVAL ANALYSIS
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Background: Primary diffuse large B cell lymphoma of bone is an extremely rare condition that is usually confused with other primary injuries of the bone.
Aims: we have thus conducted this retrospective analysis of our non Hodgkin lymphoma database to determine the clinicopathological and survival characteristics of this unusual presentation.
Methods: DLBCL patients treated at Cairo Oncology Centre (Cairo, Egypt) in the period between 2000-2008 were reviewed. Eligible patients were those who had complete information on date of diagnosis, histopathological and immunohistochemical confirmation of the diagnosis and received CHOP-like chemotherapy. We compared the difference in systemic therapy and pathological parameters between cases presenting with and without osseous involvement. We investigated the impact of osseous involvement on progression free survival (PFS) and overall survival (OS) in a Cox regression model adjusted for age, Ann Arbor stage, performance status, extranodal involvement, presence of B symptoms, IPI score and treatment.
Results: 240 DLBCL patients were included in the analysis fulfilling the inclusion criteria. Of which 21 patients only have definite radiological evidence of bone involvement (8.75%). Bone involvement was isolated in 5 cases, associated with nodal involvement in 4 cases and associated with both nodal and extranodal localization in 12 cases. Median age for the whole group is 53 years while for the bone involvement group it was 54 years. At a median follow up period of 13 months, the median PFS for the whole group was 79 months, for the osseous involvement group it was 34 months. Cases with bone involvement were more likely to have advanced stage (P=0.048), bone marrow involvement (P=0.004), higher IPI (P=0.043). Based on univariate analysis, bony involvement alone was not significantly associated with shorter PFS (P=0.202).while bone involvement was not significantly associated with other adverse clinicopathological factors (elevated LDH, B symptoms or bulky disease).
Summary / Conclusion: According to our data, patients with DLBCL presenting with bony involvement more often present with advanced stage, shorter median PFS and higher risk disease and should be considered for more aggressive treatment.

B1630

EVALUATION OF EFFICACY AND SAFETY OF BENDAMUSTINE TREATMENT IN OSAKA LYMPHOMA STUDY GROUP(OLSG) OF JAPAN

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Background: Bendamustine was approved in Japan in December, 2010 and used for the patients with relapsed or refractory indolent lymphoma.
Aims: In this study, we evaluated efficacy and safety of bendamustine in the practical use.
Methods: We analyzed the clinical data of 122 patients who were treated with bendamustine from December, 2010 to March, 2012 in OLSG.

Overall Response Rate (ORR)	BR combination (N=87)	B monotherapy (N=35)
CR/CRu	52 (59.8%)	15(42.9%)
PR	19 (21.8%)	8 (22.9%)
SD	8 (9.2%)	3 (8.6%)
PD	8 (9.2%)	8 (22.9%)
Unknown	-	1 (2.9%)
Prior B regimen numbers: ORR	*P<0.0001*	
1: CR+PR	38 (92.7%)	3 (100%)
1: SD	2 (4.9%)	-
1: PD	1 (2.4%)	-
2: CR+PR	14 (93.3%)	9 (69.2%)
2: PD	1 (6.7%)	4 (30.8%)
≥3:CR+PR	19 (61.2%)	11 (57.9%)
≥3: SD	6 (19.4%)	3 (15.8%)
≥3: PD	6 (19.4%)	4 (21.1%)

Results: The patients' ages were 36 and 90 (median: 68). The ratio of male to female was 52.5:47.5. The histological diagnoses were follicular lymphoma in 70.5% and mantle cell lymphoma in 18.9%. Bendamustine was used in combination with rituximab in 71.3% of cases. The other cases were treated with bendamustine monotherapy. The completion of the planned regimen (six cycles) was done in 29.5%, while the other caseswere discontinued therapy due to the adverse events (36.0%), PD (22.1%) and achievement of CR (24.4%). Regarding response, 59.8% of CR and 21.8% of PR were achieved in combination with rituximab, while 42.9% of CR and 22.9% of PR were achieved with bendamustine monotherapy. CR+PR were achieved in 93.2%, 82.1% and 56.0% of the patients whose prior treatment regimen number were one, two andmore than three respectively. The progression-free survival of the patients treated with bendamustine-rituximab combination was significantly better than bendamustine monotherapy (P=0.0457).The multivariate analysis showed that three factors (sex, serum LDH level and prior treatment regimen number) were prognostic; female, low LDH and one or two prior regimen were favorable factors. Regarding toxicity, the hematological adverse event (HAE)(Grade 4) was observed in 71.3% and non-HAE (Grade3, 4) was observed in 12.6% with bendamustine-rituximab combination, while in 60.0% and 11.4% respectively with bendamustine monotherapy. Furthermore, febrile neutropenia (Grade3, 4) was observed in higher number of cases with bendamustine-rituximab combination than with bendamustine monotherapy.
Summary / Conclusion: Bendamustine was quite effective for the patients with relapsed or refractory indolent B-cell lymphoma. The early use of bendamustine-rituximab combination was supposed to be more effective. However, the combination use caused the more severe toxicity. It is necessary to clarify the best dosage of bendamustine in combination with rituximab expecting the best efficacy and safety in the future study.

B1631

PREDICTING FACTORS FOR GLUCOCORTICOID INDUCED-DIABETES MELLITUS IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS WHO RECEIVED R-CHOP CHEMOTHERAPY

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Background: Glucocorticoids are widely used in treatment of patients with lymphoma in combination with chemotherapy agents, but development of hyperglycemia is one of the major problems. Avoidance of unnecessary tests for screening of glucocorticoid-induced diabetes mellitus (GDM) might improve the quality of life during chemotherapies for patients with lymphoma.
Aims: To evaluate predicting factors for GDM in patients with diffuse large B cell lymphomas (DLBCL) who received R-CHOP chemotherapy.
Methods: A total of 46 patients with DLBCL who received chemotherapy with R-CHOP regimen in University of Tsukuba Hospital from November 2006 to June 2012 were analyzed. Patients with previous diagnosed DM were excluded. Diagnosis of DM was based on the American Diabetes Association's criteria with, fasting plasma glucose (FPG) □ 126 mg/dL or a random plasma glucose □ 200 mg/dL accompanied by classic symptoms of hyperglycemia. Metabolic syndrome was defined by the criteria of the International Diabetes Federation, with body mass index (BMI) > 30 kg/m^2 and any two of the following: 1) raised triglycerides > 150 mg/dL; 2) reduced HDL cholesterol < 40 mg/dL in males, < 50 mg/dL in females, or specific treatment for this lipid abnormality; 3) systolic blood pressure (SBP) > 130 mm Hg or diastolic BP (DBP) > 85 mm Hg. All described values of Hemoglobin A1c (HbA1c) in this study are expressed in HbA1c, NGSP. Univariate analysis was perfromed by chi-square test, and linear logistic regression analysis was used for multivariate analysis.
Results: Total number of patients diagnosed as GDM during R-CHOP chemotherapy was 14 of 46 patients (30.4%). Median total cycle number of chemotherapy was6, and 9 of 14 patients with GDM (64.2%) were diagnosed during the 1st cycle. 3 of 14 patients with GDM were managed with insulin, 1 was with exercise and diet therapy, and rest of patients received no therapy. No acute complications of hyperglycemia were observed. Pre-chemotherapy factors significantly associated with GDM were turned out to be HbA1c level more than 5.8 percent (*P*<.05), metabolic syndrome (*P*<.05), and previous history of hypertension (HTN) or elevated BP (SBP> 130 mmHg, DBP> 85 mmHg) at the start of R-CHOP regimen (*P*<.05) with univariate analysis. By the multivariate analysis, HbA1c level more than 5.8 percent (*P*<.05, RR = 5.4, CI: .1.29- 22.6) and history of HTN or elevated BP (*p* =.054, RR = 4.33, CI: .97- 19.3) were the independently significant factors associated with GDM.
Summary / Conclusion: DLBCL patients with HbA1c level more than 5.8 percent or elevated BP or history of HTN at the start of R-CHOP regimen would be possible indications to check plasma glucose level during the chemotherapy to seek out GDM.

B1632

PROGNOSTIC SIGNIFICANCE OF LOW LYMPHOCYTES COUNT IN THE PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA (DLBCL)

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Background: Lymphocytes are target for immunochemotherapy and their number is an indicator of immunological status. It was previously reported that low lymphocyte count has a prognostic significance in the patients with diffuse large B cell lymphoma (DLBCL).
Aims: The present study was designed to investigate the clinical and prognostic significance of low lymphocyte count in our group of patients with DLBCL.
Methods: We retrospectively analysed prognostic significance of low lymphocytes count at the time of diagnosis in 277 DLBCL patients. There were 203 nodal and 74 extranodal DLBCL. Only the patients treated with immunochemotherapy (CHOP or CHOP-like chemotherapy plus rituximab) were included in the study. Cut off for low lymphocytes count was determined by ROC analysis.The prognostic values of absolute lymphocyte count with respect to overall survival (OS) and progression-free survival (PFS) were evaluatedby Chi-Square test and two-tailed log-rank test.Correlation of lympho-

cytes count with clinical parameters was also analysed.
Results: Median of lymphocyte count was 1.4x10^9/L (range 0,2-4.9x10^9/L). However, ROC analysis showed that optimal cut off of low lymphocyte count with the best sensitivity and specificity is 1.3x10^9/L. According to ROC analysis low lymphocyte count was found in 121 (43.32%) patients. There was significant statistical correlation between low lymphocyte count and elevated LDH (P=0.01) and "bulky" disease (P=0.001). Low lymphocyte count (1.3x10^9/L) was in significant correlation with event-free survival (EFS, P=0.014) and overall survival (OS) (P=0.04). Namely, median of survival of the patients with normal lymphocyte count was not achieved and in the group of the patients with low lymphocyte count is 23,9 months. On the contrary, low lymphocyte count was not associated with therapy response (P=0,089). A multivariate analysis revealed that only IPI remained associated with OS (P<0.01; HR, 4.286; 95% CI, 1.998 to 9.194). Similar results were found when nodal and extranodal lymphoma were analysed separately.
Summary / Conclusion: Low lymphocyte count is in significant correlation with EFS and OS of patients with CDLBL and can be used as prognostic factor in DLBCL.

B1633
MYOCET IN LYMPHOMA THERAPY: AN OBSERVATIONAL STUDY IN WESTERN AUSTRIA.
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Background: Anthracyclines play the major role within polychemotherapy. Liposomal non-pegylated doxorubicin (Myocet) is characterized by a better cardiac tolerance.
Aims: Aim of the study is the characterization of benefit and side effects of COMP in lymphoma, a CHOP-like regimen containing Myocet in real-life-setting.
Methods: 121 patients (m/w 73/48) were analyzed retrospectively by firstly characteristics of patients, and secondly details of COMP-like therapy including outcome and side effects.
Results: Median age, 73 years (26-88); adverse performance status (ECOG ≥2), 46 patients (38%); any cardiac comorbidity, 73 patients (60%). Histology of lymphoma: DLBCL 61%; MCL 12%, follicular lymphoma 9%; CLL 7%; peripheral T-NHL 7%; other NHL 4%. 89 patients (74%) received Myocet-based therapy (±rituximab) in first line, 16 (13%) in second line, and 16 (13%) in third or higher line. Median 6 cycles (1-8) were administered. 7 patients were withdrawn prematurely, 12 patients (11%) died during therapy, among them three with cardiac fatality. The response rate (CR+RR) in evaluable patients was 66% (70/106 pts) and 75% (6/8 patients) in B- and T-NHL, respectively. Response was associated with therapy line (74% in first vs 47% in higher line, P = 0.007). Grad III/IV neutropenia was found in 45%, whereby this was not dependent on therapy line. Survival data will be presented at the conference.
Summary / Conclusion: Myocet-based therapy is highly effective in therapy of lymphoma in difficult situations. Cardiac toxicity (seen during therapy at 11 patients) was not associated with preexisting cardial comorbidity.

B1634
PRELUDE: A PHASE 3 STUDY IN PROGRESS TO INVESTIGATE THE PREVENTION OF RELAPSE IN DIFFUSE LARGE B-CELL LYMPHOMA USING DAILY ENZASTAURIN
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Background: Despite the advent of rituximab-based immunochemotherapy, treatment outcomes for patients with high-risk (International Prognostic Index [IPI] score 3-5) diffuse large B-cell lymphoma (DLBCL) continue to be suboptimal with relapse rates at 2 years of 25% or more, even for patients in remission after first-line therapy. Overexpression of protein kinase C (PKC)β, a protein involved in the B-cell receptor signaling pathway, appears to be a poor prognostic marker in patients with DLBCL (Shipp et al. *Nat Med*. 2002;8:68-74). Enzastaurin, a potent, selective inhibitor of PKCβ, has demonstrated clinical activity in a subset of relapsed patients with DLBCL and has a favorable safety profile.
Aims: Based on these results, a Phase 3 trial was initiated to investigate the efficacy of enzastaurin in patients who have achieved complete remission after standard first-line therapy.
Methods: Patients had histologically confirmed DLBCL with IPI score of 3-5 at diagnosis and had achieved a complete response or complete response-unconfirmed to cyclophosphamide, doxorubicin, vincristine, and prednisone, plus rituximab therapy. Patients were randomized in a 2:1 fashion to receive enzastaurin 500 mg daily (after a 375-mg loading dose 3 times daily on Day 1 only) or placebo. Treatment continued until patients developed progression of disease, unacceptable adverse events, or completed 3 years of therapy. All patients were followed for recurrence of disease and survival until death or study closure, whichever occurred first. The primary endpoint was overall disease-free survival (DFS). The trial was designed to have 80% power to detect a hazard ratio of 0.68. The secondary endpoints were event-free survival (EFS), EFS rate at 2 years, DFS rate at 2 years, overall survival, safety, health-related quality of life using the FACT-Lym, health status using the EQ-5D scale, assessments of biomarkers, and pharmacokinetics.
Results: Trial in progress.
Summary / Conclusion: The trial completed patient enrollment (N=757) in April 2010. Two interim analyses were performed by an independent data monitoring committee, with the recommendation to continue the trial. Final results will be analyzed after the last treated patient has been followed for 3 years.

B1635
FOLLICULAR LYMPHOMA IN FIRST RELAPSE: ANEMIA AND HIGH ERYTHROCYTE SEDIMENTATION STRONGLY PREDICT THE OUTCOME
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Background: The prognosis of patients with follicular lymphoma (FL) significantly improved after adding rituximab in treatment plan of these patients, both for newly diagnosed and relapsed patients. FLIPI and FLIPI2, widely accepted prognostic indices in FL were primarily designed for patients with newly diagnosed disease. Nowadays, the precise risk assessment also in relapsed FL patients seems to be necessary, since lot of treatment strategies are available for these patients, less or more agressive.
Aims: The aim of this study was to analyze the prognostic value of routinely determined clinical and laboratory parameters in patients with first relapse of follicular lymphoma.
Methods: The retrospective analysis was performed on 60 patients with diagnosed first relapse of FL grade I, II or IIIa, in the period February 2002-April 2010. In the first line, the patients were treated with R±CHOP or R±CVP. All the patients in the first relapse were treated with fludarabine based regimens (FC, FND), of whom 33 patients in combination with rituximab. The characteristics in first relapse examined as possible risk factors were age, higher hystological grade in relapse, presence of B symptoms, presence of "bulky" tumor (>10 cm in diameter), spleen enlargement, high FLIPI score, anemia (Hgb<12 g/dL), LDH level and erythrocyte sedimentation rate (ESR). Receiver operating curve was used to determine the optimal cutoff value for ESR in prediction of overall survival (OS) for our group of patients. Survival functions were estimated using the Kaplan-Meier method and compared using the log-rank test. A multivariate analysis was performed to evaluate the potential predictive value of the examined characteristics as a risk factor.
Results: The median follow up was 32 months (range 4-115 months). In first relapse, 24 (40%) patients were older than 60 years. Higher histological grade in relapse, B symptoms, bulky disease, spleen enlargement, high FLIPI score, anemia, elevated LDH and ESR>25 mm/h were present in 17 (28.3%), 41 (68.3%), 23 (38.3%), 32 (53.3%), 36 (60%), 20 (33.3%), 26% (43.3%) and 33

(55%) patients, respectively. The patients with B symptoms, high FLIPI score, anemia, and ESR>25 mm/h had significantly worse OS (P=0.000; P=0.001; P=0.003; P=0.000, respectively), while there was a trend toward worse OS in elderly patients (P=0.065) and patients with elevated LDH (P=0.091). Multivariate analysis identified anemia (P=0.034) and ESR>25 mm/h (P=0.005) as independent risk factors for poor outcome. Based on cumulative score of unfavorable prognostic factors identified in multivariate analysis, 2-years OS was significantly better (P=0.024) in patients who didn't have unfavorable factors (2-years OS 81.8% pts), compared to patients with 1 (2-years OS 60.9% pts) or 2 (2-years OS 40% pts) risk factors.
Summary / Conclusion: Modern clinical researches are having the aim to individualize treatment approach based on risk for poor outcome. Our findings suggest that some FL patients in first relapse require more effective treatment. Having in mind that more aggressive treatments such as high dose therapy with stem cell transplantation are associated with higher toxicity, the optimal approach according to risk has to be defined in new prospective studies.

B1636
ORBITAL AND OCULAR ADNEXAL MALT (MUCOSA-ASSOCIATED LYMPHOID TISSUE) LYMPHOMAS, TEN YEAR EXPERIENCE
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Background: Orbital and ocular adnexal Non Hodgkin Lymphomas (NHL) consist 2% of all NHL. The most frequent hystological sybtipe is extranodal marginal zone B cell lymphoma, MALT (Mucosa-Associated Lymphoid Tissue). Autoimmune inflamatory disorders as well as chronic infections are important ethiological factors. Various local disease signs and simptoms can occur a long period of time prior to diagnosis. Immunohistochemical markers CD5 or CD43 (sialophorin) are important negative predictive factors. Various treatment modalities are available.
Aims: To investigate clinical and laboratory parameters of patients with ocular adnexal MALT lymphoma (OAML), to compare efficiency between different therapy modalities and to investigate disease outcome.
Methods: Seventeen patients with OAML, diagnosed in Clinic of Hematology, Clinical Centre of Serbia between 2003 and 2013, were inrolled. We researched epidemiologic-demographic, clinical and laboratory characteristics on presentation, importance of Helicobacter Pylori (Hp) infection, clinical stage of disease and prognostic value of CD43. Efficiency of various therapy modalities was compared.
Results: Highest disease incidence rate was in eight decade, it is almost 2.5 times more frequent in male population. Overall median age is 66 years (range 36-79), males 67.5 years (36-79), females 57 years (48-77). No significant statistic difference between age at diagnosis and patients gender was confirmed. Local signs and symptoms of the disease were present much earlier prior to diagnosis (median 8.9 months, range 3-36). Seven patients (53%) had orbital lymphomatous involvement, 4 (23%) conjuctival, 2 (12%) lacrimal gland, one (6%) eyelid and one (6%) uveal involvement. The most frequent sign on presentation was swelling of orbital tissue, conjuctiva or eyelid (7 patients, 33%). Observed laboratory parameters on presentation showed low disease activity: median sedimenation rate 12mm/h (range 2-24mm/h), mean lactat dehydrogenase 333.76U/l (range 200-409U/l), median C reactive protein 1.49mg/l (range 0.20-9.50mg/l) and median beta-2 microglobulin 1.96mg/l (range 1.39-5.60mg/l). A significant presence of Hp infection (66.67%) was recognized. Predictive significance of CD43 was not confirmed. CD5 was negative in all cases. All patients have had localised disease and were staged as IE CS (Ann Arbor lymphoma staging system). One patient had B simptomatology on presentation. Ten patients, aged ≥60 had low intermediate risk (International Prognostic Index, IPI), six aged <60 had low risk and one low intermediate risk (age adjusted IPI, aaIPI). In our group, 5 year progression free survival (PFS) is 60%. There was no significance in PFS between initialy used treatment modalities, surgery vs. chemiotherapy (P=0.9942), surgery vs. radiotherapy (P=0.8296) and chemotherapy vs. radiotherapy (P=0.9191). All patients after initial or relapse treatment achieved disease remissions. No significance was observed between cumulative radiotherapy dosage and treatment outcome. Seven patients (41.17%) had relapse. One patient died due to non-hematologic complications.
Summary / Conclusion: Our results confirms that OAML has good overall therapy response, regardless of initial or relapse treatment modality, as well as good progression free survival and overall survival rate.

B1637
HEPATITIS B REACTIVATION IN PATIENTS WITH NON HODGKIN LYMPHOMA CD 20+ IN MAINTENANCE THERAPY WITH RITUXIMAB
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Background: Anti CD20 antibody (Rituximab) based chemotherapy regimens increase the HBV reactivation risk although sporadic HBV reactivation cases are reported in patients on maintenance with Rituximab single therapy too.
We evaluated how many HBV reactivation occurred among patients Hepatitis B core antigen positive (HBcAB +) and Hepatitis B surface antigen negative (HBsAg-) who received Rituximab single therapy during maintenance.
Aims: The aim of this study is to assess the prevalence of HBV reactivation among patients HBcAb +/ HBsAg – during the maintenance therapy with Rituximab.
Methods: In our Unit, 88 patients with non Hodgkin Lymphoma CD20+ received maintenance therapy with Rituximab (schedule: 375 mg/mq every 3 months for 2 years) from January 2007 to February 2013.
Patients were treated with different chemotherapy regimens: 40% (35/88) with R-CHOP; 52% (46/88) with R-FN; 3%(3/88) with R-F; 5% (4/88) with R-Leukeran. None of these patients received prophylactic therapy with lamivudine during induction or maintenance.
All the patients were given blood tests for HBV (HBsAg; HBsAb; HBeAg; HBeAb; HBcAb) before starting maintenance therapy and liver function tests before each administration of Rituximab.
Results: 20% of the patients (18/88) were HBcAb positive.
64% of the patients (56/88) completed the maintenance treatment: one of thesepatientsoccurred the HBV reactivation.
36% of the patients (32/88) arestillintherapy with Rituximab and 9% of them are HBcAb positive (3/32): all thesepatientsare at risk for HBV reactivation too.
Summary / Conclusion: Inpatients HBcAb +/ HBsAg – treatedwithRituximab in single therapy is indicated the prophylaxiswithlamivudine.
In our observational study the HBcAb +/ HBsAg- patients didn't receive prophylactic therapy with lamivudine during the maintenance therapy with Rituximab and the HBV reactivation occurred in one patient HBcAb+/HBsAg- three months after the end of the maintenance therapy (1/18).
More ambitious prospective studies are required to establish theclinical utility-of prophylactic therapy with lamivudine during the maintenance therapy with Rituximab.

B1638
GOOD PROGNOSIS IN PRIMARY HEPATIC LYMPHOMA WITH OR WITHOUT HCV INFECTION
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Background: Primary hepatic lymphoma (PHL) is an uncommon lymphoid tumor frequently associated with a poor prognosis. PHL was first described in 1965 by Ata *el al* and in 1986 Caccamo et al defined PHL as a lymphoma localized and limited to the liver without extrahepatic involvement. To date, less than 150 cases have been published.
Aims: We report 11 patients with PHL diagnosed from 1995 to 2011 in our center, with a study of the viral status and the result of cytotoxic treatment.
Results: Eleven patients with PHL were identified. The disease occurred in middle-aged men (median age: 58 years). The main presenting complaint was right upper quadrant abdominal pain (4/11 patients). Tumor markers (α-fetoprotein and CEA) were normal in 8 patients tested. Liver scans demonstrated either a solitary nodule or multiple lesions. Pathologic examination revealed diffuse large B cell lymphoma in six patients, one case of follicular lymphoma, one of small lymphocytic lymphoma and one case of T cell lymphoma. Eigth patients (72%) were HCV-positive. Eigth patients received chemotherapy with CHOP regimen (6CHOP, 2 R-CHOP), two patients received R-FN, while a patient with a single focal lesion received surgical treatment. The complete remission rate was 100% (11/11); one of these patients, who had HCV-related cirrhosis, died because of hepato-renal syndrome, and another one died because of Acute Myeloid Leukemia.
Summary / Conclusion: The outcome of patients with PHL who are treated with combination chemotherapy seems excellent. The frequent association of PHL with HCV infection suggests a possible role of this virus in lymphomagenesis. HCV- infection does not appear to influence the outcome of therapy.

B1639
MODIFIED VIGEPP PROTOCOLE AS A NEW SALVAGE REGIMEN FOR RELAPSED REFRACTORY LYMPHOMA PATIENTS
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Background: Salvage chemotherapy followed by high dose therapy and autologus stem cell transplantation(ASCT) is the standart treatment for relapsed/refractory Hodgkin and Non Hodgkin lymphoma patients.Response of these patients to salvage chemotherapy protocols predict outcomes after ASCT.Currently, the optimum salvage therapy is still not known; platinum,mitoxantron,ifosfamide or gemcitabine based regimens can be preferred according

to the patient characteristics.
Aims: We aimed to share our results with a new salvage regimen 'modified ViGePP protocole' in relapsed/refractory Hodgkin/Non Hodgkin lymphoma patients.

Diagnosis(n) NHL HL	6 16
Median age	39(17-63)
Gender(male/female)	12/10
Stage before ViGePP(n:) Stage 1 /2/3/4	1/5/7/9
Disease status before ViGePP (n) Relapse Primary refractory	14 8
Number of salvage treatment before ViGePP(n) 1 line 2 line >2line	4 12 6
Number of ViGePP courses(n) 1/2/3	2/18/2
Response to 2 cycles ofViGePP (n) CR/PR/Refractory	2/10/7
Auto transplant before/after ViGePP(n) **Allo transplant after ViGePP(n)**	3/9 3
Current status (alive /exitus)	15/7

Methods: We retrospectively analyzed 22 relapsed /refractory Hodgkin/Non Hodgkin lymphoma patients who were treated with modified 'ViGePP' salvage regimen' in Ankara Oncology Hospital. Demographic features and clinical variables of patients are summarized in Table 1. All of the patients had been treated with two or three cycles of modified ViGePP chemotherapy either as first line salvage regimen or after multiple lines of different regimens.Three patients were treated with this regimen for relapse after ASCT.Chemotherapy protocol consisted of vinorelbine 25 mg /m^2 gemcitabine 800 mg /m2 in 1. and 8.days, oral (PO) prokarbazin 100 mg/m^2 1-7 days and oral prednisone 60 mg /m^2 1-15.days. Response assesment has been performed after 2 courses of regimen except 3 patients who had only 1 course
Results: Median time period between diagnosis to ViGePP treatment was 32 (6-179) months. Overall response rate after 2 courses of chemotherapy was 60% (12/20, CR(n:2)+PR(n:10)). Treatment related mortality was 13% (3/22).We observed 18% grade 3 neutropenia,31% grade 4 neutropenia,22 % grade 3 thrombocytopenia, 13 % grade 4 thrombocytopenia.There were no nonhematological toxicities over grade 1.Ten patients were mobilized with filgrastim alone (n:5), after ViGePP regimen(n:4) or after plerixafor (n:1).Median time from the beginning of the treatment to ASCT was 4.5 (2-10) months. Nine patients who had ASCT has been followed in remission (1 PR/8 CR).Three patients who had relapsed after ASCT were treated with ViGePP followed with allogeneic SCT.One year overall survival of all patients was 74%±11%.
Summary / Conclusion: ViGePP salvage regimen has similar response rates with other chemotherapy protocoles without high treatment related toxicity rates.Although our number of patients mobilized with this regimen are low ,we think that it has high mobilization success rates.So we conclude that,it can be used easily as a bridge to transplantation for relapsed/refractory Hodgkin /Non Hodgkin lymphoma patients.

B1640
DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) COEXISTENT WITH HEPATITIS C INFECTION; SINGLE INSTITUTIONAL EXPERIENCE FROM EGYPT
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Background: Hepatitis C is a major health problem in Egypt and many European and North African countries; and cases of DLBCL co existing with HCV positive infection are increasingly encountered.
Aims: So, we have conducted this retrospective analysis of our non hodgkin lymphoma database to clarify the clinicopathological and survival characters of cases with coexistent HCV infection and DLBCL.
Methods: DLBCL patients treated at Cairo Oncology Centre (Cairo, Egypt) in the period between 2000-2008 were reviewed. Eligible patients were those who had complete information on date of diagnosis, histopathological and immunohistochemical confirmation of the diagnosis and received CHOP-like chemotherapy. We compared the difference in systemic therapy and pathological parameters between cases that are hepatitis C antibody (HCV Ab) positive and cases that are not. We investigated the impact of HCV positive status on treatment toxicity and progression free survival (PFS) and overall survival (OS) in a Cox regression model adjusted for age, Ann Arbor stage, performance status, extranodal involvement, presence of B symptoms and treatment.
Results: 230 patients were included in the analysis fulfilling the inclusion criteria. 17 patients were confirmed to be HCV Ab positive whilst the rest of the cases were either negative or unknown. At a median follow up period of 13 months, the median PFS for the whole group was 12.03 months, while for the HCV Ab +ve group it was 8.48; the median OS for the whole group is 13.2 months while for the the HCVAb +ve subgroup it was 8.48 months. ther ewas no statistically significant correlation between HCV positive status and any adverse prognostic indicator like extranodal presentation (P=0.34),B symptoms(P=0.33),elevated LDH (P=0.54) or age>60 years (P=0.32). Based on univariate analysis, HCV Ab +ve status was not associated with shorter PFS (P=0.65). Treatment was tolerated in the majority of HCV Ab +ve patients with only 2 patients showing grade 2 liver dysfunction with treatment.
Summary / Conclusion: According to our data, patients with DLBCL coexistent with HCV infection should be managed in the same way as other DLBCL cases .however, more frequent monitoring of liver function and PCR status and multidiciplinary discussion with the hepatologists is required .

B1641
SAFETY OF BENDAMUSTINA INCLUDING DHAP REGIMEN AS SALVAGE THERAPY IN MULTIRESISTANT LYMPHOMA PATIENTS
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Background: The management of patients (pts) with lymphoma recurring after stem cell transplantation or multiply relapsed disease remains challenging. Many studies have demonstrated the efficacy and safety of Bendamustine combinations in heavily pretreated pts.
Aims: Our study was designed to assess the safety of Bendamustine including DHAP regimen as salvage therapy in pts with refractory/relapsed lymphomas.
Methods: Ten patients were treated at 4-weekly intervals with Bendamustine 90 mg/mq on days1,2; Cisplatin 100 mg/mq over 24 hours on day 2; Cytarabine 2000 mg/mq (two doses) on day 3; Dexamethasone 40 mg on days 1–4 with or without Rituximab 375 mg/mq on day +4. Palonosetron was given as antiemetic prophylaxis on days1,3. A daily G-CSF was admistered starting by day +6 in 4 patients while a pegilated G-CSF was used in 6 patients on day +5. A total of 22 courses were administered and each patient received at least 2 cycles of therapy. The patient's characteristics are shown in table 1. At the time of enrollment 8 pts had a progressive disease.
Results: Chemotherapy-induced grade 1 nausea and vomiting were observed in 4 pts, mainly on days 3 and/or 4. Grade 3 and 4 haematological toxicity consisted of anaemia (3 pts), neutropenia (7 pts) and thrombocytopenia (6 pts). Severe neutropenia and thrombocytopenia were recorded mainly between days +10 and +14 with haematological engraftment (Neutrophils>500/mmc and Platelets>20.000/mmc) after a median of 4 days (range 3-6 days) from the nadir. Three patients had a febrile neutropenia requiring hospitalization and two died because of K. Pneumoniae sepsis. No grade 3 and 4 extra-haematological toxicity was observed. Eight patients are still on therapy.

PARAMETERS		N°
Histological subtypes	HL	4
	DLBCL	1
	PTCL	1
	SMZL	2
	RICHTER	1
	FL	1
Primary Refractory Disease		5
Relapse After ASCT		2
Prior Lines of Chemotherapy≥2		3
Disease Status at Enrollment	Progressive Disease	8
	Partial Response	2
Age Median (range)	53 years	(26-77)

Summary / Conclusion: In our experience, despite patients had multidrug-resistant disease heavily pretreated, the addition of Bendamustine to DHAP regimen seems safe with an acceptable toxicity profile if compared with an historical control group of patients treated at our Institute with DHAP alone. The two patients died due to a sepsis had a primary refractory disease relapsed after ASCT. Survival and response rate analysis, as well as the mobilizing potential of this Bendamustine combination can not be evaluated because of the small number of patients and the short follow-up. We are increasing our series and we hope to be able to present these results further on.

B1642

SPINAL CORD COMPRESSION AS THE INITIAL MANIFESTATION OF B- CELL LYMPHOMA: A CLINICO PATHOLOGICAL REVIEW OF 11 CASES

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Background: In patients with non-Hodgkin's lymphoma (NHL), inaugural spinal cord compression is rare and thought to occur in less than 5% of cases. Even in major centers experience of this entity is modest and optimal treatment thus remains unclear. We retrospectively studied 11 patients who had B-cell lymphoma revealed by spinal cord compression symptoms.
Aims: The aim of this study is to study the epidemiological features and clinical outcome of B-cell lymphoma revealed by spinal cord compression.
Results: We reviewed departmental records covering the 6 years period from 2006 to 2012 and we identified 11 patients presenting with spinal cord compression as their first manifestation of NHL.
They were 6 men and 5 women in this series. Patients ranged from 32 to 82 years of age with a median of 62 years old. Tumor lesions involved the thoracic spine in 4 cases, and the lumbar spine in 7 cases. At presentation 6 patients were non-ambulatory. Dual sphincter impairment was found in 4 patients. Bladder dysfunction was noted in 3 cases only. The pathology study showed two cases with low-grade lymphoma: lymphocytic lymphoma and follicular lymphoma and 9 cases with diffuse large B cell lymphoma. On admission; there were 3 cases in PS=0-1, 4 cases in PS=2 and 4 cases in PS=3. Serum lactate dehydrogenase (LDH) was normal in 7 cases and high in 4 cases. Seven had advanced stage at diagnosis, while 4 had limited disease: including three with localized epidural lymphoma and one with primary bone lymphoma. Nine patients underwent laminectomy for decompression and tissue diagnosis, after which 2 underwent radiotherapy, 2 underwent chemotherapy, and 7 underwent combined-modality treatment. The functional outcome was improvement in all cases, no patient worsened after surgery. Tow patients had autologous peripheral stem cell transplantation. All patients had early physiotherapy and achieved functional independence at the community ambulation level, even if paretic at presentation. The overall survival (OS) at 1 year was 81%. No difference in OS was noted between localized and advanced disease.
Summary / Conclusion: B-cell lymphomas are an uncommon cause of spinal cord compression. Functional outcome can be quite favorable, as can tumor outcome. Residual sensory deficits greater than motor deficits are not uncommon. The clinical and functional response of such patients to treatment and their more favorable overall prognosis emphasis the importance of an accurate histological diagnosis, full disease staging and the subsequent initiation of appropriate therapy.

B1643

EPIDEMIOLOGICAL DATA AND CLINICAL FEATURES OF 205 NON-HODGKIN LYMPHOMA PATIENTS AT A SINGLE CENTER IN TURKEY

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Background: There has been major progress in the diagnosis, classification, and treatment of nonHodgkin lymphoma (NHL).The distribution of NHL in various geographic regions might differ and certain subtypes might prevail in one area.
Aims: There is no data about the epidemiology of NHL in Turkey. We retrospectively determined the annual incidence of hospital-based NHL in Turkey based on patient registration data in our center in northwestern Turkey.We also report on clinical features, treatment modalities and outcomes, survival, prognostic factors.
Methods: We evaluated 205 NHL patients diagnosed between 2002-2011. Our hospital has been the only tertiary referral center for hematological diseases for a mixed rural and urban population of 616000 people for longer than 16 years (316000 males, 300000 females). Patients' demographic and clinical features, treatment modalities, and responses were recorded.The current World Health Organization (WHO) classification was adopted for histopathological diagnosis. Response to therapy was based on 2007 International Workshop Criteria. For survival analysis, time from diagnosis until the end of follow-up or time to death were considered. Informed consent was obtained.
Results: Of 205 NHL patients,121 (59%) were males and 84 (41%) were females (M/F=1.44).During the study period,the annual incidence rate for all NHL was 3.33/100000.The annual incidence in women was 2.8/100000, and in men it was 3.83/100000.At the end of 2011,the overall prevalence of NHL was 33.3 per 100000 population aged >16 years.The prevalence in men (38.3/100000) was higher than the prevalence in women (28/100000).The mean age was 58.4 years (range:15-87).Seventy patients (34.9%) had B symptoms, 39 (19%) had bulky disease,and 99 (48.3%) had extranodal involvement.An intermediate-to-high risk International Prognostic Index (IPI) score was present in 39% of the patients.Diffuse large B cell lymphoma (DLBCL) was the most common subtype (106 patients,52%). Other most frequent histologic subtypes were follicular (18 patients,8.8%), peripheral T-cell (15 patients,7.4%), and small lymphocytic (14 patients, 6.9%) lymphomas. For remission induction, CHOP and CHOP-like regimens were used in 66 patients (32.2%), while R-CHOP was used in 61 patients (30%). For DLBCL patients, in intent-to-treat analysis, the overall response and complete remission rates were, respectively, 60.4% and 43.4%. During a 10-year period, 78 NHL patients died. The median survival was 41 months. Five and 10-year survivals were, respectively, 45% and 25%. NHL patients with B symptoms (28 vs. 60 months,P=0.05), splenomegaly (26 vs. 60 months,P=0.048), intermediate-to-high IPI scores (20 vs. 86 months,P=0.002), high β-2 microglobulin (26 vs. 96 months,P=0.024), and those with bone marrow involvement (26 months vs. unreached,P=0.005) at initial diagnosis had significantly worse prognosis than others. NHL patients who obtained complete remission with first line therapy had significantly longer median survival than patients who obtained partial remission or who were refractory (p values<0.001).
Summary / Conclusion: The incidence of NHL according to our hospital-based data was similar to the incidence in western registries. The most frequent histopathologic subgroup was DLBCL. NHL patients who had poor prognosis were those with B symptoms, intermediate-to-high IPI scores, and bone marrow involvement at initial diagnosis.

B1644

PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) HAVING DEVELOPED MALIGNANT LYMPHOMAS. COMPLETE REMISSION OF LYMPHOMA FOLLOWING RITUXIMAB-CONTAINING THERAPY, BUT NOT OF SLE.

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Background: The development of malignant lymphomas, generally of the non-Hodgkin type (NHL), and with a preference to diffuse large cell B lymphomas (DLCBL), in systemic lupus erythematosus (SLE), has been proven and analyzed in an exhaustive recent literature. The combination of germline and somatic mutations, persistent immune overstimulation and the impairment of immune surveillance facilitated by immunosuppressive drugs, is thought to be at the origin of the increased lymphoma genesis. However the treatment and course of such affected patients is less known, and prognosis is generally estimated as poor.
Aims: Out of more than 500 patients with complete/incomplete lupus and secondary antiphospholipid syndrome (APS) seen and treated at the institutional Day Hospital between 1982 and 2009, 9 developed lymphomas (5 DLCBL, 1 Hodgkin's, 1 follicular lymphoma and 1 indolent lymphocitic lymphoma).
Methods: Eight patients were treated with Rituximab-containing regimen
Results: All patients achieved complete remissions (CR) with a follow-up comprised between 18 and 190 months. In a patient with DLCBL was documented relapse,that was fatal.Two patients achieved complete remission (CR) of both diseases. In the other 5 lupus serology (ANA, APA) persisted, with occasional lupus flares and vascular complications.
Summary / Conclusion: While eradication of the last cancer stem cell is tantamount to cure in neoplastic disease, persistent autoantigenic overstimulation may contribute to the refractoriness of autoimmunity. This analysis proposes the institution of clinical trials with the use of rituximab and stem cell transplantation in the treatment of autoimmune diseases.

B1645

FOLLICULAR LYMPHOMA IN SITU: SERIES OF 8 CASES

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Background: Follicular lymphoma in situ (FLIS or follicular lymphoma-like cells of uncertain biological significance) is a rare lymphoproliferative disorder defined as colonisation of germinal centers by Bcl2 and CD10 overexpresing B cells. Follicular architecture is usualy preserved, so immunohistochemistry (in particular Bcl2 and CD10) and/or molecular work-up is needed for diagnosis. Clinical significance of FLIS is still obscure. Some cases are found either prior or next to overt lymphoma, or sinchronously with other solid tumors, while some are incidental findings.

Aims: Here we present a series of 8 FLIS cases diagnosed in our institution during the period from July 2010 to January 2013.
Methods: H&E and immunohistochemical staining were performed on routine fomalin-fixed paraffin-embeded lymph node specimens. Immunohistochemical panel included Bcl2 and CD10 markers. t(14;18) FISH analysis was performed using Vysis LSI BCL2 Dual Color Break Apart Rearrangement Probe. Clinical data was available in 5 cases.
Results: One patient had a history of microsatelite instable colonic carcinoma and squamous cell carcinoma of the lung. Both paratracheal lymph node biopsy (performed for staging of lung cancer) and peribronchial lymph nodes in subsequent lobectomy specimen revealed lymph node involvement by FLIS. Two patients had overt lymphoma: one had FL lymphoma and FLIS in the same lymph node, while the other had FL transformed to DLBCL with FLIS in the otherwise uninvolved lymph node. Remaining 5 patients underwent diagnostic biopsies due to lymphadenopathy. The first patient had concomitant pneumonia and elevated LDH of 418 U/l (normal LDH <243 U/l). The second patient with subsequent diagnosis of viral hepatitis C had splenomegaly, pancytopenia, and elevated LDH (470 U/l). The third patient had hepatosplenomegaly, weight loss, fever and malaise, he died of unknown causes 1 month later. The remaining two patients had only lymphadenopathy and one had elevated LDH (254 U/l). On gross evaluation lymph nodes in these 5 cases measured from 0.9 cm to 1.9 cm in greatest dimension. FLIS-involved lymphoid follicles were populated by homogeneous centrocyte-like cells with strong immunohistochemical Bcl2 and CD10 expression while demonstrating fairly preserved tissue architecture. Two out of 5 cases also demonstrated Bcl2 break by FISH analysis.
Summary / Conclusion: Our case series represents different clinical scenarios in which FLIS can be encountered. Though sometimes FLIS was just an incidental finding in cases of synchronous or metachronous lymphoma or solid tumor, 5 out of 8 cases demonstrated that FLIS alone can cause clinicaly suspicious lymphadenopathy leading to lymph node biopsy. To detect FLIS, routine minimal immunohistochemical panel should include Bcl2 and CD10. Close clinical follow up may be indicated for the early diagnosis of overt lymphoma in FLIS cases.

B1646
LYMPHOMA (HODGKIN AND NON-HODGKIN): EPIDEMIOLOGICAL FEATURES IN THE SOUTH EAST OF ALGERIA
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Background: In Western countries: non Hodgkin lymphoma (NHL) represents approximately 85% of lymphomas, their incidence is increasing, and almost 50% are over 70 years old.
The Hodgkin lymphoma (HL) represents approximately 15% of lymphomas: incidence rates have been stable over last 20 years, nearly 70% are less than 50 years at diagnosis, and many people have even less than 30 years.
Aims: Also the goal of this work is a comparative epidemiological study of lymphoma (HL and NHL) recruited in our area (south east of Algeria) over 4 years in the service and to extract their characteristics.
Methods: This is a retrospective comparative epidemiological study, based on the clinical data of patients (pts) diagnosed from January 2009 to December 2012. The diagnosis was confirmed by histological and immunohistochemical study.
Results: During this period, 253 patients with lymphoma were diagnosed, 127 NHL and 126 LH (50% each).The NHL are composed of aggressive NHL: 88 cases (69%), the most common sub type is diffuse large B-cell lymphoma (DLBC: 68%) and T/TNK (19%).The indolent NHL: 39 cases (30.7%); the most common is follicular lymphoma Comparing the epidemiological results found in the NHL with those in the HL we found: Cases/year: 32 versus 31. Median age: 54 versus 29 years. Frequency peak: (50-60) and (65-75), versus (20-30), knowing that 68% of pts with NHL are under the age of 60 years and75% of HL's pts have less than 50 years. Sex ratio (M/F): 0.98 versus 0.89. Notion of cancer in family: 12% versus 3%. Exposure to agriculture products: 9% versus 3%.
Summary / Conclusion: The epidemiological profile of lymphoma over 4 years in the south east of Algeria has some particularities: The LH and NHL; each occupies 50% of all diagnosed lymphoma. A parallel increase in the number of cases for the NHL and HL. The HL affects the young (median age = 29 years, 75% have less than 50 years) while the NHL is more observed in average age (median age=54 years, 68% are under the age of 60 years), this may be related to our young population. The majority of NHL diagnosed are aggressive (69%) with a predominance of DLCB lymphoma (68 %), and predominance of follicular and PCB (43% each) in indolent lymphoma. These data join the literature. These particularities are relevant and they must be the subject of deeper epidemiological and etiological investigations.

B1647
COMPARATIVE STUDY THE EFFICACY OF DIFFERENT ERYTHROPOIESIS-STIMULATING AGENTS IN ANEMIC PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS
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Background: Anemia in lymphoproliferative disorders (LPD) patients is a frequent symptom and can decrease the efficacy of antitumor chemotherapy, survival rate and overall quality of life (QoL). Pathogenesis of anemia in LPD patients is based on suppression by proinflammatory cytokines, decreasing erythroid precursor's sensitivity to serum erythropoietin and effect of chemotherapy. Therefore erythropoiesis-stimulating agents (ESA) are used as a pathogenetic therapy of anemia in patients with LPD which significantly increase hemoglobin, reduce and prevent RBC transfusions and improve QoL.
Aims: To study the efficacy of different ESA in anemia patients with LPD.
Methods: To our interventional prospective study were included the LPD patients (n=114): low-grade non-Hodgkin's lymphoma (n=17), chronic lymphocytic leukemia (n=26) and multiple myeloma (n=71). The median age of patients was 67 years (range 24-85). All patients had been received two or more cycles of antitumor chemotherapy before they were administrated ESA treatment. Every patient was observed anemia with initial Hb ≤10.0 g/dl. RBC transfusions were administrated to patients whose Hb concentration was <8.0 g/dl until level of Hb was increased up to 8.0-9.5 g/dl. We compared the efficacy of different ESA: Epoetin alfa (n=59), Epoetine beta (n=29), Darbepoetin alfa (n=26). All ESA were administrated to the patients subcutaneously, Epoietine alpha and beta – 150 IU/kg body weight 3 times a week and Darbepoietine alpha – 6.75 μg/kg b/w once per 3 weeks. The target Hb level was 11 g/dl. The planning duration of ESA treatment was within 16 weeks. Positive response was estimated as increasing Hb concentrating ≥2.0 g/dl or achieving target Hb level (11 g/dl).
Results: In the whole group of LPD patients mean baseline Hb concentration was 8.66±1.63 g/dl (3.7-10.0 g/dl). Before ESA-treatment 29 patients had received 2-12 units (median 3) of RBC transfusions during last 2-6 months because of low Hb (3.7-8.0 g/dl). The period of ESA-treatment was from 4 to 24 weeks (mean 9.6±4.6 weeks). During the study period 12 patients (41.4%) followed RBC transfusions after ESA-treatment and 17 patients (58.6%) showed transfusion-independency and 8 new patients began receiving transfusion first time as a result of the anemia progressing. On the whole we observed positive response in 78 patients (68.4%), their Hb concentration increased from baseline to 12.1±1.2 g/dl (11.0-15.7 g/dl; P<0.001) and in group patients with positive response reduced such symptoms as: feeling fatigue, weakness all over, having trouble starting things because of tiredness, depression, drowsiness, giddiness, headaches, pain in thorax and dyspnea. The comparison of efficacy ESA-treatment showed insignificant difference between all ESA. So patients received Epoetin alfa positive response observed 40/59 patients, Epoetin beta positive response – 20/29 and Darbepoetin alfa positive response – 18/26, that is showed similar results (67.8%, 68.9%, 69.2%, respectively) without statistical difference (P>0.1).
Summary / Conclusion: In this study was shown that all ESA is effective reducing symptoms of anemia, increasing Hb in anemic patients with LPD and there wasn't found out significant differentiation between all these ESA.

B1648
MANAGEMENT OF STAGE IA DIFFUSE LARGE B CELL NON-HODGKIN'S LYMPHOMA (DLBL): IS RITUXIMAB REALLY NECESSARY?
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Background: Stage IA DLBL is a distinct entity with a favourable prognosis, where a combination of chemotherapy and local radiotherapy afforded durable remissions in the pre-rituximab era. We set out to review our experience of the treatment of this early stage of DLBL.
Aims: To assess the response and relapse rate of stage IA DLBL treated with chemotherapy and local radiotherapy alone, no patient received rituximab in line with national policy.
Methods: We undertook retrospective case notes review of patients treated with radiotherapy alone and those who received both chemotherapy with 3 cycles of cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP) and radiotherapy.
Results: Thirty patients were included, treated between 2005 and 2012. The mean age at diagnosis was 78.3yrs (Range 39-95), 23 aged >70yrs. Twenty-seven patients underwent the full course of intended treatment. Of those that did not complete, two discontinued due to intolerance and one declined any therapy prior to commencement. Site of disease frequently involved the head and neck (20 patients), while torso, abdomen and limbs were next most frequent. A greater proportion of deaths occurred in the head and neck group than any other (not all related to lymphoma or treatment). Radiotherapy alone was chosen for 11 patients usually due to frailty, of whom 3 relapsed and 8 died

subsequently (2 from lymphoma). Of the combined modality group (16 patients), there were no relapses and three deaths, none lymphoma related. None of these patients received rituximab. Dosage of radiotherapy was 30Gy in ten fractions in 22 patients (3 relapses), while 6 patients received 35Gy (1 relapse) [patient performance status was often poor thus mandating the use of abbreviated fractionation schedules for many]. Two thirds of patients who relapsed after radiotherapy did so within the first 12 months following treatment.
Summary / Conclusion: Radiotherapy alone or chemo/radiotherapy has shown to be effective therapy for patients with stage IA DLBL. Our series show good tolerability of therapy among an elderly group with an acceptable proportion of relapses with radiation alone and none in the combined modality group. It does not appear that rituximab is mandated in this group of patients.

B1649

REARRANGEMENTS OF BCL-2, BCL-6, CYCLIN-D1, IGH, P53 AND C-MYC AS PROGNOSTIC MARKERS IN REPRESENTATIVE TURKISH DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS

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Background: Diffuse large B-cell lymphoma (DLBCL) form a highly heterogenous type with different clinical, morphological, immunological and cytogenetic features, treatment responses, and prognosis. During the last decade, most studies dealing with the heterogeneity of DLBCL have focused on genetic and molecular analyses.
Aims: In this study, we aimed to determine the frequency of BCL-2, BCL-6, CYCLIN-D1, IGH, P53 and C-MYC rearrangements in DLBCL and to assess their prognostic impact.
Methods: A total of 44 patients of DLBCL classified according to WHO classification between 1996 and 2011 were chosen for the study. The samples prepared from the paraffin blocks of lymph nodes were analyzed by fluorescence in situ hybridization (FISH).
Results: Twenty-four of the patients were male and 20 were female and age at diagnosis ranged from 27 to 77 years. Advanced stage (III/IV) was observed in 22 cases (50%). 30 patients (68%) presented with high serum lactic acid dehydrogenase (LDH) levels and 15 patients had high serum B2-microglobulin levels. 28 of the patients with available data had a favourable risk group by the IPI. Succesful FISH analysis was performed in all patients. Rearrangement of BCL-6 was found in 27 patients (61.4%), C-MYC in 14 (31.8 %), P53 in 10 (22.7%), BCL-2 in 8 (18.2 %) and CYCLIN-D1 in 4 (9.1%). Furthermore, 18(41%) of cases showed rearrangements of more than 1 gene. Univariate analysis showed that the IPI score (P=0.013), stage (P=0.025), albumin level (P=0.007) and LDH level (P=0.025) were significantly associated with overall survival Patients with rearrangements of BCL-6 (P=0.053), C-MYC (P=0.483) and P53 (P=0.877) tended to have shorter survival times while patients with rearrangements of BCL-2 (P=0.302) were in a trend for better overall survival.
Summary / Conclusion: The presence of different genetic rearrangements in the same lymphoid tissue samples highlight the complex nature of molecular events in DLBCLs, which is a reflection of the morphologic and clinical heterogeneity of this disease. Our initial results are consistent with the previous literature that showed a different prognostic impact for gene rearrangements. The present study will be extended by including more number of patients with longer follow-up duration.

B1650

PRIMARY BONE LYMPHOMA: CLINICOPATHOLOGICAL CHARACTERISTICS AND TREATMENT RESULTS FROM SINGLE CENTER EXPERIENCE

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for less than 2 percent of all lymphomas in adults, 3 percent of primary bone tumors and 3 to 5 percent of all extranodal non-Hodgkin lymphomas. Men are diagnosed slightly more frequently than women. The vast majority of patients present over the age of 30 years.The radiographic appearances of PBL are variable. Despite this variability, the presence of a solitary, permeative, metadiaphyseal lesion with a layered periosteal reaction on plain radiographs and a soft-tissue mass on MR images, especially in a patient older than 30 years, is highly suggestive of lymphoma. The case for a diagnosis of primary bone lymphoma is further strengthened if the soft-tissue mass and marrow changes are associated with surprisingly little cortical destruction. Histopathologically, the majority of PBL cases have been diffuse large B-cell lymphoma (DLBCL). The patients with PBL treated with combined modality therapy were found to have a superior outcome and a significantly better survival, than the patients treated with single modality therapy.
Aims: To analyze the clinicopathological features of primary bone lymphoma, the role of MR in evaluation of response and correlation between the treatment modality and the outcome.
Methods: Totally 11 patients diagnosed with the primary bone lymphoma, were treated in Institution for radiology and oncology of Serbia in period from 2008.y to 2012.y. The treatment modality, the response and its duration (follow up) were registered for all the patients. We used bone scintigraphy for surveying the entire skeleton and magnetic resonance (MR) imaging to determine the exact extent of local involvement. Whole body CT was performed for each patient in initially staging and in response evaluation of initially pathological findings. MR imaging was used to assess the local outcome of treatment. PET/CT was done for some patients.
Results: Median age of patients was 38 years (range, 27-62). There were 9 male and 2 female patients. Three (27%) patients had B symptoms. Almost all patients were diagnosed with diffuse large B cell lymphomas except one with follicular B cell gradus 3A histology. The most frequent primary site of lymphoma was femur (4 patients). Other primary affected sites were: ossis ilei (3), tibia (2), humerus (1) and thoracic vertebra (1). 91% patients presented with Ann Arbor Stage I or II disease. Regional lymphadenopathy was registered in four patients. 82% patients were treated with combined modality therapy. Eight patients were treated with chemotherapy followed by radiotherapy. One patient was treated by surgery after chemotherapy, due to pathologic fracture. In the combined modality therapy group, the patients received IV to VI cycles of R-CHOP and in the only chemotherapy group, VI to VIII cycles of R-CHOP. Only one patient with high Ki 67 received R-EPOCH regimen. The median follow up was 18 months (range, 6-54). The overall response rate (ORR) for all the PBL patients was 100% as none of the patients showed PD during initial treatment. Seven patients (64%) achieved CR and 4 patients (36%) achieved PR. During response evaluation in all patients, NMR showed disappearing of soft tissue component with persistence of some bone lesions and minimal changes during time. FDG-PET revealed CR in four patients and all patients were alive at the time of the last follow-up and all remain in achieved response.
Summary / Conclusion: Our acquired data demonstrate and confirm that the primary lymphoma involving bone has a good prognosis. But, in our patients, we did not confirm earlier results of protocol influence (combined or single modality therapy) on the outcome. As bone changes that are seen on NMR images are pretty stabile during the treatment period and long time after completion of the treatment, it is difficult to achieve adequate response evaluation using NMR imaging. The need for the further multicenter studies (taking into account the rarity of the disease) in order to define the optimum treatment modality and better response evaluation is emphasized.

B1651

HIV-RELATED HEMATOLOGIC MALIGNANCES PRE-HAART (HIGHLY ACTIVE ANTIRETROVIRAL THERAPY) ERA AND HAART ERA: EXPERIENCE IN ONE CENTRE

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Background: Highly Active Antiretroviral Therapy (HAART) has had a dramatic effect on the natural history of HIV-infected patients. The incidence of AIDS-defining cancers has declined. However, non AIDS-defining cancers have gradually emerged.
Aims: Analyze the clinical and biological features and the outcome of hematologic malignancy among people with HIV infection or AIDS in the era pre-HAART and post-HAART.
Methods: We conducted a retrospective review of HIV-infected patients with hematological malignancies treated in our institution. Pre-HAART era included patients who did not receive HAART.Histological diagnosis was based according to WHO criteria. HAART was defined as a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor along with a backbone of at least two nucleoside reverse transcriptase inhibitors.
Results: Nineteen patients were evaluated, 10 of them belonged to pre-HAART era, and 9 of them belonged to HAAT era. The median age was of 37.3 years, and there was a male predominance. HAART era: the median duration from the diagnosis of HIV infection to development of hematological malignancy was 3 years (range, 0 to 11 years). The histological diagnoses were the following: 3 Hodgkin lymphoma (HL), 5 Non- Hodgkin lymphoma (NHL) and 1 myelodysplastic syndrome. Four of 8 lymphomas had stage II disease and 4 had stage III/IV disease. The 4 patients with stage II disease were concomitantly diagnosed of HIV infection. All patients with advance disease had a previous diagnosis of AIDS, two of them with poor adherence to HAART.
HAART was associated with standard chemotherapy in 8 cases. Five patients died mainly because of chemotherapy-resistant disease. At a median follow-up of 7 months (range, 2 to 48 months), two years progression-free survival (PFS) was 50%. Pre-HAART era: the median time from the diagnosis of HIV infection to development of hematological malignancy was 9 years (range, 2 to 16 years). The histological diagnoses were the following: 3 Hodgkin lymphoma (HL), 7 Non- Hodgkin lymphoma (NHL) and 1 acute myeloid leukemia of intermediate risk. Seven of 10 lymphomas had stage III/IV disease. All of them received standard chemotherapy except one patient with a central nervous system lymphoma. Six patients died: 3 died of lymphoma, 2 patients died after the first cycle of chemotherapy (1 neutropenic sepsis and 1 subdural hemorrhage), and 1 patient died of secondary wasting-HIV syndrome. At a median follow-up of 13 months (range, 1 to 96 months), two years progression-free survival (PFS) was

52%.
Summary / Conclusion: It has decreased the time from HIV-infection diagnosis to hematologic malignancy diagnosis from 9 years to 3 years after HAAR. The reasons for this finding remain unclear. Lymphoproliferative disease was diagnosed at early stage in the HAART era, if the adherence is successfully. Chemotherapy-resistant hematological disease remains the mainly cause of death.

B1652
ONLY RITUXIMAB IN THE EARLY TREATMENT OF INDOLENT LIMPHOMAS RELAPSED AFTER AUTOTRANSPLANTATION
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Background: In the last years the indolent lymphoma has benefited of transplantation procedures.High dose therapy folowed by autotransplantation has been used as salvage or first line tratment for indolent lymphomas.
Aims: The problem is the managment of the relapse of the disease in post-transplant, were as patients higly treated.We reported a single center exsperience in which patients whit indolent lymphomas relapsed after autotransplantatin,were treated only with immunotherapy.
Methods: From january 2005 we have autotransplantated,in our division,23 indolent lymphomas; 15 follicular,7 mantle cells and 1 marginal lymphoma. 13/23 (56%) patients have relapsed by a median PFS of 12 months (range 3-87). All patients received strict follow up with CT and PET and were treated early. 9/13 patients (70%) relapsed (8 follicular,1 mantle cells lymphoma) were treated with 4 weekly doses of rituximab 375 mg/msq for 1 month and then revaluated. If CR have started, maintenance with rituximab 375 mg/msq every 2 months for 2 years. 7/9 patients (80%) revaluated after 4 weekly doses have documented the CR and began rituximab maintenance. All patients who responded had a follicular lymphoma.
Results: With a median follow up of 36 months, 6/7 (85%) patients are in CR and in 4/9 (44%) we have documented grade IV hematologic toxicity (neutrophils<500/mm3) quikly resolved with G-CSF treatment.
Summary / Conclusion: In conclusion, for patients with follicular lymphoma, a strictly follow up may consnent a rapid treatment in relapsed patients after the autologus transplantation and the only immunotherapy may be sufficient to obtain a new CR consolidated by maintenance cycles every 2 months.We need a larger cohort and follow up longer to confirm these data.

B1653
LONGER MEDIAN TIME TO DIAGNOSIS FOR CASES WITH NON HODGKIN LYMPHOMA PRESENTING WITH PLEURAL EFFUSION.
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Background: Non Hodgkin lymphoma presenting with pleural involvement is a rare presentation that is usually confused with other pleural inflammatory and malignant pathologies with consequent diagnostic dilemmas and delay.
Aims: we have thus conducted this retrospective analysis of our non Hodgkin lymphoma database to determine the clinicopathological characteristics of this presentation.
Methods: Non Hodgkin lymphoma (NHL) patients treated at Cairo Oncology Centre (Cairo, Egypt) in the period from 2000-2008 were reviewed. Eligible patients were those who had complete information on date of diagnosis, histopathological and immunohistochemical confirmation of the diagnosis. We compared the difference in clinicopathological parameters between cases presenting with and without pleural involvement.
Results: In the period from 2000-2008; 380 Non Hodgkin lymphoma patients were included in the analysis fulfilling the inclusion criteria. Of which 26 patients only have definite radiological evidence of pleural involvement (6.8%). Pleura was the only site of disease in 2 cases, associated with nodal involvement in 16 cases and associated with both nodal and extranodal localization in 8 cases (of which 3 cases have pulmonary parenchymal involvement).of the nodal sites involved with the pleura, mediastinal nodes were involved in 13 cases. DLBCL was the diagnosis in 18 cases (69%), small lymphocytic lymphoma in 5 cases and follicular, Mantle cell and anaplastic T cell lymphoma one case each. Cases with pleural involvement were more likely to have higher LDH (P=0.005) and aggressive histology (0.05). Median time from initial presentation to established diagnosis is 2 months; pleural fluid aspirate was used in 10 cases; of which 6 cases needed further confirmation by a tissue biopsy.
Summary / Conclusion: According to our data, patients with NHL presenting with pleural involvement have a longer time to diagnosis with more diagnostic dilemmas, FNA from the pleural fluid should not be routinely considered as the diagnostic procedure of choice for cases with suspected NHL but rather tissue biopsy from accessible sites should be the standard.

B1654
BLASTIC PLASMOCYTOID DENDRITIC CELL NEOPLASM (BPDCN): SINGLE CENTER EXPERIENCE WITH TWO CASES IN ONE YEAR
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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare, highly aggressive hematopoietic malignancy that is characterized by cutaneous infiltration with or without bone marrow involvement and further leukemic spread. Its overall incidence is very low, accounting for 0.44% of all hematologic malignancies. The leukemic form of the disease is an extremely rare situation, representing <1% of all cases of acute leukemia. BPDCN predominantly affects males, with a sex ratio of 3:1, and generally occurs in the elderly.
Aims: The aim of this presentation was to evaluate symptoms, signs and outcome of two cases of BPDCN.
Methods: Between February 2012 and January 2013, we identified 2 patients with BPDCN presenting with skin lesions. Data regarding clinical presentation, diagnosis, staging, treatment and outcome was collected.
Results: First patient was female, 78 years-old, the other one was male, 75 years-old. At diagnosis both had asymptomatic skin lesions. The female patient presented with a cutaneous lesion on her right shoulder since the last month. Laboratory data disclosed anemia (hemoglobin: 11, 3 g/dl) thrombocytopenia (139×10^9/L) and morphologically immature atypical cells in the peripheral blood. Bone marrow aspiration showed 5% infiltration of immature blastic cells with the following immunophenotype: CD45(+), CD123(+), CD85k(+), CD33(-), CD14(-), CD16(-), CD19(-), CD5(-), CD10(-), CD20(-)CD56(+)20%, CD4(+), NG2(+). No chromosomal alterations were detected by cytogenetic analysis of the bone marrow. She had axillary, jugular, submandibular, and supraclavicular lymphadenopathy. Cutaneous, lymph node and bone marrow biopsies, all confirmed the diagnosis of BPDCN. She was treated with Cy-VAD (cyclophosphamide, vincristine, adriamycin and dexamethasone). She achieved CR, continued with induction 2 chemotherapy with Vepesid-Aracytin and died 4 months later of multi-organ failure. The male patient had a generalized purplish dermal rash arising from the head to the lower extremities that presented one week before and progressed very rapidly. Laboratory data revealed anemia (hemoglobin: 10, 9 g/dl), thrombocytopenia (100×10^9/L), WBC: $8.30*10^3$/μL with 42% of morphologically immature atypical cells. Bone marrow aspiration showed 88% infiltration of immature blastic cells with the following immunophenotype: CD45 (+) low, CD43 (+), CD123 (+), CD56 (+), CD4(+), CD34 (-).Cytogenetic analysis showed deletion of the long arm of chromosome 12 - deletion of ETV6 gene and deletion of the long arm of chromosome 17- deletion of P53 gene. Computed tomography scans did not disclosed any pathologic lymphadenopathy. Histopathology of skin lesions showed infiltration of blastic cells. Immunohistochemical analysis confirmed the presence of cells with the same immunophenotypic features. He started chemotherapy with Zavedos and Aracytin (3+7) and he is now in CR after this induction.
Summary / Conclusion: We present two cases of a rare clinical entity with cutaneous and bone marrow infiltration with blastic plasmocytoid dendritic cells. The diagnosis relied on the immunophenotypic features of the malignant cells, particularly with the presence of CD4 (+) and CD 56(+). Several treatment options have been used so far, all with poor results. The ALL-type treatment regimen that seems to result to a better outcome according to a recent publication resulted to a very short survival. Unfortunately neither of them could proceed to bone marrow transplantation, which is a better therapeutic option for younger patients with good performance status.

B1655
IMMUNOCHEMOTHERAPY-INDUCED CARDIOVASCULAR COMPLICATIONS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA: RESPONSE TO ZOFENOPRIL
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Background: The aim of this study was to determine the protective effect of zofenopril in immunochemotherapy-induced cardiomyopathy. The natural history of immunochemotherapy-induced cardiomyopathy, as well as its response to cardiovascular therapy, remains poorly defined. Hence, evidence-based recommendations for management of this form of cardiomyopathy are still lacking. Zofenopril proved to be effective in patients with coronary artery disease and myocardial infarction, thanks to its unique effective mechanism of action for improving blood pressure control, left ventricular function and myocardial ischemia burden, as well as angiotensin-converting enzyme inhibition. Rituximab is a monoclonal antibody to CD20 that has activity in leukemia and lymphoma.
Aims: This study aims to describe the complications and outcomes of a subset of patients with diffuse large B-cell lymphoma who were treated with immunochemotherapy.
Methods: Patients with diffuse large B-cell lymphoma in whom rituxmab, doxorubicin, cyclophosphamide, vincristine, and prednisolone therapy was planned were enrolled in the study. We included in the study 14 patients in zofenopril and 10 patients in control groups. In the zofenopril group, 7.5 mg twice-daily

oral zofenopril was given during 3 months. The patients were evaluated with echocardiography before and after chemotherapy. Left ventricular ejection fraction (EF) and systolic and diastolic diameters were calculated.
Results: At the end of 3 months of follow-up, 1 patient in the zofenopril group and 2 in the control group had died. Control EF was below 50% in 2 patient in the zofenopril group and in 3 in the control group. The mean EF of the zofenopril group was similar at baseline and control echocardiography (64.6 vs. 64.8, respectively; P=0.2), in the control group the mean EF at control echocardiography was significantly lower (62.7 vs. 49.2; P<0.001). Both systolic and diastolic diameters were significantly increased compared with basal measures in the control group. In Doppler study, whereas E velocities in the zofenopril group decreased, E velocities and E/A ratios were significantly reduced in the control group.
Summary / Conclusion: Prophylactic use of zofenopril in patients with diffuse large B-cell lymphoma receiving immunochemotherapy may protect both systolic and diastolic functions of the left ventricle.

B1656
A RARE CAUSE OF GENERALIZED LYMPHADENOPATHY: ROSAI DORFMAN DISEASE
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Background: Rosai Dorfman Disease is a rare benign disease characterized by generalized lymphadenopathies usually involving cervical lymph nodes. Its association has been reported with other autoimmune conditions and malignancies at the time of diagnosis or during its course. Anemia, polyclonal gammapathy and high sedimentation rate are remarkable findings in the laboratory investigations. Diagnosis is made by biopsy of the involved lymph node. Although the condition is a benign entity presenting with spontaneous remissions, it also may be lethal in the case with multi organ involvement associated with other autoimmune conditions.
Aims: We present here four cases followed in our center with diagnosis of Rosai Dorfman Syndrome.
Results: All of the cases presenting to our center with cervical lymphadenopathy were male and their median age was 49 years (range: 41 – 80 years). Anemia compatible with anemia of chronic diseases was found in all cases at the time of presentation. In regard to laboratory investigations, all cases had high levels of globulin, C-reactive protein and high rate of sedimentation, and polyclonal gammopathy.Diagnoses of all patients were made with biopsy of lymphadenopathy.In regard to follow-up of the patients, anemia worsened and increased level of creatinine was found after follow-up of 4 years without treatment. Biopsy results of the patient for whom renal biopsy was performed because of suspicion of renal involvement is still being waited, and steroid treatment was scheduled to the patient after the result of the biopsy. Hodgkin lymphoma developed in the course of one patient. Complete remission occurred with 4 courses of ABVD chemotherapy.New mass lesion was found in lung parenchyma on imaging studies following 6th course of ABVD chemotherapy.This patient whose result of lung biopsy has come as pulmonary adenocarcinoma is still receiving chemotherapy in the oncology unit for lung cancer. Steroid treatment was given to two patients with complaints of fever, weight loss, fatigue due to anemia (hemoglobin level was 8 g/dL and 6 g/dL, respectively). Fever response occurred with steroid treatment in both patients and their symptoms were controlled. High rate of sedimentation and high level of globulin normalized in the patients with anemia resolved. In one patient receiving steroid treatment, chronic renal failure developed due to accompanying cresenteric glomerulonephritis. The patient is still on chronic hemodialysis program. Steroid treatment is still continuing in both patients.
Summary / Conclusion: Although Rosai Dorfman syndrome is rare, lymphadenopathy should be kept in mind in differential diagnosis. It should be remembered that although it is a benign condition, it may be associated with other autoimmune conditions or malignancies at the time of diagnosis or during its course, and that it should be treated for symptoms due to such associated conditions and the patients should be followed for this.

B1657
POSITRON EMISSION TOMOGRAPHY SCAN FOR FOLLICULAR LYMPHOMA: GOOD CORRELATION WITH CLINICAL AND HISTOLOGIC FEATURES AT DIAGNOSIS
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Background: To date, positron emission tomography (PET) scan is not the standard in the staging at diagnosis of follicular lymphoma. Its use is limited to those cases in which residual disease is crucial for therapeutic decisions and only if transformation to a high-grade lymphoma is suspected.
Aims: Our aim is to demonstrate a positive correlation between the standardized uptake value (SUV) at biopsy site detected by PET scan with pathologic (Ki67 and follicular lymphoma histologic grade) and clinical (LDH, beta-2-microgloblin, Ann Arbor stage and Follicular Lymphoma International Prognostic Index -FLIPI) features at diagnosis.
Methods: We retrospectively detected in the last 3 years 10 patients in whom node biopsies were performed taking into account the maximal SUV detected in the PET scan at diagnosis. The statistical analysis was made with SPSS version 20 for MAC-OS; descriptives and one-tailed Pearson's correlation test were calculated.
Results: The mean at diagnosis for SUV at biopsy site was 9.29 (range 4-24), for LDH the mean was198.90 U/L (range 135-290), for beta-2-microglobulin 3 mg/L (range 2-6) and for Ki67% index was 22% (range 5-50%).
Three (30%) patients had a histologic grade1, 6 (60%) cases were grade 2 and 1 patient (10%) was grade 3a. Six cases (60%) were stage IV of Ann Arbor, 3 (30%) were stage III and 1 (10%) case was stage II. The one-tailed Pearson's correlation test showed correlation (r>0) of SUV at biopsy site with beta-2-microglobulin (r=0.667; P<0.05), FLIPI (r=0.744; P<0.05), histologic grade (r=0.797; P<0.05) and Ki67 (r=0.885; P<0,05). We did not find correlation with serum LDH (r=0.120; P=0.37).
Summary / Conclusion: The SUV measured in the PET scan at biopsy site correlates with almost all the daily used pathologic and clinical parameters. The serum lactate dehydrogenase (LDH) is not frequently elevated in low-grade lymphoma, this may be the reason why no correlation was found. The most clinically important correlation detected with SUV is the histologic grade.(figure 1). The follicular lymphoma histologic grade has a prognostic value and also a therapeutic implication. The Pearson's r=0.8 reveals a lineal dependence between both variables. In practice, the computed tomography scan is used for the staging of follicular lymphoma but with this radiologic technique it is impossible to detect which lymph nodes have a higher histologic grade. Many patients are misdiagnosed as follicular lymphoma when in fact they already have a high-grade lymphoma. With this little approach we will consider using prospectively PET scan as guidance for node biopsy at diagnosis and make a new statistical analysis with a higher number of patients.The SUV measured in the PET scan at biopsy site correlates with almost all the daily used pathologic and clinical parameters. The serum lactate dehydrogenase (LDH) is not frequently elevated in low-grade lymphoma, this may be the reason why no correlation was found. The most clinically important correlation

B1658
ERDHEIM-CHESTER DISEASE PRESENTED WITH CENTRAL NERVOUS SYSTEM INVOLVEMENT: A CASE REPORT
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Background: Erdheim–Chester disease (ECD) is non-Langerhans form of histiocytosis of unknown origin. It is rare disease. The clinical picture may vary from asymptomatic bone lesions to multisystemic and life-threatening forms with poor prognosis, especially in case of specific central nervous system (CNS) or cardiovascular involvements .It is mainly diagnosed by typical pathologic features with the biopsy specimen displaying the xanthomatous or xanthogranulomatous infiltration of tissues by $CD68^+$ $CD1a^-$ S-100± spumous histiocytes, which distinguished from Langerhans cell histiocytosis. Although steroids has been the most common medical treatment in this disease an optimal therapeutic strategy remains to be defined. Interferon α-2a (IFN-a) has recently demonstrated valuable results in ECD, specisificly with CNS involvement.
Aims: Herein, we present a 35–year-old patient with ECD who had intra cerebral tumor-like lesion, which have occasionally been reported.
Results: A previously healthy 35–year-old woman, except visual impairment in left eye due to cataract for last 2 years, presented to the neurology department with complaints of weakness and headache 5 months ago. Headache was throbbing type on temporofrontal region and last in 5 minutes. Any pathological sign was found on physical examination. Cranial magnetic resonance imaging (MRI) revealed a 3x3.5 cm mass on the level of left basal ganglion, which was heterogenic contrasted on central part. Due to edema and mass, 3. Ventricular and the left frontal horn of lateral ventricular were compressed. Dekzamethazon treatment was initiated. The patient was referred to neurosurgery department for biopsy from that mass. The biopsy revealed infiltration of $CD\ 68^+$ $S\ 100^+$ $CD1a^-$ non-Langerhans histiocytes which were consistent with ECD. The patient was referred to our department. The physical examination of the patient was normal. Although deksamethazon dose was lowered the patient was still receiving it about one month. Laboratory studies revealed an elevated C-reactive protein (>4.4 mg/L) and erythrocyte sedimentation rate (45 mm/hour), and decreased hemoglobin level (10.1 g/dl) which was compatible with iron deficiency anemia. The other laboratory studies including urea and electrolytes, lipids, urinary studies, liver function and antinuclear antibodies were all within normal limits. Radiologic studies including direct x ray and 99Technetium (Tc) scintigraphy of long bones, thoracal and abdominal computed tomographics and heart echocardiography were normal. Dexamethazon was stopped and 3 million units in 3 days for a week interferon α-2a and oral iron treatments were started. Interferon treatment was well tolerated. There was a marked regression on cranial MRI scan after 3 months. The mass was almost completely disappeared and the impression of the mass and edema were not seen.
Summary / Conclusion: As a result, IFN-a was a very effective treatment in our ECD patient with cranial mass, which is rarely seen.

Stem cell transplantation - Experimental

B1659

USAGE AND MIXED CHIMERISM STATUS OF RECIPIENT FREE EDGE FINGERNAILS FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTS

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Background: based on microsatellite(short tandem repeat, STR) genotyping is the standard method for donor cell engraftment monitoring after allogeneic hematopoietic stem cell transplantation (allo-HSCT). A standard chimerism analysis need pre-transplant blood specimens to identify the STR genotype of the patient's own. But sometimes we can't get the pre-transplant blood specimens, and hair follicles and oral mucosa were not the ideal choice due to hair loss and bleeding in oral mucosa.
Aims: To inspect whether DNA extracted from free edge fingernails specimens from patient after allo-HSCT could be used for STR genotyping and chimerism analyzing, and to observe the chimerism status in fingernails after allo-HSCT.
Methods: Peripheral blood, bone marrow, oral mucosa and free edge fingernail specimens were collected from patients after allo-HSCT and their donor. Genomic DNA was extracted and 15 STR loci genotyping and chimerism analysis was performed.
Results: For the first group which including 12 patients, pairs of fingernail and oral mucosa specimens were collected within one month after allo-HSCT and were comparative analyzed. For the second group which including 13 patients, chimerism status in fingernail samples were analyzed 3 months or longer after allo-HSCT, and 3 patients underwent repeated testing at different times. The results showed that for the first group, 4 oral mucosa specimens showed donor chimerism with varying degrees, but no donor chimerism was detected in all of 12 fingernail specimens. For the second group, 6.7% to 82.6% donor chimerism was detected in fingernail specimens in 5 out of 13 patients. For the 3 patients underwent repeated testing, donor chimerism was continued negative in one cases, but continued positive in the other 2 cases.
Summary / Conclusion: Free edge fingernail samples of patients within one month after allo-HSCT can be used for STR genotyping and chimerism analysis and better than oral mucosa samples. Donor derived skin cells chimerism can be formed and persist in some patients, and this indicated that there are cells in allo-HSCT donor graft can differentiate into skin cells.

B1660

GUT MICROBIOTA TRAJECTORIES AND IMMUNE SYSTEM RECOVERY IN CHILDREN UNDERWENT TO ALLOGENIC HEMATOPOIETICS STEM CELL TRANSPLANTATION (HSCT)

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Background: Allogeneic HSCT is a potential curative therapy for children with hematologic disorders. The main complications of HSCT are represented by infections and acute graft-versus-host disease (aGVHD), an immunological disorder which could be lethal and limits the use of this procedure. The gut microbiota (GM) evolves as an integral component of the human immune system (IS) and the developmental trajectory of GM represents a crucial factor in the process of immune recovery after HSCT. Human beings share a close mutualistic relationship with the GM, which is crucial for a balanced IS development. Specific intestinal bacterial groups are directly involved in this process, modulating a variety of T cell functions, as Th17, Th1, Th2 and regulatory T cells.
Aims: Characterization of the compositional and functional modifications of GM in children undergone allogeneic HSCT and correlation with the specific patterns of immunological reconstruction after ablative regimen.
Methods: Paediatric patients undergoing allogeneic HSCT at the Pediatric Oncology and Hematology Unit "Lalla Seràgnoli", Sant'Orsola-Malpighi Hospital, Bologna have been enrolled. For each pts blood and faecal samples were collected before HSCT and once every 2 weeks after the day of engraftment for 100 days. Phenotyping and identification of lymphocytes subpopulations was carried out by flow cytometry including CD3+CD4+ and CD3+CD8+ T cells, naïve T cells, memory T cells, B cells, Tregs, NK, Th17, Th1 and Th2.
HTF-MicroBi.Array has been used to rapidly and reliably characterize the GM, it is a validated tool, with a limited detection capacity (30 bacterial groups).
Results: 7 pediatric patients have been considered, 4 of which presented aGVHD (grade II-IV) within 100 days from HSCT. One of them had a intestinal GVHD of grade IV. HSCT deeply revolutionize GM, which progressively recover an "healthy-like" asset within 100 days from HSCT. This process is temporarily compromised by aGVHD. In the 4 patients which developed aGVHD, the appearance of aGvHD seems to temporarily compromise the rebuilding of the ecosystem. The non-aGVHD pts recover a "healthy-like" gut microbiota structure after-HSCT within 100 days, while the pts with GVHD showed a lower level of *Clostridiales* and a significant increase of *Streptococcus* at the onset of aGVHD
Summary / Conclusion: HSCT deeply rearranges the gut microbiota profile, which progressively recover an healthy-like asset within 100 days from HSCT. The results of this preliminary longitudinal study suggest a link between aGVHD and the trajectory of the re-building process of the symbiotic relationship between host and intestinal bacteria.

B1661

NAÏVE AND CENTRAL MEMORY CD4+ T CELLS RATIO AND GVHD

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Background: Despite the data now available, the exact pathophysiology of graft versus host disease GVHD is still poorly understood. However it is now widely accepted that GVHD is mediated by T lymphocytes and that the process has three stages, from activation of antigen-presenting cells (APCs) to destruction of the target organ. Compared with other transplants, PBSCs contain 10 times as many T lymphocytes; However, following their use for several years, a genuine divergence has been demonstrated in terms of the incidence of acute and chronic GVHD . Phenotype characterisation of T lymphocyte subpopulations is a recent concept, to elucidate their role in the development of GVHD, T cell depletion studies have been performed.In a mouse model, selective depletion of naive CD4(+) T lymphocytes (TNs) in situations of major or minor antigen incompatibility lead to transplant acceptance without GVHD and transfer of antiviral immunity .Naive subpopulations are identified by a positive reaction of CCR7, CD62L and CD45RA , and in adults, 40 to 50% of peripheral T lymphocytes have a memory phenotype. The retrospective study conducted by our team in 2006 concerning the impact of the graft composition on the development of GVHD demonstrated that patients receiving a graft containing a CD4(+)CCR7(+) ratio of > 70% developed early and severe GVHD. However, this ratio has no influence on graft acceptance, the incidence of infectious complications or recurrence.
Aims: To assess the impact of reducing the proportion of the naive and central memory CD4 T cell subpopulation (CD4(+)CCR7(+)) to 50% on anti-infectious immune response
Methods: 8 peripheral blood stem cell (PBSC) donors were analysed cultured in the presence of pp65 antigen recombinant protein of CMV, and ADV before and after partial and selective depletion of the haematopoietic stem cell (HSC) graft CD4(+) CCR7(+) ratio to 50%. The anti-infectious response is assessed using three methods: Elispot, tritiated thymidine (3H) and CFSE. The negative controls are represented by analysis against HTLV-1 antigen
Results: This study demonstrated the feasibility of CD4(+)CCR7(+) double labelling for partial and selective depletion. A CD4(+)CCR7(+) ratio calculated at 50% does not impair the integrity of a PBSC graft's defences against infection. This result also provides proof of concept prior to a future therapeutic trial on pre-graft immunomodulation.
Summary / Conclusion: T lymphocyte subpopulation phenotype characterisation and T cell depletion studies represent a major advance in terms of our understanding of GVHD; the CD4(+)CCR7(+) ratio - the level of which is still to be determined - may represent a new foundation upon which we can build.

B1662

IMMUNOMODULATION OF MESENCHYMAL STEM CELLS IN CHRONIC GRAFT-VERSUS-HOST DISEASE

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Background: Chronic graft-versus-host disease (cGVHD) is one of the principal causes of mortality in long-term survivors of allogeneic stem cell transplantation. The immunopathogenesis is unclear, we know that cGVHD is TH-2 mediated, but there are a lot of cytokines that must be studied to clarify the immune response. We know that Mesenchymal Stem Cells (MSCs) possess the ability to impart immunomodulatory effects.
Aims: In this preliminary prospective study, serum and lymphocytes subsets of cGVDH patients after MSC infusion were assessed.
Methods: Patients received intravenous infusion of MSC and were evaluated after7, 20, 42 and 56 weeks. We monitored the immunophenotype profile of lymphocyte subsets and cytokines using Flow Cytometer.
Results: The number of CD19+ cells increased on week 20 after therapy.

CD16+CD56+ subset was increased on the first 7 weeks and remain until week 20 after therapy. Differences were seen on cytokines evaluated, especially on IL-10, IL-2 and TNFa.
Summary / Conclusion: The infusion of MSC appears to have had an impact on cytokines and lymphocytes population. The results are encouraging in that there is possibility for effective therapy using mesenchymal stem cells for patients with cGVDH

B1663
COMPARABLE EFFECTS OF PIOGLITAZONE AND PERINDOPRIL ON CIRCULATING ENDOTHELIAL PROGENITOR CELLS, INFLAMMATORY PROCESS AND OXIDATIVE STRESS IN PATIENTS WITH DIABETES MELLITUS
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Background: Comparable effects of pioglitazone and perindopril on circulating endothelial progenitor cells, inflammatory process and oxidative stress in patients with diabetes mellitus
Aims: Endothelial progenitor cells (EPCs) originating from the bone marrow play a significant role in neovascularization of ischemic tissues and in re-endotheliazation of injured blood vessels. The purpose of this study was to investigate if the administration of pioglitazone or perindopril in diabetic patients can modify the number of EPCs in the peripheral blood and alter the endothelial function and inflammatory status of these patients.
Methods: Fifty type 2 diabetic patients were recruited and were randomly assigned to receive either pioglitazone (15mg/day) or perindopril (4mg/day) for a one-month period. In both groups blood sample were drawn on admission (baseline) and after one month of drug's administration. Blood samples were taken in order to count EPCs and inflammation markers such as C-reactive protein (hsCRP), vascular endothelial growth factor (VEGF) and asymmetric dimethilarginine (ADMA). Circulating EPCs were defined by the surface markers CD34+/KDR (CD34 and VEGFR2 expressing cells) and analyzed by flow-cytometry. Moreover the endothelial function of the patients was evaluated both on admission and after treatment with flow mediated dilation (FMD).
Results: We have found that neither pioglitazone (P=0.09), nor perindopril (P=0.5) affected the number of EPCs. Importantly, we have shown that pioglitazone reduced CRP (P=0.04) and ADMA levels (P=0.002). In addition, pioglitazone improved FMD (P=0.04) and increased plasma concentrations of VEGF (P=0.01). On the contrary perindopril had no significant effect on CRP levels (P=0.07), FMD (P=0.23) as well as on ADMA levels (P=0.09). However, perindopril administration increased significantly plasma levels of VEGF (P=0.03). Moreover, both agents did not differ regarding to their effect on ΔEPCs (P=0.34), ΔFMD (P=0.70), ΔVEGF (P=0.27) and ΔCRP (P=0.85). Interestingly, we have found that perindopril had a superior effect than that of pioglitazone considering ΔADMA levels (P=0.01), despite the non significant (post administration) effect on ADMA levels resulting solely.
Summary / Conclusion: Based on the existing data, it seems that both treatments did not affect significantly number of circulating endothelial cells. Importantly, pioglitazone affected beneficially all the study parameters. On the other hand, perindopril increased significantly only VEGF levels, while CRP, FMD and ADMA levels were unaffected. Interestingly, we found that although perindopril had no significant effect on ADMA levels, this effect was greater when compared to that of pioglitazone. Considering our results, we provide also evidence for a possible addition of perindopril to the conventional treatment with pioglitazone which could affect further the neovascularization and reduce oxidative stress in these patients.

Stem cell transplantation - Clinical

B1664
NON CRYO-PRESERVED AUTOLOGOUS PERIPHERAL BLOOD HEMATOPOIETIC STEM CELL TRANSPLANTATION(HSCT) FOR HEMATOLOGIC MALIGNANCIES IN SHIRAZ,SOUTH OF IRAN
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Background: Hematologic malignancy currently represents the main indication for HSCT. clearly, autologous and allogeneic HSCT are established therapies in many of hematologic malignancies. High dose therapy(HDT) supported by autologous HSCT are the preferred choice for lymphoproliferative disorders and multiple myelom.
Aims: In this study we report our experiences in non cry- preserved autologous stem cell transplantation of hematologic malignancies in south of Iran.
Methods: Between Jan 2004 and JAN 2013, 300 patients with diagnosis of hematologic malignancies including Hodgkin's lymphoma(HD) , non-Hodgkin's lymphoma(NHL),Acute myelogenous leukemia (AML)and multiple myeloma(MM)underwent autologous peripheral blood stem cell transplantation in our center in Shiraz university of medical sciences. Patients were treated by intensive chemotherapy followed by reinfusion of non- cryopreserved autologous stem cells. The pretransplant conditioning chemotherapy regimen were CEAM (lomustin 150- 200 mg/m^2, etoposide 1000 mg/m^2, cytarabine 1000-1200 mg/m^2 and melphalan 140 mg/m^2) for Lymphoma,Busulfan 14 mg/kg and etoposide 1000 mg/m^2 for AML and melphalan 140-200 mg/kg for multiple myeloma patients. All apheresis products were kept in a conventional blood bank refrigerator at 4°C for 2-4 days before infusion.
Results: During this time, 86 HD patients with median age 27 years (range;7-51),72 NHL patients with median age 29 years(range;18-64), 92 MM patients with median age 55 years(range;31-70)and 50 AML patients with median age 26(range ;17-51) underwent autologous peripheral blood stem cell transplantation. The median time to platelet count > 20×10^9/L was 15 days (range ;9-39).The median time to ANC > 0.5×10^9/L was 12 days (range ;8-32).All patients have engrafted and there were not graft failure in this study group. 100 days transplant related mortality rate was 2.2% in MM, 3.5% in HD, 4.2% in NHL and6% in AML group respectively.
Summary / Conclusion: Our data reflects the important role of HDT followed by HSCT in improvement of outcome for a variety of hematologic malignancies in our center. We concluded high dose therapy rescued with non- cryopreserved auto SCT is safe and effective method that is feasible in our patients.

B1665
ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR CHRONIC MYELOGENOUS LEUKEMIA IN THE ERA OF IMATINIB - A SINGLE CENTER EXPERIENCE
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Background: Tyrosine kinase inhibitors (TKI) have revolutionized treatment of chronic myeloid leukemia (CML). Allogeneic hematopoietic stem cell transplantation (alloHCT) was standard first-line therapy for CML, but it is now reserved for patients (pts) whose disease does not respond optimally to TKI. Whether this strategy of delayed transplantation will increase the risks of late relapse and mortality is not known.
Aims: To evaluate the experience of a single center in the treatment of CML with alloHCT in the Imatinib era and how relapse was managed.
Methods: Retrospective analysis of clinical registries databases of a cohort of CML allografted between 2000-2012pts. Outcomes of interest were transplant-related mortality (TRM), incidence of aGvHD, cGvHD, relapse and relapse-related mortality (RRM) and overall survival (OS)-estimated from the transplant date. Statistical analysis was performed with SPSS®v21
Results: 45 patients (pts) with CML received alloHCT between 2000-2012; median age at transplant 40 years (range 20-58).Median time from diagnosis to transplant was 20 months (range 3-167).Treatment prior to alloHCT: TKI (17 pts), acute leukemia induction (10 pts), acute leukemia induction+TKI (1 pts), others without TKI (17 pts). Disease state at alloHCT: 1st chronic phase (CP; 27 pts), 2nd CP (12 pts), 3rd CP (1 pt), accelerated phase (AP; 2 pts) and blastic phase (BP; 3 pts). Donors were matched in 39 (35 related/4 unrelated), mismatched in4, haploidentical in1 and syngeneic in 1. 29 received a myeloablative regimen (BuCy2+/-ATG) and 16 a reduced conditioning regimen (FluBu+/-ATG). Pts were transplanted with unmanipulated peripheral blood stem cells in 41, cord blood cells in 1 and bone marrow progenitor cells in 3. GvHD prophylaxis was: CsA (14pts), CsA+MTX (14pts), CsA+MMF (10pts), Tac+MMF (3pts), Tac+MTX (4pts). Chimerism at day 100: complete chimerism (30 pts), mixed chimerism (11 pts), graft failure (1 pts); 3 pts were not evaluable (1 syngeneic

and 2 deaths before day 100). Disease status at day 100: persistent disease in 12 and complete molecular response in 31. 40% developed grade II/IV aGvHD and 42% cGvHD. Cumulative incidence of relapse was 44.5% at 5 years; median time from alloHCT to relapse was 9 months (range 6-59 months). Post alloHCT disease progression in 25 pts (12 with post alloHCT persistent disease and 13 relapses): hematological (6 pts), cytogenetic (7 pts), molecular (10 pts), extramedullary (1 pt), hematological and extramedullary (1 pt). They received: donor lymphocyte infusion (DLI; 2 pts), TKI (4 pts), TKI+DLI (9 pts), acute leukemia induction (6 pts: alone-3; plus DLI-1; plus DLI and TKI-2), 2nd alloHCT (2 pts: alone-1, plus DLI and TKI-1), TKI and radiotherapy (1pt), tapered immunosuppression (1 pt). Complete molecular response attained in 13, major molecular response in 3 and 9 failed. Median follow up for 32 alive pts was 86 months (8-155). Cumulative incidence of 90-days, 1 year, and 3 years TRM was 4%, 11% and 11%, respectively. Cumulative incidence of 5-years RRM was3,7% and 37,5% for pts allografted in CP and advanced phase, respectively. 5-year OS of 72,4%, with rates of 86,2% and 50% for pts allografted in CP and advanced phase. At the last molecular follow-up complete molecular remission was achieved in 29 of 32 pts (90,6%).

Summary / Conclusion: AlloHCT value keeps supported by the quality of molecular remissions and continues an option for those who cannot tolerate TKI or when CML progresses on TKI therapy highlighting the fact that transplantation outcome in advanced phase is poor. Post alloHCT therapy with TKI needs to be further investigated.

B1666

DO EOSINOPHILS HAVE A ROLE IN GRAFT-VERSUS-HOST DISEASE?

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Background: Eosinophilia and eosinophilic tissue infiltration have been observed in patients with acute and chronic graft-versus-host disease (GVHD). It is unknown if eosinophils are involved in GVHD or are mere bystander cells.

Aims: The purpose of this study was to determine if eosinophils were activated in the blood of patients with GVHD, if the pattern of activation was the same in acute and chronic GVHD, and how systemic corticosteroid therapy might impact of the activation state of eosinophils.

Methods: Adult hematopoietic stem cell transplant recipients (n=37) with and without GVHD were investigated with respect to absolute and relative eosinophil counts and expression levels of 14 surface markers by 4-color flow cytometry. Multivariate analysis of pattern recognition (Partial Least Squares Projections to Latent Structures-Discriminant Analysis) was used to segregate the various patient subgroups.

Results: Multivariate analysis revealed that eosinophilic data could separate patients with acute GVHD (aGVHD) from those with chronic GVHD (cGVHD): aGVHD patients had lower levels of the integrins CD11c and CD18 compared to those with cGVHD. Eosinophils from patients whose GVHD was diagnosed within 100 days post-transplant also differed phenotypically from GVHD diagnosed after 100 days. Finally, systemic corticosteroid therapy drastically altered the phenotype of eosinophils in patients with GVHD, but not in those without GVHD. Thus, corticosteroid-treated aGVHD and cGVHD patients' eosinophils had significantly lower levels of the surface molecules CD9, CD11c, CD44, CD49d, and CCR3, as well as lower relative counts of blood eosinophils. In addition, decreased levels of CD18 were seen in steroid-treated cGVHD patients.

Summary / Conclusion: Eosinophils are likely to play a role in GVHD since they display an activated phenotype. Moreover, the different phenotypes exhibited by eosinophils in acute and chronic GVHD suggest that blood eosinophils receive different signals of activation from the tissues in these two forms of GVHD. Possibly, eosinophilic phenotypes could be used to facilitate the diagnosis of GVHD.

B1668

THE PREVALENCE AND PROGNOSTIC VALUE OF CONCOMITANT EOSINOPHILIA IN CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Chronic graft-versus-host disease (cGVHD) is a common and often severe condition affecting long-term survivors of allogeneic hematopoietic stem cell transplantation (ASCT). New theories suggest that impaired thymic function with defective immune reconstitution may contribute to cGVHD by a defective peripheral immune regulation with cytokine dysregulation and a succeeding imbalance in the T helper cell (Th) cytokine secretion pathways. In this context, cGVHD has been associated with an activation of the Th2 pathway where certain Th-type 2 cytokines, e.g. interleukin (IL)-5, also act as potent eosinophilopoietic factors. Hence, eosinophilia, which may be present at the time of cGVHD diagnosis, might act as a surrogate marker of activation of the Th2 pathway, which has been proposed to be associated with a more favorable prognosis among cGVHD patients. Though several studies have focused on the prognostic impact of eosinophilia on transplant outcome, the prognostic significance of eosinophilia after myeloablative ASCT and the relationship between cGVHD and concomitant eosinophilia remain to be established.

Aims: The primary objective of this study was to identify pre-transplant factors associated with the development of cGVHD with concomitant eosinophilia. Secondly, to determine the prevalence and clinical impact (overall survival, non-relapse mortality and relapse incidence) of the condition in a large population of patients who have undergone myeloablative ASCT with a long follow-up.

Methods: Data were collected retrospectively from 142 patients, all of whom developed cGVHD after having received myeloablative ASCT with unmanipulated grafts. An absolute eosinophil count of $\geq 0.5\times10^9$/L was defined as eosinophilia (EO) and analyzed at the time of cGVHD onset.

Results: There were 28 EO+ patients (19.8 %) at the time of cGVHD onset. The median follow-up time starting at the onset time of cGVHD was 5.8 years [CI: 3.2 – 6.8 years]. Relapse was observed for 27 patients. Twenty-five patients died from other causes than relapse. The remaining 90 patients were event-free at the end of follow-up. We identified significant differences among disease-, cytomegalovirus serostatus-, cGVHD onset-, and "ongoing steroid treatment"-groups at the time of cGVHD diagnosis. We observed no significant association between eosinophilia and the grade of cGVHD; neither when assessed according to the revised Seattle criteria nor when the high risk features of thrombocytopenia and extensive skin involvement were analysed. Importantly, we observed no significant association between cGVHD with concomitant eosinophilia and long-term clinical outcomes. Furthermore, subgroup analyses performed in order to assess the influence of ongoing steroid treatment at the time of cGVHD onset revealed a considerable confounding effect. Therefore, we suggest that positive results found in previously published studies may have been the result of the confounding effect of ongoing steroid treatment on both eosinophilic levels and clinical outcomes.

Summary / Conclusion: Based on this study we advocate that conclusions regarding the prognosis of cGVHD with concomitant eosinophilia after ASCT should be drawn with caution.

B1669

INFECTIOUS COMPLICATIONS FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) AND PROPOSAL OF EVIDENCE-BASED KOREAN GUIDELINES FOR PREVENTING INFECTIOUS COMPLICATIONS IN HSCT RECIPIENTS.

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Background: Infectious complications are a major issue after HSCT and remain a major cause of transplant related morbidity and mortality.

Aims: We analyzed the recent trends of infectious complications following HSCT based on a Korean Blood and Marrow Transplant Registry (KBMTR) data and proposed Korean guidelines for preventing infectious complications in HSCT recipients.

Methods: We collected 36 previous guidelines (9 international and 27 Korean individual HSCT centers) and reviewed their qualities. We also investigated incidence and causative organisms of infectious complications in1,667 patients (1,145 alloHSCT, 522 autoHSCT) undergoing HSCT in Korea during recent 5 years. On the basis of these data, 9 PICO questions were confirmed.

Results: Median follow-up time was 14 ms (0-66 ms). There were 768 episodes of infection in 456 patients after HSCT. Bacteria accounted for 59.6% of the major causative pathogens (fungus: 21%, virus: 17% and TBc: 2.2%). Infectious complications comprised 52.4% of the cause of death after HSCT. G(-) Bacterial infection is the most common cause of death in all HSCT patients G(-) bacteria: 25.1%, G(+): 21.2%, fungus: 24.7%, virus: 2.7%, TBc: 1.6%, unspecified infection: 24.7%. Routine antimicrobial prophylaxis is recommended for all adult HSCT patients. In Korea, 6.0% of HSCT recipients and 3.0% of donors had HBV infection before HSCT. Almost 5% of recipients new-

ly developed HBV infection after HSCT. Routine screening for HBV viral status in donor and recipient is recommended and tests should be repeated during follow-up for recipients. In all alloHSCT patients, 2.4% showed EBV-related PTLD. Routine screening and follow-up test for EBV viral load should be considered. Viral-associated hemorrhagic cystitis occurred in 2% of HSCT patients (87.9% BK/12.1% adenovirus-related). Almost 2% of HSCT patients had a history of active (n=12) or previous TBc (n=22) infection before HSCT and 0.8% of recipients newly diagnosed as TBc after HSCT. Korean HSCT centers revealed to have several different strategies for the vaccination after HSCT. A considerable rate of vaccine-preventable diseases such as varicella zoster or influenza viral infections occurred in HSCT patients, especially in adults. Therefore, we recommend routine immunization schedule with available vaccines for HSCT patients.

Summary / Conclusion: Infection is still one of the main complications after HSCT and highly contributes to mortality in HSCT recipients. Proposed guidelines may help to reduce the infectious complications after HSCT patients.

B1670

ALLOGENEIC TRANSPLANTATION AFTER REDUCED INTENSITY CONDITIONING WITH FLUDARABINE/CYCLOPHOSPHAMIDE IS FEASIBLE AND EFFECTIVE FOR HIGHLY RESISTANT INDOLENT AND AGGRESSIVE LYMPHOID MALIGNANCIES.

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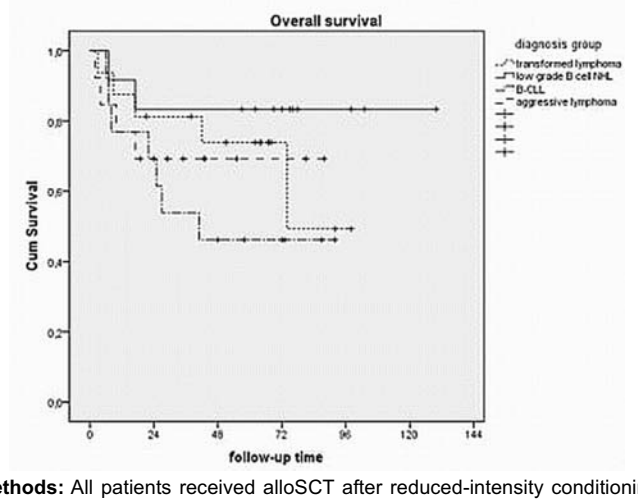
Background: Allogeneic stem cell transplantation (alloSCT) is a potentially curative treatment strategy for relapsed lymphoid malignancies.

Aims: We studied outcomes of 54 patients with relapsed or refractory indolent as well as aggressive lymphoid malignancies, who received alloSCT after reduced intensity conditioning in our center. Although the patient group reported here is heterogeneous as to lymphoma type and previous treatments, all patients had at least stable disease before transplantation and received the same conditioning regimen consisting of fludarabine and cyclophosphamide.



Methods: All patients received alloSCT after reduced-intensity conditioning with fludarabine and cyclophosphamide between July 2001 and November 2010 at the VU University medical center. AlloSCT was applied because of relapse after autologous stem cell transplantation (autoSCT) or because no further therapy was expected to lead to cure or a meaningful remission. The conditioning regimen consisted of fludarabine 25 mg/m^2 i.v. and cyclophosphamide 500 mg/m^2 i.v., both from day -7 until -3. Patients received an unmanipulated peripheral blood stem cell graft, targeting at a minimum of 4.0x10^6 CD34+ cells/kg patient body weight at day 0. Data were collected using retrospective chart review and analyzed using the SPSS statistical package (version 20.0). Overall survival (OS) and event free survival (EFS) were estimated using the Kaplan Meier method and compared using the log-rank test.

Results: A total of 54 patients were treated between July 2001 and November 2010. Median follow up of all patients alive at last follow up is 67 months (range 19-130 months). Patients were diagnosed with B-CLL (n=13), indolent B cell lymphoma (n=12), aggressive lymphoma (n=13) and 16 had DLBCL classified as transformation of indolent lymphoma. Thirty-two patients (59%) previously received rituximab. Immediately (in the first 0-3months) after conditioning and transplant, remission status had improved in 21 patients, all without donor lymphocyte infusions (DLI). During follow up six additional patients achieved CR without further therapy. Four patients relapsed within 6 months, and seven relapsed more than 6 months after transplant (range 9-58 months). Five patients required DLI during follow-up, all because of disease persistence or progression, resulting in remission in four patients. Analysis per disease category revealed a 4-year OS for B-CLL of 46% (95%CI: 19-73%), 83% for indolent B-cell lymphoma (95%CI: 62-100%), 69% for aggressive lymphoma (95% CI:44-94%) and 74% for transformed lymphoma (95%CI: 52-96%). (NS, P=0.28, see Figure 1). Four-year EFS was 46%, 75%, 55% and 67% respectively (95%CI: 19-73%, 51-100%, 24-87% and 44-91% respectively, NS, P=0.54). OS was not significantly different between patients with (n=16) and without (n=38) previous autologous stem cell transplantation. One year non relapse mortality was 11%, increasing to 16% after two years. (95%CI: 2.2-20% and 5.7-26% respectively)**Summary / Conclusion:** Reduced intensity conditioning with fludarabine cyclophosphamide is feasible with acceptable toxicity and effective for both highly resistant indolent and aggressive lymphatic malignancies, even after previous autoSCT. Due to the excellent anti-B-cell/lymphoma activity, fludarabine-cyclophosphamide decreases tumor load during alloSCT, gaining time for the development of a graft versus lymphoma effect, which was also apparent after DLI.

B1671

IMPROVED OUTCOME AND NON-RELAPSE MORTALITY AFTER ALLO-HSCT FOR CHILDREN AND ADOLESCENTS WITH HEMATOLOGICAL DISEASES: A THREE DECADE ANALYSIS FROM A SINGLE INSTITUTION

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Background: Allogeneic hematopoietic stem cell transplantation (allo-SCT) is a curative treatment for many pediatric patients with hematologic malignancies, bone marrow failure syndromes, and some inherited disorders. Over the past decades, major landmarks and advances have been achieved in the field of allo-SCT with respect to conditioning regimens, choice of stem cells source, as well as supportive care.

Aims: With this background, we conducted a study to assess the outcome of pediatric transplanted in a single institution over a 3 decades period.

	Total	1983-1999	2000-2010	p
N	250	101	149	
Sex M	153 (61%)	55 (54%)	98 (66%)	0.09
Median age	9.1 [0.7-17.9]	11.4 [0.7-17.9]	8.2 [0.7-17.6]	0.001
Disease:				
ALL	118	51	67	0.56
AML	63	28	35	
JMML	8	2	6	
Bone marrow failure	10	4	6	
MDS	8	4	4	
NHL	18	6	12	
CML	7	3	4	
MPD	3	0	3	
CMML	4	0	4	
Other	5	2	3	
Risk of disease				
High risk	61 (26%)	30 (31%)	31 (22%)	0.25
Standard risk	175 (74%)	65 (69%)	110 (78%)	
Myeloablative regimen	227 (91%)	101 (100%)	126 (84%)	9.10^{-5}
TBI-based regimen	102 (41%)	80 (79%)	72 (48%)	2.10^{-4}
BM	194 (78%)	97 (96%)	97 (65%)	2.10^{-10}
PB	16 (6%)	2 (2%)	14 (9%)	
BM+PB	1	1 (1%)	0	
CB	39 (16%)	1 (1%)	38 (25%)	
MSRD	15 (6%)	10 (10%)	5 (3%)	3.10^{-2}
MSUD	42(17%)	9 (9%)	33 (22%)	
MRD	128 (50%)	71 (70%)	55 (37%)	
MUD	67 (27%)	11 (11%)	56 (38%)	

Methods: All patients who received allo-SCT in our pediatric hematology department between 1983 and 2010 were included in this analysis. This period was *a priori* divided into two parts namely 1983-1999 and 2000-2010, the latest period corresponding to the initiation and development of cord blood allo-SCT and introduction of the so-called reduced-intensity conditioning (RIC) regimens in some subgroups. The main characteristics of the patients and the most relevant aspects of allo-SCT are shown on Table 1. The use of a myeloablative conditioning (MAC) regimen was predominant between 1983 and 1999 while RIC regimen represented 16% of the procedures after 2000 (P=9.10^{-5}).Bone marrow was the major stem cell source between 1983-1999 compared to 2000-2010 (96% vs 65%, P=2.10^{-10}). After 2000, cord blood was introduced as stem cell source for 25% of cases and peripheral blood stem cell for 9%.

Results: Overall survival (OS) was significantly higher in the second period (64% versus 52%, P=0.03). Also, non-relapse mortality (NRM) at 5 years was significantly lower after 2000 (29% versus 9%, P=0.0002). The cumulative incidence of relapse was 25% between 1983-1999 and 34% between 2000-2010 (P=0.11). Concerning the cumulative incidence of mortality due to relapse or progression was not statistically different between the 2 periods (20% vs 26%, P=0.26). Finally, between the 1983-1999 period and the 2000-2010 period, the cumulative incidence of death related to GVHD was significantly reduced (13% versus 3%, P=0.001).

Summary / Conclusion: In conclusion, this large analysis performed over a 3 decades period confirms that major advances in terms of outcome were achieved over the time in the pediatric allo-SCT population. The safety of the

procedure and the overall outcomes have significantly improved, despite the increasing use of alternative stem cell sources such as cord blood cells and HLA-matched unrelated donors.

B1672

THROMBOTIC MICROANGIOPATHY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION – A RETROSPECTIVE ANALYSIS OF 31 CASES

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Background: Transplantation associated thrombotic microangiopathy (TA-TMA) can be a devastating complication of allogeneic hematopoietic stem cell transplantation (aHSCT). The incidence of TA-TMA in aHSCT recipients is reported to be very variable. This is mainly caused by the lack of a standardized definition of disease and the broad variability of the severity of TMA. Currently, the mechanisms causing this disease are still not well known. Treatment guidelines are not available.
Aims: The aim of our analyses was to evaluate the efficacy of different treatment strategies for TA-TMA.
Methods: We retrospectively analyzed 31 (f/m = 11/20) cases with TA-TMA. All patients received allogeneic hematopoietic stem cell transplantation at our hospital between 1997 and 2011. Patients suffered from AML (11), ALL (8), CML (6), NHL (3), Multiple Myeloma (2) and CLL (1). Follow up period was closed on 31.12.2012. Median follow up after transplantation was one year (range 86 to 2460 days). TMA has been defined as follows: elevated LDH, thrombocytopenia below 50 G/L or reduction of platelets of more than 50% and fragmentocytes in the peripheral blood smear.
Results: Median time for occurrence of TMA after aHSCT was 138 days (range 16 to 1862). Median duration of TMA was 72 days (range 11 to 238). Most of the patients (27/31; 87%) were treated with calcineurin – inhibitors before TMA occurred. Eight patients (26%) died from TMA, but in our cohort only two patients (6%) survived until the end of surveillance period. Most of them died from relapse, infectious complications or chronic GvHD. Treatment modalities used were plasmapheresis, defibrotide, rituximab or combinations of each. In all cases calcineurin-inhibitor treatment was stopped. Four patients were treated with supportive care only. Remission rates for defibrotide, plasmapheresis, rituximab and supportive care were 43%, 40%, 50% and 100% respectively. Median duration of TMA was 68 days (defibrotide), 168 days (rituximab), 61 days (plasmapheresis) and 70 days (supportive care). Achieving remission of TMA, duration of TMA and survival was not statistically different compared in the three treatment groups except the fact that TMA duration was longer in the rituximab group (mean 137 vs. 75 days, P=0.0246, unpaired t-test). This may be caused by the fact, that rituximab was used late in the treatment course (median 38 days (rituximab) vs. 10 days (defibrotide) vs. 8 days (plasmapheresis) after onset of TMA).
Summary / Conclusion: Treatment of TA-TMA is still challenging. None of the treatment regimens used can be considered as standard treatment. If there is no organ complication stop of calcineurin inhibitors may be sufficient, but for patients with life-threatening complications this may not be appropriate. According to the existing literature plasma exchange is no longer recommended except in patients with - very rarely found - ADAMTS13 activity below 5%. Rituximab seems not to be useful as a rescue medication. Only defibrotide may be able to shorten the course of TMA, but has no influence on inducing remission or survival. Elucidation of the pathophysiology of TA-TMA is mandatory. Especially the role of complement activation may be a cornerstone for defining new treatment options.

B1673

KIR AND CYTOKINE GENE POLYMORPHISMS AS INFLUENCE FACTORS FOR EARLY AND LATE COMPLICATIONS AFTER HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Immunology of transplant is very complex and yet not well known.
Aims: This involve polymorphism of HLA, minor histocompatibility antigens , KIR (activatory and inhibitory) allele, cytokine(homeostatic ,inflammatory, anti-inflamatory) ,chemokines and their receptors.other non-HLA encoded genes.HLA matching can have dualistic effect on transplant outcome:it reduces rejection but conversely,it may promote other HLA-restricted mechanisms of allograft injury.
Methods: We have assesed eight pairs for allogeneic bone marrow transplantation for hematological malignancies..All related donors and recipients had 100% HLA alleles match(HLA A,B,C,DRB1,DQB1,DPB).All pairs were investigated for their cytokine gene polymorphisms and KIR haplotypes as long term prediction factors for graft versus host disease acute and chronic,complication of vascular origin(veno-oclusive disease,thrombotic microangiopathy) ,recurrence of the disease and develope of a second malignancy. HLA typing was performed by high resolution sequence specific primers(SSP) method using Dynal (KIR Genotyping SSP Kit)high resolution SSP.HLA alleles ambiguities were resolved by Sequencing Based Typing(SBT)Allele SEQR,ABBOTT.Cytokine gene polymorphisms were performed by Dynal(Cytokine Genotyping SSP Kit)PCR SSP9(IL1 alpha,IL1 beta,IL1R,IIL1R alpha,IL2,IL4,IL4Ralpha,IL6,IL10,IL12,gammaIFN,TGF beta and TNFalpha).KIR genotyping for both donors and recipients were revealed using PCR SSP (Dynal)We try to correlate with leukocytes ,platelets recovery presence of schistocytes,appearence of reccurrence.
Results: Short,our results:-following genes were identified: IL1alpha,IL1beta,IL1ra,gammaIFN,TGFbeta1,IL4,IL6,IL10;44%TNF alpha and IFN gamma,56%TGFbeta.-KIR genotype: B in 12 patients,A,in 4 patients. HLA typing at 4 and 8 digits reveal as alleles secreted but not stable at the cell surface,find at soluble form,allel which contains a mutation outside the coding region (null allele) or low expresed allele at the cell surface.
Summary / Conclusion: These differences in "perfect" matches,KIR genotype, the presence and fluctuation of cytokine influence the appearence and gravity of GVHD acute or chronic,vascular injury ,leucocyte and platelets recovery,recurrence of disease and a late second malignity.Of course,thats depends also from disease,conditioning regimen ,treatments before and after transplant.

B1674

IS SYMPTOM ASSESSMENT OF VALUE IN LYMPHOMA SURVIVORS AFTER AUTOLOGOUS HAEMATOPOIETIC STEM CELL TRANSPLANTATION (AHSCT)?

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Background: AHSCT offers effective control and potential cure of lymphoma patients but is accompanied by adverse effects and long-term complications, which have a negative impact on patient's quality of life (QoL). Self-reported symptom assessment may be a useful tool to identify patient benefits and risks of AHSCT at long-term follow-up.
Aims: We aimed to evaluate the symptom profile in lymphoma patients in complete remission after AHSCT at long-term follow-up and study its interference with QoL.
Methods: A total of 68 patients with lymphomas(non-Hodgkin lymphomas – 33, Hodgkin lymphoma –35) in complete remission after AHSCT with the follow-up of at least 12 months were enrolled in the study. Median follow-up period was 18 months. Mean age – 37 yrs (SD=12); male/female 33/35. For symptom assessment the Comprehensive Symptom Profile for patients with Lymphomas (CSP-Lym) was used. The CSP-Lym is developed to assess the profile of 41 lymphoma specific symptoms. Symptom severity and percentages of patients with moderate-to-severe (ratings 3-5) symptoms were evaluated. QoL was measured by the SF-36 questionnaire. For comparisons Mann-Whitney test was used.
Results: The majority of patients experienced fatigue (75%), decreased work energy (66.6%), feeling of constant tiredness (62.5%), shortness of breath (62.5%), and sleepiness (58.3%). Other common symptoms were exertion sweatiness (54.2%), insomnia (50%), numbness (45.8%), rapid heartbeat (45.8%), and concentration loss (45.8%). In most cases these symptoms were mild (ratings 1–4). Twenty five percent of patients experienced at least one moderate-to-severe (ratings 3-5) symptom; half of them suffered from moderate-to-severe fatigue. A small proportion of patients (8.3%) exhibited more than 7 moderate-to-severe symptoms. QoL parameters were significantly lower (P<0.001) in the patient group with moderate-to-severe symptoms as compared with the ones who did not have moderate-to-severe symptoms.
Summary / Conclusion: Lymphoma patients in complete remission after ASHCT with the follow-up of at least 1 year experience treatment-related symptoms. In the majority of cases they were of mild severity. The subgroup of patients with moderate-to-severe symptoms was identified; these patients experienced significant QoL impartment. Clinical self-reported symptom assessment in lymphoma patients in complete remission after ASHCT provides an important opportunity to promote proactive management of transplant-related side effects and maintain or improve their QoL.

B1675

BUSULFAN/CYCLOPHOSPHAMIDE VS. BUSULFAN/FLUDARABINE CONDITIONING FOR ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH AML/MDS: A SINGLE-CENTRE RETROSPECTIVE ANALYSIS

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Background: There has been some controversy over whether busulfan/fludarabine (BuFlu) can be a substitute for busulfan/cyclophosphamide (BuCy) conditioning for allogeneic haematopoietic stem cell transplantation (allo-HSCT) in patients with acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS). In many earlier retrospective, nonrandomised studies, BuFlu was less

toxic and resulted in similar or better survival compared with BuCy. In a recent randomised controlled study, however, BuFlu conditioning was associated with inferior survival outcomes and no benefit in non-relapse mortality.
Aims: We compared these two myeloablative conditioning regimens in patients with AML and MDS.
Methods: Patients with AML/MDS who underwent allo-HSCT between 2002 and 2012 at Chungnam National University Hospital were enrolled. Between 2002 and 2006, patients received busulfan (3.2 mg/kg per day for 4 days) plus cyclophosphamide (60 mg/kg per day for 2 days). Between 2007 and 2012, patients received busulfan (same dose and schedule) plus fludarabine (40 mg/m^2 per day for 5 days). Primary end points were survival outcomes and secondary end points were toxicities.
Results: Eighteen and 56 patients received BuCy and BuFlu, respectively. The median age was 40.5 years (range, 19-51) and 46 years (range, 18-63), respectively, and the median follow-up duration was 63 months (range, 1-148) and 21.5 months (range, 4-64), respectively. Primary graft failure was not observed in either group. During the follow-up period, no statistically significant differences in overall survival (OS), relapse-free survival (RFS) or event-free survival (EFS) were observed between the two groups (OS at 5 years, 72.2% *vs.* 77.0%, *P*=0.580; RFS at 5 years, 70.6% *vs.* 81.1%, *P*=0.322; EFS at 5 years, 66.7% *vs.* 68.0%, *P*=0.692). Stem cell source, donor type and HLA matching did not influence OS. Acute graft-versus-host disease (GVHD) was less common in the BuFlu group (27.8% *vs.* 3.6%, *P*=0.002), whereas incidence of chronic GVHD was similar in the two groups (77.8% *vs.* 73.2%, *P*=0.700). Hepatic sinusoidal obstruction syndrome (SOS) was less common in the BuFlu group (16.7% *vs.* 1.8%, P=0.015). Non-relapse mortality (NRM) did not differ between the two groups (NRM at 5 years, 15.0% *vs.* 15.1%; *P*=0.529).
Summary / Conclusion: BuFlu conditioning has similar efficacy compared with BuCy conditioning and is associated with fewer cases of acute GVHD and SOS in patients with AML/MDS.

B1676
NEW PROPOSED GUIDELINES FOR FASTER IDENTIFICATION OF SUCCESSFUL MYELOID AND ERYTHROID ENGRAFTMENT IN HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background: After hematopoietic stem cell transplantation (HSCT), prediction of engraftment relies on recovery of peripheral blood cell counts. Over the past decades, the performance of hematologic analyzers has evolved with improved sensitivity and creation of new cellular parameters, and better resolution of various cell populations in the blood. Since these new parameters are directly correlated to the presence of immature elements in the blood, their main proposed application has been evaluating the level of hematopoiesis. Previous studies have demonstrated earlier increases in immature reticulocyte fraction (IRF) by 48 hours on average prior to days required for ANC to reach the cut-off value of 500/μL in patients with successful engraftment. However, these studies did not address the question of possible false positive results in the cases without engraftment using that specific cutoff value and did not identify lineage specific engraftment.
Aims: The objective of this study is to identify possible improved laboratory guidelines for confirming engraftment post-HSCT and identify lineage specific parameters that could predict myeloid and erythroid engraftments, separately. Another objective of this study is to analyze the effect of various clinical characteristics to the myeloid and erythroid engraftment day.
Methods: We evaluated blood cell parameters including complete blood count (CBC), differential counts, and various reticulocyte parameters every work day in 115 patients who received HSCT (allogeneic, n=93; autologous, n=22) in the purpose of identifying possible improved laboratory guidelines for engraftment prediction. The analyzed reticulocyte parameters were reticulocyte count (%), mean reticulocyte volume (MRV), immature reticulocyte fraction (IRF), high light scattering reticulocyte (HLR), and absolute count of HLR (#HLR). The earliest engraftment guidelines for confirming erythroid and granulocytic lineage specific engraftment separately were searched. Using these guidelines, we analyzed the effect of various clinical characteristics including graft type, conditioning regimen, ABO compatibility and disease types to the myeloid and erythroid engraftment day.
Results: Days to while blood cell (WBC) count over 100 cells/μl with more than 2 fold increase from nadir after transplantation (proposed new WBC guideline) preceded absolute neutrophil count (ANC)>500 cells/μl by 1.7 days. Among erythroid parameters, the earliest marker for erythroid engraftment was High light Scattering Reticulocyte (HLR)>0.1 (proposed new red blood cell guideline), which preceded RET>1% and IRF>0.5 by 3.9 and 1.6 days, respectively. Among the clinical parameters compared, those with statistically significant influence on myeloid engraftment were donor type (P=0.009) and conditioning intensity (P=0.009). As for erythroid recovery, ABO incompatibility was the only significant factor. The erythroid engraftment days after HSCT from ABO major mismatched donor were 20.7±17.1 days and delayed than that from minor mismatched donor (P=0.004) and ABO matched donors (P=0.001). Especially, the erythroid engraftment days from ABO major mismatched donors to blood type O recipients were much more delayed to 45.9±37.8 days. All 2 cases which did not show engraftment after HSCT by conventional parameters did not show engraftment by the new guidelines also.

Table 1. Clinical factors showing significant difference in time of achieving granulocytic and erythroid engraftment by new WBC (>100/ μL plus > 2 fold increase from nadir after D0) and RBC guidelines (HLR>0.1).

Clinical factors	N	Days to achieve new WBC guideline mean ± S.D. (median, range)	ANOVA	Multiple comparison by T-test*	Days to achieve new RBC guideline mean ± S.D. (median, range)	ANOVA	Multiple comparison by T-test
Donor type (n=100)							
Conventional (conv)	61	10.7 ± 1.9(.8-16)	0.009	conv vs. alter 0.870	14.2 ± 10.4(.8-64)	0.564	
Alternative (alter)	27	10.3 ± 1.6(.8-13)		alter vs. auto 0.063	13.7 ± 6.3(.8-34)		
Autologous (auto)	22	9.3 ± 1.7 (3-13)		conv vs. auto 0.007	11.7 ± 2.5(.7-19)		
Conditioning intensity							
MAC	46	10.7 ± 1.7(.8-15)	0.009		14.8 ± 9.5(.8-64)	0.498	
RIC	42	10.5 ± 1.8(.8-16)		RIC vs. Auto 0.034	13.6 ± 9.0(.8-63)		
Auto	22	9.4 ± 1.7(.8-15)		MAC vs. RIC 0.007	11.7 ± 2.5(.7-19)		
ABO incompatibility			0.001			0.001	
Autologous	22	9.4 ± 1.7(.8-15)		match vs. auto 0.001	11.7 ± 2.5(8-34)		major vs. auto 0.003
Minor incompatibility	21	9.9 ± 1.6(.8-13)		match vs. minor 0.034	12.0 ± 4.5(.7-29)		major vs. match 0.005
Major incompatibility	19	10.2 ± 2.3(.8-16)			20.7 ± 17.1(.9-64)		major vs. minor 0.004
Compatible	48	11.1 ± 1.6(.8-15)			12.4 ± 4.0(.7-19)		

Summary / Conclusion: In conclusion, our guidelines may ensure engraftment a few days earlier than the conventional parameters, which may help clinicians for decision-making on rescue therapy earlier.

B1677
THE EFFECT OF SIROLIMUS BASED REGIMEN ON IMMUNE RECONSTITUTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION
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Background: Reconstitution of the immune system following allogeneic stem-cell transplantation (allo-SCT) is a complex process that requires successful engraftment of the hematopoietic stem cell, as well as adequate thymic function. Although GVHD control, improvements in antibiotic spectra, and circumspection in the use of immunosuppressants have helped, too many patients still die of infections because of insufficient immunologic recovery. Sirolimus has been used alone and in combination with calcineurin inhibitors for prevention of allograft rejection after solid organ transplantation. In the field of hematopoietic stem cell transplantation, the combination of sirolimus and tacrolimus has also resulted in a low incidence of acute GVHD and reduced transplant-related toxicity.
Aims: We evaluated the effect of sirolimus and tacrolimus as a acute GVHD prophylaxis on the immune reconstitution after allogeneic stem cell transplantation compared to a historical control.
Methods: This study included 24 patients received the combination of sirolimus and tacrolimus and 21 patients received the combination of tacrolimus and methotrexate (MTX) for historical control. They were received G-CSF mobilized peripheral blood stem cells after myeloablative conditioning with intravenous busulfan and fludarabine.
Results: The incidence of acute GVHD in patients with sirolimus based regimen was lower. And the incidence of CMV or EBV reactivation in the same group was higher. The recovery of CD4$^+$ T cells and natural killer (NK) cells seemed to be more delayed in recipients with sirolimus based regimen compared to those in patients with tacrolimus and MTX at 1 month after transplantation (8.8±1.6 vs 14.9±5.8 for CD4$^+$ T cells, 35.7±6.4 vs 53.4±19.6 for NK cells). However, there was no significant difference between in the recovery of CD4$^+$ T cells and NK cells at 3 months after transplantation. In the aspect of humoral immunity, there was a trend to be lower in immunoglobulin-A and IgM levels during 1 month and 3 months after transplantation in patients with sirolimus based regimen. These differences were overcome around post-transplant 6 months. And regulatory T cells (CD4+CD25+Foxp3+) seemed to be higher in patients with sirolimus based regimen.
Summary / Conclusion: The sirolimus based regimen is associated with well-controlled acute GvHD. Although the correlation between the increased risk of infection and delayed immune reconstitution was not confirmed, the regimen might be cause of increasing the risk of opportunistic infection. Therefore we need an effort of early tapering of immunosuppressants and a careful monitoring for opportunistic infections in patients received sirolimus based regimen.

B1678
HIGH–DOSE THERAPY WITH AUTOLOGOUS TRANSPLANT SUPPORT IN 176 PATIENTS WITH MULTIPLE MYELOMA (MM) OVER 12 YEARS (1.999 – 2.011)
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Background: High dose therapy (HDT) with autologous hematopoietic stem cell (HSC) is still effective treatment of MM in young pts under 65 years of age. On 12 years period (January 1999 to December 2011), 176 pts with MM underwent HDT with autologous HSC that represent 39% of all autologous transplantation (176/449) in our center during this period.
Aims: This retrospective study analyzes the results of 176 pts.
Methods: It includes 88 males and 88 females (sex-ratio: 1); median age 52 years (24-65). Durie and Salmon stage III is observed in 155 pts (88%) with stage IIIB in 27 pts (15%), one pt with plasma cell leukemia; the distribution of monoclonal protein type IgG 105 pts (88%), IgA 38 pts (21,5%) light chain 23 pts (13%), no secretion 6 pts (3,5%), IgD 2 pts and no determined 2 pts. Initial standard therapy include 4 to 6 cycles of VAD in 152 pts; association Thalidomide–Dexamethasone (TD) in 11 pts; CTD in 6 pts, Velcade –Thalidomide 6 pts. Complete remission (CR) was observed in 67 pts (38%), partial response in 74 pts (42%) and refractory disease in 35 pts (20%). Two HDT regimen was used: MEL200 protocol (Melphalan 200 mg/m²) for 125 pts (71%) and BU-MEL (Busulfan 12mg/kg, Melphalan 140mg/m²) for 51 pts (29%). Peripheral HSC was collected by cytapheresis after mobilization by G-CSF alone, median CD34 + cells infused was4, 22 x 10^6/kg (0, 65 – 19). Median follow up is 42 months (6 -153)
Results: Early deaths accured in 9 pts (5%) about whom 7 by TRM. Within 167 appraisable pts, 132 pts (79%) observed CR, 31 pts (18,5%) a good response and 4 pts (2,5%) still on refractory disease after graft. HDT with autologous transplant improve CR rate from 38% to 79%. Also refractory disease rate decrease from 20% to2,5%. Progression disease observed in 121 pts (73%) during follow up with 74 pts (61%) at first 24 months after graft and the same frequency is noted with the two protocol regimen of HDT (MEL 200 and BU-MEL): 68,5% and 61% respectively. 81 pts (48,5%) are alive : 46 pts (27,5%) with persistent response and 35 pts (21%) with relapse; 86 pts (51%) died, of progression disease for 82 pts and other reason for 4 pts. The estimated overall survival is 46% at 5 years and 22, 5% at 12 years with a median survival at 58 months.
Summary / Conclusion: Our results are comparable with others studies who treat with VAD and found median survival at 55 months.

B1679
SEQUENTIAL INTENSIFIED CONDITIONING FOLLOWED BY EARLY TAPERING IMMUNOSUPPRESSANTS AND DONOR LYMPHOCYTE INFUSION IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR REFRACTORY/RECURRENT LYMPHOBLA
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Background: Adult T lymphoblastic lymphoma is an aggressive form of non-Hodgkin lymphoma occurring in predominantly adolescent and young adult men. High-dose chemotherapy followed by autologous or allogeneic hematopoietic stem cell transplantation (allo-HSCT) as consolidation for patients in first complete remission is controversial. Refractory/recurrent T lymphoblastic lymphoma has poor response to conventional chemotherapy.
Aims: To prospectively evaluate the efficacy of sequential intensified conditioning followed by early tapering immunosuppressants and donor lymphocyte infusion (DLI) in allo-HSCT as salvage therapy for refractory/recurrent T lymphoblastic lymphoma.
Methods: Fourteen patients with refractory/recurrent T lymphoblastic lymphoma (male in 12 cases and female in 2 cases) from September 2010 to December 2012 were enrolled in this prospective study, with median age 33 (range 16-42) years. Twelve patients received related and 2 unrelated donor transplantation, and 13 patients received HLA-matched and 1 HLA-mismatched transplantation. The sequential intensified conditioning included fludarabine 30mg/m^2/d and cytarabine 2g/ m^2/d (on days -10 to -6),4.5 Gy total body irradiation/day (on days -5 and -4), cyclophosphamide 60 mg/kg/d and etoposide 10mg/ kg/d (on days -3 and -2). Cyclosporine A was rapidly withdrawn in a stepwise fashion if acute graft-versus-host disease (aGVHD) did not develop at day +30. Granulocyte colony-stimulating factor (G-CSF) mobilized donor lymphocytes (absolute neutrophil count: 1.0×10^6/kg, once a month, 4 doses totally) would be infused in patients without II° or more than II° aGVHD by day + 60 post-transplantation. Once patients developed GVHD after DLI, DLI would stop and methylprednisolone was added to the regimen.
Results: All patients achieved complete remission by day +30,and none of patients died of regimen-related toxicities. With a median follow-up of 26 months post-transplantation (range: 2-29 months), 3 patients relapsed and one died of relapse. Till now, 13 patients were alive and one died. The 2-year cumulative overall survival and disease-free survival post-transplantation were 65% and 51%, respectively. The 2-year cumulative incidence of relapse was 14%.
Summary / Conclusion: Our data indicate that sequential intensified conditioning and rapid tapering immunosuppressants as well as DLI in the early stage after allo-HSCT might be an effective method for refractory/recurrent T lymphoblastic lymphoma.

B1680
MYELOABLATIVE OR NON-MYELOABLATIVE? TIME TO ENGRAFTMENT UNYIELDING
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Background: It is commonly felt that non-myelablative conditioning before stem cell transplantation leads to less profound myelosupression and faster engraftment than it is observed after classical myeloablative conditioning. Published results indicated faster engraftment after non-myeloablative conditioning in some selected cohorts of patients.
Aims: We performed a retrospective study to compare engraftment times in unselected patients undergoing allogeneic stem cell transplantation after myeloablative and non-myeloablative conditioning. The question was if type of conditioning determines time to engraftment in clinical practice.
Methods: We retrospectively studied group of 76 patients transplanted in the period from 2009 to 2012 in our institution. 45 patients received myeloablative (MAC) and 31 non-myeloablative (nMAC) conditioning. All but 1 transplantation were performed using peripheral blood as stem cell source. Patients receiving myeloablative reduced intensity conditioning (eg. Fludarabine/Busulfan 12.8 mg/kg or Fludarabine/Treosulfan) were not included the study. Conditioning regimen most frequently used in MAC was Busulfan 12.8 mg/kg with cyclophosphamide 120 mg/kg. In nMAC two most common regimens were Fludarabine 150 mg/m^2 plus Busulfan 6.4 mg/kg or Fludarabine 150 mg/m2 with Melfalan 140 mg/m^2. All patients undergoing transplantations from unrelated donors received antilymphocyte globulin at total dose of 7.5 mg/kg. Prevailing graft versus host disease prophylaxis regimen in both groups consisted of cyclosporine and short course methotrexate. Median age was 35.1 years for MAC and 48.7 years for nMAC. The MAC and nMAC groups were identical in terms of the number CD34+ cells transplanted. They were also comparable in proportion of unrelated donors, stage of the disease at transplantation (early vs. late) and proportion of patients receiving G-CSF after transplantation.
Results: Time to neutrophil engraftment was 18 (17.8) days in MAC and 17 (16.9) days in nMAC (P=0.39). Platelet engraftment was observed on day 13 in MAC and on day 14 in nMAC group (P=0.82). The number of days with leucopenia < 1.5 G/L was 16.3 days in MAC and 16.6 days in nMAC (P=0.82). Age of the patients in the whole population as well as in MAC and nMAC groups had no influence on time to neutrophil and platelet engraftment or duration of leucopenia.
Summary / Conclusion: Our data show identical time to engraftment after myeloablative and non-myeloablative conditioning in patients receiving hematopoietic stem cell transplantation for hematologic malignancies. In clinical setting type of conditioning does not influence engraftment time.

B1681
OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HCT) FOR PATIENTS WITH ACUTE LYMPHOID LEUKEMIA (ALL) WITH ACTIVE DISEASE: EXPERIENCE OF A SINGLE EUROPEAN INSTITUTION
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Background: In primary refractory or advanced relapsed ALL, allogeneic-HCT is associated with three-year leukemia-free survival rates of 12 to 23 %.This is clearly superior to chemotherapy alone, which achieves no cures at this stage of the disease. Treatment options for these patients (pts) are limited and a HCT may provide a realistic chance for long term disease remission.
Aims: We report the outcome of ALL pts with active disease treated with Allogeneic-HCT.
Methods: A retrospective cohort from including clinical registries' databases for pts with diagnosis of relapsed/refractory ALL, with 31 fulfilled inclusion criteria pts from 1990 to 2011. Outcomes of interest were transplant-related mortality (TRM), incidence of acute and chronic graft-versus-host disease (GVHD), incidence of relapse and overall survival (OS) - which were estimated from the date of transplant. Parametric and non-parametric tests were used appropriately. Kaplan Meir curve was used for OS assessment. Statistical analysis was carried out using SPSS v19.0 for Mac (IBM, 2010, USA) and statistical significance was considered for P<0.05.
Results: The median age was 17 (range 2 – 48) years. Median follow up for alive pts was 6 months (08 pts; range 0.373 – 11.627). The cumulative incidence of 90-days, 1 year, and 3 years TRM was 75%, 12.5%, and 12.5%, respectively (P<0.059). 39.4% of patients developed grade II-IV acute GVHD and 23.3 % suffered from chronic GVHD. The actuarial incidence of relapse was 68.4% at 1 year and 21.1% at 3 years and 10.5% at 5 years. Actuarial OS was

estimated to be 63.6 % at 1 year, 21.2% at 3 years, and 15.2% at 5 years (P<0.001).
Summary / Conclusion: In summary, TRM and acute GVHD in first years after HCT remains considerable, but HCT in this setting seems to be a good choice for ALL pts with active disease. Indeed, actuarial OS 3 years post HCT in our cohort seems to encourage this approach in this setting.

B1682
EFFICACY OF HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION (AUTO-HSCT) IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA (PMLBCL)
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Background: Primary mediastinal large B-cell lymphoma (PMLBCL) is a unique type of B-cell lymphoma the WHO classification of Lymphoid Malignancies. It constitutes 6–10% of all diffuse large B-cell lymphomas (DLBCL), occurring more often in young females. PMLBCL has been postulated arisefrom a putative thymus medulla B-cell. PMLBCL is characterized by a locally invasive anterior mediastinal mass, often producing cough, chest pain, dyspnea, and superior vena cava syndrome. Most PMLBCL patients have stage I–II, bulky disease, with pleural or pericardial effusions in third of cases. In the absence of prospective studies, the optimal treatment of PMBCL is still a matter of debate. Uncertainties exist with regard to the intensity of primary chemotherapy, the role of consolidating involved-field radiotherapy, high-dose chemotherapy and the impact of an additional treatment with rituximab.
Aims: To evaluate the role of an early treatment intensification (induction CHT, autoHSCT and RT) in patients with primary PMLBCL presenting with adverse prognostic factors.
Methods: We analyzed outcomes of first-line chemotherapy, including first-line CHT with Rituximab (R-MACOP-B) and auto-HSCT in patients with PMLBCL. 38 patients with PMLBCL (13 m. and 25 f.), admitted to Hertcen Oncology Center from 2006 to 2012 are included in this study. The average age of PMLBCL patients was 34,3 years (20-64, median 37). In all cases the diagnosis was confirmed by immunomorphology. Samples were obtained by mediastinotomia (N15), percutaneous needle biopsy (N12), thoracotomy (N6) or neck/supraclavicular lymph node biopsy (N3), partial or total excision of mediastinal tumor (N2). At the time of diagnosis 32 patients had I-II stage disease, 5 - had stage III and 1 patient had VI stage disease according to Ann Arbor staging system.Local infiltration of adjacent tissues and organs was defined in the majority of patients (47%>lungs, 58%>pleura, 22%>thoracal wall), 44% presented hydrothorax and/or hydropericard. Superior vena cava syndrome observed in 69% of patients, thrombosis in 64%. Bone marrow involvement was not observed. Elevated LDH levels were found in 42% of patients. First-line chemotherapy including Rituximab received 27 patients (R-CHOP-1, R-CHOEP-1, R-MACOP-B-25), treatment without Rituximab (MACOP-B) – 9 patients. Patients with partial remission (N19) received radiotherapy on residual tumor. HDCT followed by autoHSCT was performed in 14 patients with PMLBCL to consolidate the first partial/complete remission. The source of hematopoietic progenitors was peripheral blood in all patients. The number of CD34+ cells in the autograft averaged 17.5x10^6/kg (4.3-52.1x10^6/kg). Pretransplantation conditioning was carried out according to BEAM protocol. After HDCT followed by autoHSCT 10 PMLBCL patients received radiation therapy on residual disease.
Results: Currently 32/38 (84,2%) patients with PMLBCL remain in first prolonged complete/partial remission. Average time of observation is 42,3 months (from 3 to 78). One early and two late relapses occurred after HDCT followed by autoHSCT. One patient died from concurrent disease in partial remission during radiotherapy. One patient had refractory disease.
Summary / Conclusion: The optimal treatment of PMBCL is still under investigation. Intensive CHT regimens combined with RT and treatment with Rituximab could possibly hold over the use of HDCT with autoHSCT in the low risk patients. In the high-risk group of patients, achieving partial remission, early intensification treatment including HDCT and autoHSCT could result in higher frequencies of complete remissions and improve patients survival. In patients with refractory disease autoHSCT is not considered to be sufficiently effective.

B1683
DOES SIZE MATTER? RESULTS OF ALLOGENEIC TRANSPLANTS AT A SMALL NEW ZEALAND CENTRE.
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Background: Reduced intensity conditioning (RIC) protocols for allogeneic haematopoietic stem cell transplants have become commonplace treatments for patients with haematological disease during the last fifteen years, extending allogeneic transplant to older or less fit patients. Outcomes in patients with different diseases and with different schedules vary. Doctors and patients want to know what they can expect in the centre where treatment is undertaken and that outcomes are equivalent to published results, especially if transplant activity is at the minimum level required by FACT.
Aims: We aimed to examine our total allogeneic transplant activity in adults and outcomes of that activity since RIC protocols were first introduced in our centre.
Methods: Clinical and Laboratory data were collected on all adult patients who received allogeneic SCT at Christchurch Hospital during 2000 – 20012. A programme of tandem auto/RIC allo transplant for patients with myeloma in first response who had sibling donors was active during this period. Outcomes including overall survival (OS) and time to treatment failure due to relapse or death in remission were compared between patients receiving myeloablative conditioning (MAC) and RIC protocols.
Results: Over 12 years between 4/2000 and 5/2012, 118 adult patients had allografts, 55 with MAC, 63 with RIC. The annual allograft rate was 1 per 100,000 of the population. During the same period 296 patients received autografts. The mean age of patients receiving MAC (35 years, range 18.2-55.8) was lower than that of those receiving RIC (49.8 years, range18.5-63.6). More MAC patients had acute leukaemia than RIC patients (71% v 23%) and fewer had myeloma (5% v 37%). 25% of patients in both groups had unrelated donors. Fewer MAC patients received peripheral blood stem cells rather than bone marrow stem cells (56% v 92%). MAC and RIC patients had similar comorbidity scores whereas EBMT score was 1 – 2 points higher for RIC patients. There was no difference between the two groups in the incidence of graft versus host disease requiring systemic treatment. 5yr OS and 5yr failure free survival was greater for MAC patients than for RIC patients (71% v 48%, P=0.02; 67% v 32% P=<0.01). Relapse rates were reduced in MAC patients, whereas there was no difference in the rate of death in remission between the groups. Within each conditioning group, OS was poorer in patients older than the median age for that group. OS was reduced in those with higher EBMT scores and there was a trend for poorer OS with increasing HSCT comorbidity index. OS was unaffected by donor source or patient/donor CMV status. The sub group of older MAC patients had the same mean age as the sub group of younger RIC patients and had the same outcome.
Summary / Conclusion: Allograft activity is lower in our centre compared with Australasia generally but meets the FACT activity level standard. RIC protocols have been followed by long term survival in many patients ineligible for MAC protocols. Results at our centre are comparable with international outcomes. Our patient outcomes correlate with their age, rather than with their conditioning protocol.

B1684
MESENCHYMAL STEM CELLS TRANSFUSION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: RESULT OF A SINGLE CENTER
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Background: Patients may present with different kinds of refractory complications after allogeneic hematopoietic stem cell transplantation (allo-HSCT), including graft-versus-host disease(GVHD), poor graft failure(PGF) , bronchiolitis obliterans(BO) and so on. In recent years, mesenchymal stem cells(MSCs) transfusion becomes a potential treatment for allo-HSCT patients.
Aims: The aim of this work is to analyse the outcome of patients treated in our institution with MSCs after allo-HSCT .
Methods: Eight patients were included in this study and median age was 25 years(range 14-44). Two patients were diagnosed of severe aplastic anaemia(SAA) and the remaining were haematological neoplasias(4 acute leukemias, 1 lymphoma and 1 myelodysplatic syndrome). Patients with haematological neoplasia were in the state of complete remission (CR) when HSCT was carried out and all the 8 patients received graft from HLA-identical relative donors.The indication for MSCs use was refractory acute GVHD-1, refractory extense chronic GVHD-4, PGF-2 and BD-1. Patients received bone marrow derived MSCs from a third party donor at a dose of 1×106/kg for 2 to 3 cycles, with an interval of 28 to 30 days.

Table1.

complication	complete response	patial response	no response
acut GVHD n=1	0	1*	0
chronic GVHD n=4	0	4**	0
PGF n=2	2	0	0
BD n=1	1	0	0
Total n=8	3	5	0

*This patient relapsed and died.
** Two out of patients reached PR of cutaneous and oral GVHD but no response of ocular GVHD. The patient with lymphoma developed PTLD.

Results: A total of 17 infusions have been performed and no infusion-related adverse reaction was found. Patients with cutaneous, astro-intestinal, liver and oral GVHD showed partial response, but for ocular GVHD, no response was displayed. Patients with PGF were responsive to the first infusion of MSCs within one week and their blood routine turned to normal 3 months after the second. Patient with BD obtained complete response after 2 cycles of MSCs and she didn't need to depend on oxygenotherapy any more. In the median follow-up of 19 months(3-30months), one patient with acute leukemia developed disease relapse and died, and the patient with lymphoma developed posttransplant lymphoproliferative disorders(PTLD) and gained CR after 4 cycles of rituximab Table1.
Summary / Conclusion: Administration of MSCs was safe for treatment of severe complications after allo-HSCT. MSCs may be a promising therapy for refractory cutaneous , astro-intestinal, liver and oral GVHD, PGF and BD, but ocular GVHD.

B1685

POOR PREDICTION OF POST-INFUSION HEMOGLOBIN RISE IN PEDIATRIC RECIPIENTS OF UNMANIPULATED BONE MARROW HARVEST

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Background: Hemopoietic stem cell transplantation is a commonly undertaken and potentially curative procedure for many children with underlying benign and malignant disorders. Matched sibling donors are preferred but when this option is unavailable a matched unrelated donor is commonly selected from adult bone marrow registries. In children, bone marrow harvest rather than peripheral stem cell collection is often favored, as there is a lower risk of graft versus host disease. The combination of a larger donor and bone marrow as the source of stem cells results in a relatively high cell dose with an excess of red cells, particularly in younger children. Provided there is no recipient-donor major ABO mismatch, these units are often infused un-manipulated, and the recipient risks iatrogenic polycythemia with potentially deleterious consequences. As a result, we use the formula outlined below to predict the increment in hemoglobin concentration based on the recipient's weight and the quantity of red cells in the product. If the predicted Hb rise is >4g/dL red-cell reduction is undertaken pre-infusion. To date, this formula has not been validated in the literature.
Aims: Our aim was to compare the predicted rise in hemoglobin concentration with the actual value measured in recipients in order to ascertain the robustness or otherwise of the formula.
Methods: Patients' age, gender, weight, baseline hemoglobin and bilirubin were recorded, along with marrow volume and hematocrit and the predicted hemoglobin increment calculated according to the following formula: Predicted Hb rise = (Marrow Vol [mls]/Recipient Wt [Kg] x 3) x (Marrow Hct/0.6). We then measured post-infusion hemoglobin at 24 and 48 hours, and compared this to the predicted increment. A post-infusion bilirubin was also checked to exclude hemolysis as an explanation for any discrepancy. The predicted versus actual increment was statistically compared using the independent t-test. 42 patients were included in the study, 29 males and 13 females. The patients were aged from 4 months to 18 years old (median 7.5 years). The patients weighed from 5.8kg to 82.0kg (median 25.9kg). All patients received un-manipulated bone marrow.
Results: The average pre- and post-infusion hemoglobin were 9.6 g/dL and 11.5 g/dL respectively. The average measured increment in hemoglobin concentration was 1.95 g/dL. The average predicted increment in hemoglobin concentration was 2.89 g/dL, a difference approaching 1 g/dL. No patients had clinically significant rises in serum bilirubin to explain a discrepancy between predicted and measured hemoglobin increments. No statistically significant correlation was demonstrated between observed and predicted Hb increment.
Summary / Conclusion: In pediatric recipients of bone marrow harvest from allogeneic donors, the above formula does not accurately predict for post-infusion hemoglobin concentration and may lead to unnecessary red-cell depletion pre-infusion. Further analysis is required to modify this formula to improve its predictive value.

B1686

THE EFFECT OF NUMBER OF APHERESIS SESSIONS REQUIRED TO ACHIEVE STEM CELL DOSE ON EARLY AND LONG TERM ENGRAFTMENT IN AUTOLOGOUS STEM CELL TRANSPLANT FOR LYMPHOMA

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Background: The dose of CD34+ peripheral blood stem cells (PBSCs) infused during autologous stem cell transplantation (ASCT) correlates with time to engraftment. The number of apheresis sessions required to achieve a minimum cell dose varies between patients, affected by factors such as peripheral blood CD34 count, previous lines of chemotherapy, therapy with nucleoside analogues and radiation. It is unknown whether engraftment differs among patients whose stem cells have been difficult to mobilize, and require multiple days to collect.
Aims: To examine the effect of the number of apheresis sessions required to achieve a stem cell dose for reinfusion, on short-term and long-term engraftment in ASCT
Methods: The ASCT database at Princess Margaret Hospital was interrogated to identify lymphoma patients with stem cell collections between 2007-2011. Data were collected retrospectively on patient characteristics, cell dose, number of days required for stem cell collection, days to engraftment and blood counts at day 100 and 1 year post-transplant. Patients were excluded if they did not proceed to or relapsed within 1 year of ASCT or did not have cell counts available at PMH at follow-up.
Results: 143 patients were included with relapsed/refractory Hodgkin Lymphoma (n=54), aggressive B and T cell lymphomas (n=60), and Mantle Cell Lymphoma (MCL) in first remission (n=29). 71% of patients received 2 prior lines of therapy and 21% received radiotherapy prior to stem-cell collection. Intensive therapy for relapsed HL/NHL included high dose etoposide and melphalan; patients with MCL age <61 years also received total body irradiation (TBI). An adequate PBSC collection needed to proceed to ASCT was >2 x 10^6 CD34 cells/kg: 67% of patients required 1 apheresis to collect sufficient cells. Median peripheral blood CD34 count prior to collection was 85 CD34 cells/uL (range 4-1112). Median PBSC dose infused was 7.05x10^6 CD34 cells/kg (range 2.56-30.22). Median number of days to engraftment of neutrophils (>0.5x10^6/uL) and platelets (>20x10^6/uL unsupported by transfusion) was 11 and 12 days respectively. At day 100, proportion of patients with haemoglobin, neutrophils and platelets below normal were 67%, 29%, 36% respectively. At 1 year, patients with low haemoglobin, neutrophils and platelets were 15%, 20% and 34% respectively. Univariable analysis demonstrates PBSC dose, peripheral blood CD34 count, age and number of aphereses significantly influenced early neutrophil and platelet engraftment, and most counts at Day 100 and 1 year. Number of prior lines of therapy influenced only platelet engraftment, but not later counts. Peripheral CD34 counts strongly correlated with stem cell dose collected. The addition of TBI did not influence early or late engraftment. On multivariable analysis PBSC dose significantly influenced early neutrophil and platelet engraftment as well as haemoglobin and platelet counts at day 100, but only neutrophil count 1 year post-ASCT. Number of aphereses to obtain adequate PBSCs did not affect engraftment time or blood count recovery at any of the later time points evaluated. Time to early neutrophil and platelet engraftment predicted counts at day 100, which in turn predicted counts 1 year post-ASCT.
Summary / Conclusion: We conclude stem cell dose is the most important factor influencing early and late blood counts post-ASCT regardless of the number apheresis required to achieve this. Neutrophil and platelet counts demonstrate a temporal relationship with early engraftment predicting higher counts at Day 100, and in turn 1 year.

B1687

HEALTH-RELATED QUALITY OF LIFE AMONG EGYPTIAN CHILDREN RECEIVING AUTOLOGOUS STEM CELL TRANSPLANTATION.

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Background: Stem cell transplantation (SCT) is an aggressive medical procedure associated with high mortality and morbidity rates. It is the best line of therapy after disease relapse or failure of the conventional treatment. It may add a severe stress to the patient with profound disruption for the entire family. Thus, it places each family member at risk for psychological maladjustment and decrease in quality of life.
Aims: The aim of this study was to describe the quality of life and behavioral adjustment of receivers of autologous stem cell transplantation and their family members before the procedure and six months after transplantation.
Methods: It compromised 27 children with age range of 4-18 years (19 male and 8 female). Sixteen patients had Hodgkin's lymphoma, 5 patients had Non-Hodgkin lymphoma, 2 patients had acute myeloid leukemia, one patient with Wilm's tumor and another one with metastatic neuroblastoma.
We used peds QL cancer module-child report version 3 and 4 for assessment of health related quality of life (HRQOL) of children and QOL scale-family for assessment of HRQOL of the parents.
Results: Many children and adolescent have been found to exhibit decline in social competence and self-concept or to experience social isolation and other emotional difficulties that require psychological intervention.
Symptoms of psychological distress usually appear immediately after SCT and increase reaching the peak during the period of hospitalization and then improve gradually. Stem cell recipients showed variable degree of life satisfaction after stem cell transplantation, while their parents appeared more anxious and stressed after the procedure.
Summary / Conclusion: Assessment of Quality of life is an important indicator to the patient's psychosocial aspects after the procedure.

B1688

PRE-EMPTIVE TREATMENT OF CYTOMEGALOVIRUS INFECTION IN PEDIATRIC PATIENTS WHO UNDERWENT HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Cytomegalovirus (CMV) remains a serious problem after hematopoietic stem cell transplantation (HSCT). It's associated with significant morbidity and mortality if not treated promptly.
Aims: To investigate the incidence of CMV infection and outcome we retrospectively analyzed 79 pediatric patients received HSCT monitored by CMV polymerase chain reaction (PCR) and CMV pp65 antigenemia. The influence of recipient and donor CMV serostatus on the risk for development of CMV disease was studied. The characteristics of patients with CMV infection and their outcome were described.
Methods: All consecutive patients, who underwent miyeloablative allogeneic (n=60) or otolog (n=19) stem cell transplantation were included in this analysis. Data were available on demographic characteristics, underlying diseases, donor and recipient CMV serostatus, occurrence of GVHD and treatment (i.e. initiation, duration, type and dosage of drugs used) and the ganciclovir formulation (i.e. valganciclovir or ganciclovir), CMV DNA load, CMV pp65 antigenemia measurements and general laboratory parameters. Patients were routinely screened 1 or 2 times a week using CMV pp65 antigenemia and quantitative CMV-PCR for CMV DNA. CMV monitoring begins on day -9 pre HSCT until day 120 and thereafter at any time point when the patients attended the outpatient clinic until immunosuppression was discontinued. Patients with 2 positive results for CMV DNAemia received induction therapy using ganciclovir at a dose of 5 mg/kg twice daily for 7–14 days and continued until 2 consecutive negative results were obtained. If antigenemia decreased or converted to negative, maintenance therapy with ganciclovir was given at 5 mg/kg/day for another seven days. If antigenemia still remained positive after 7–14 days, ganciclovir was continued until the antigenemia test became negative. Acyclovir prophylaxis was withheld during ganciclovir treatment. If the patient must continue the therapy at home then the therapy was switched to valganciclovir.
Results: A total of 79 patients (47 male / 32 female) at risk of CMV reactivation were analyzed. The median age was 107 months (range= 9-241 months). Patients had acute leukemia (n=29), chronic myelogenous leukemia (CML) (n=7), solid tumor (n=13), haemoglobinopathy (n=11), primary immune deficiencies (n=2), hystiocytic disorders (n=6), metabolic disorders (n=2), aplastic anaemia (n=9). The median age of patients at the time of transplant was 107 months (range= 9-241 months). All allogeneic transplant patients received grafts from a matched sibling donor. The source of stem cell was peripheral blood cells in 14 cases, bone marrow in 64 cases. All stem cell products were infused without T-cell depletion. The median follow-up time was 32 months (min 1- max 80) post transplant. Graft-versus-host disease was positive in 26 patients (acute:20, chronic:2, acute+chronic:4). 96% of patients were CMV seropositive before transplantation (recipient or donor seropositive); in 3 cases, both the recipient and the donor were CMV seronegative. CMV infection was detected in 9 patients (11.5%). Both CMV antigenemia and PCR were positive in 9 patients; in 2 patients a positive CMV PCR together with negative antigen assay was found. The median time for CMV reactivation was 45 days (min 30 – max 162) post HSCT. The median value of pp65 antigenemia and CMV copies on start of GCV treatment was $10/2x10^5$ PBLs (range:2-98/$2x10^5$ PBLs) and 1650 copies/10^6 PBLs(range: 619-18700 copies/10^6 PBLs). All patients obtained a complete clearance of antigenemia and/or CMV DNA. None of them developed CMV disease. The median time to the first CMV antigenemia and DNA negativity was 22 and 21 days. CMV infection relapsed in 2 patients. One of these patients (hyperimmunglobulin M syndrome +chronic GVHD) relapsed 74 days after the completion of the treatment and succumbed to bronchiolitis obliterans and multiorgan failure. The second patient (CML+chronic GVHD) relapsed 150 days after the completion of the treatment and succumbed to cryptococcus meningitis.
Summary / Conclusion: CMV reactivation and disease remains a major problem in high-risk patients undergoing allogeneic HSCT. Prophylactic acyclovir and pre-emptive ganciclovir treatments to prevent CMV disease are effective in children. Valganciclovir (VGC) has increasingly been used as prophylaxis against CMV infection after solid organ transplantation, but it's efficacy in pediatric hematopoietic stem cell transplant patients has been studied poorly. Novel prophylactic measures such as immunotherapy and valganciclovir prophylaxis need to be considered in this specific group of patients especially to prevent prolonged hospitalization.

B1689

REAL-LIFE CML PATIENTS REFERRED TO STEM-CELL TRANSPLANTATION IN TKI ERA: A SINGLE-CENTRE EXPERIENCE.

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Background: Tyrosine Kinase Inhibitors(TKIs) are first-line standard treatment for adult patients with Chronic Myeloid Leukemia (CML). Allogeneic Stem Cell Transplantation (alloSCT) is indicated for patients (pts) who do not tolerate or develop resistance to TKIs. Outcome of alloSCT in active Blastic Phase (BP) of CML remains, however, still poor.
Aims: In this retrospective study we intend to evaluate the outcome of alloSCT in a monocentric consecutive series of pts referred to alloSCT for CML in chronic or more advanced phases.
Methods: Between 2005 and 2012, 2 CML patients out of 46 patients followed in our Institution under TKI and 8 patients referred from other centers received alloSCT: 8 were in BP, 1 in chronic phase (CP) refractory to imatinib,1 in second CP after dasatinib. All patients had received TKIs treatment before transplant±associated to chemoterapy. Seven out of 8 patients in BP received allotransplant in presence of active disease; 1/8 achieved a complete hematologic response (CHR) before alloSCT. Donor sources were: 1 HLA identical sibling, 2 matched unrelated donor (MUD), 6 mismatched related donor (Haplo), 1 double Unit Cord Blood (CB). All patients received treosulphan based myeloablative conditioning. Graft versus Host Disease(GvHD) phrophylaxis administered was Rapamycin/Mycophenolate in 7 patients, Cyclosporin (CSA) in 1 patient, CSA/Methotrexate in 1 patient and Rapamycin/Cyclophosphamide in 1 patient. Table 1 summarizes pts and transplant characteristics at transplant.
Results: Median follow up of our pts is 7 months (range 2 – 45 months). Eight pts were transplanted in active BP. Four of them died for transplant-related complications (2 GvHD, 2 infections). Two of these 8 pts are alive at last follow up (1 in Complete Molecular Response (CMR) after 12 months from transplantation, 1 in relapse after 20 months).Two other pts were lost at follow-up, disease status at the last visit (5 and 9 months after transplantation) was CHR and CMR respectively. Notably, at day +30, 6/8 pts transplanted in BP 6 achieved disease remission: 3 reached CMR, 1 showed a Major Molecular Response (MMR), 1 obtained a Complete Cytogenetic Response (CCyR) and 1 had a CHR, that became a MMR at day+60.
Both patients that underwent alloSCT while in CP are still alive and in CMR.
Donor Lymphocyte Infusion (DLI) and TKI combination treatment (in one case systemic and intrathecal chemotherapy was associated) was used as a strategy to treat 2 cases of relapse after aSCT and 1 case of refractory disease. No clinically signs of GvHD and no increase of TKIs toxicity were reported. All three patients responded achieving: 2 CMR (3 months after and 2 months after) and 1 MMR (2 months after). One of these three pts experienced a recurrency 6 months after.**Summary / Conclusion:** AlloSCT can rescue a minority of pts with active BP-CML. A combination of immunotherapy and TKIs administration need more investigation strategy after alloSCT.

B1690

THE LEVELS OF TOTAL AND VIABLE CD34+ STEM CELLS DO NOT DECREASE STATISTICALLY WHEN THEY ARE CRYOPRESERVED AT -80OC AFTER OVERNIGHT STORAGE AT REFRIGERATOR

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Background: Although peripheral blood stem cells (PBSC) which are cryopreserved at -80ºC after overnight storage at refrigerator are used for stem cell transplantation, the efficacy of this method is not searched in prospective studies.
Aims: In this study we tried to identify the levels of viable and total CD34+stem cells and the effect of this on stem cell transplantation.
Methods: 43 patients (median age: 52, female/male: 20/23) with 80 PBSC products were analyzed prospectively. Total and viable CD34+ stem cells levels of the products were determined immediately after leukapheresis (group 1), after overnight storage at refrigerator (group 2) and post-thaw periods (group 3). The levels were analyzed by flowcytometry method (BD FACSAria cell sorter, BD Biosiences). We tried to determine the effect of the results on neutrophil and thrombocyte engraftments after stem cell transplantation.
Results: The levels of total CD34+ cells were not statistically different (P<0,5) between the group 1 ((664.5 [20.76 – 8420] /µL), group 2 (614.5 [21 – 7982] /µL) and group 3 (582.3 [17.04 – 4062] /µL). Also we did not find any statistical difference (p:0,08) between the groups when we compared the viable CD34+ cell levels (group 1:632 [20.12 – 8420] /µL; group2: 602.5 [21.80 – 7978] /µL; group 3: 526.8 [4.1 – 3880 /µL). Reverse relation was documented between neutrophil (12 days [9 – 19]) and thrombocyte (12days [0– 35]) engraftment periods and group 1(r=-0.41, P=0.02; r=-0.36, P=0.04,respectively) and group 3 (r=-0.51, P=0.005; r=-0.41, P=0.02, respectively) viable CD34 + cell levels but not group 2.
Summary / Conclusion: Since we did not find any statistical difference between total/viable CD 34+ stem cells and groups, freezing directly at -80ºC

after overnight storage at refrigerator method could be used safely and effectively. Group 1 and group 3 viable CD34+ stem cells levels should be evaluated carefully before stem cell transplantation because we can predict any engraftment delays by these results.

B1691

THE EXAMINATION OF CELL CHIMERISM AS A NONSPECIFIC MARKER IN POST-TRANSPLANT PERIOD BY MOLECULAR GENETICS METHODS

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Background: Allogeneic bone marrow or peripheral blood stem cell transplantation (allo-HSCT) is an important medical procedure in the treatment of malignant and nonmalignant disorders in children. After the transplantation it leads in the recipient to the development of chimerism (cells from genetically different individuals coexist in one body). The detection of only donor´s genotype in blood cells is called the complete chimerism (CC). The coexistence of recipient's and donor's haematopoiesis in different proportions (mixed chimerism – MC) augments the risk of relapse.
Aims: The early detection of mixed chimerism or microchimerism (less than 1% of recipient´s genotype) is important for individual approach to the patient care. The ability to detect early the recurrence of recipient's haematopoiesis followed by disease depends on the sensitivity of the used method.
Methods: The most of the samples are quantitatively determined by the short tandem repeat polymerase chain reaction (STR-PCR). Currently, sex-specific loci and a method based on SNP (Single Nucleotide Polymorphism) or short insertion and deletion (indel) are also used; less frequently the length polymorphisms of VNTR type (Variable Number of Tandem Repeats) are determined. The sensitivity of the used methods is the following: VNTR 1-5%, STR 1% and SNP or indels 0.01%.We have regularly received the samples of pediatric patients after allo-HSCT from The University Hospital Motol since 1992. Informed consents were obtained from all patients, or from their legal guardian.
Results: The informativity was determined in 356 children´s patients indicated for allo-HSCT during the years 1992-2012, including 112 cases of acute lymphoblastic leukemia (ALL), 49 cases of acute myeloid leukemia (AML), 58 cases of aplastic anemia, 26 cases of chronic myeloid leukemia (CML), 40 cases of immunodeficiency, 41 cases of myelodysplastic syndromes (MDS) and 30 cases of other diagnoses. It was also investigated 23 cases of maternal engraftment and 2 detections of twins zygocy. In total, 67% of transplantations were provided from unrelated donors and 33% from related donors. Patients achieved CC in a median of 21 (range, 7-543) days after allo-HSCT, 9% of all patients never reached CC and they still have MC. Altogether, 77% of all monitored patients survive and they are constantly monitored, while 23% of all patients died. Five years after HSCT 80% of patients with CC and 74% of patients with MC continue to survive. Relapse was the cause of death in 37%, infection in 23%, graft versus host disease (GVHD) in 16%, organ failure in 13% and the other causes in 11%. In 29 patients who died because of relapse MC was detected in 20 of them (69%). In the other 9 patients with relapse CC was detected. These patients died before the year 2005 when the samples were analysed only by VNTR polymorphisms, that´s sensitivity is 1-5%. According to the selected examples of the patients who died because of relapse the increasing of cell chimerism corresponds with the other results (increasing of blast number).
Summary / Conclusion: Analysis of individual haematopoietic chimerism in the time period after transplantation is a nonspecific marker for post-transplant haematopoiesis for all diagnoses and it provides diagnostic evidence for clinical decisions. With this analysis it is possible to predict negative events, such as graft rejection or relapse in the early stage of disease. Subsequently quick therapeutic intervention can efficiently reduce the number of autologous cells before clinical manifestation of the disease.

B1692

OUTCOMES OF ALLOGENEIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH ACUTE MYELOID LEUKEMIA WITH ACTIVE DISEASE: EXPERIENCE OF A SINGLE EUROPEAN INSTITUTION

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Background: Allogeneic hematopoietic stem cell transplantation (AHSCT) represents a potentially curative approach for patients (pts) with relapsed or refractory acute myeloid leukemia (AML). However, those pts are a heterogenous population with long-term survival rates after AHSCT ranging from 40 % to less than 5%.
Aims: We report the outcome of relapsed/refractory AML pts treated with AHSCT.
Methods: A retrospective cohort from including clinical registries' databases for pts with diagnosis of relapsed/refractory AML, with 61 fulfilled inclusion criteria pts from 1994 to 2012. Outcomes of interest were transplant-related mortality (TRM), incidence of acute and chronic graft-versus-host disease (GVHD), incidence of relapse and overall survival (OS) - which were estimated from the date of transplant. Parametric and non-parametric tests were used appropriately. Kaplan Meir curve was used for OS assessment. Statistical analysis was carried out using SPSS v19.0 for Mac (IBM, 2010, USA) and statistical significance was considered for $P<0.05$.
Results: The median age was 38 (range 1 – 65) years. Median follow up for alive pts was 8 months (17 pts; range 3.26 – 12.73). The cumulative incidence of 90-days, 1 year, and 3 years TRM was 50%, 45%, and 5%, respectively ($P<0.001$). 42.6% of patients developed grade II-IV acute GVHD and 76.5% suffered from chronic GVHD. The actuarial incidence of relapse was 37.2% at 1 year and 25% at 3 years and 66.7% at 5 years. Actuarial OS was estimated to be 41.7% at 1 year, 50% at 3 years, and 8.3% at 5 years ($P<0.001$). OS of pts age < 45 years or >= 45 years was 52.9% versus 47.1% for all pts ($P=0.549$).
Summary / Conclusion: In our study, TRM and GVHD in first years after AHSCT remains considerable, but AHSCT in this setting seems to be a good choice for AML pts with active disease. However, new approaches are needed to reduce TRM and relapse in this cohort of patients.

B1693

TREATMENT OUTCOME IN THE PATIENTS WITH LARGE-B-CELL LYMPHOMA (LBCL) AFTER HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Diffuse large B-cell lymphomas (DLBCL) are the most common lymphoid neoplasms. The International Prognostic Index (IPI), and even more, age-adjusted IPI (aaIPI), has proved valuable for risk stratification of patients, particularly to "tailor" more intensive therapy such as high-dose therapy (HDCT) followed by autologous hemopoietic stem cell transplantation (autoHSCT). Despite the striking advances in the treatment of DLBCL, a group of patients with high risk factors still have a poor prognosis (long-term survival estimated at only 50%). In the absence of prospective studies, the optimal treatment for high-risk patients is still a matter of debate. Uncertainties exist with regard to the intensity of primary chemotherapy, the impact of rituximab and the role of high-dose chemotherapy.
Aims: To evaluate the effectiveness of high-dose chemotherapy and autologous stem cell transplantation in large-B-cell lymphoma patients with unfavorable prognosis.
Methods: 38 patients with large B-cell non-Hodgkin's lymphoma (24 patients with diffuse large B-cell lymphoma (DLBCL) and 14 patients with primary mediastinal large-B-cell lymphoma (PMLBCL), admitted to Hertcen Moscow Scientific Research Oncology Center between 2006 and 2013, were treated with HDCT followed by autoHSCT. There were 13 men and 25 women, most of PMLBCL patients were young women (79%). The average age of DLBCL patients was 38 years (17-60, median 38), PMLBCL patients – 32 (20-43, median 32). In all cases the diagnosis was confirmed by immunomorphology. All DLBCL patients had 2 or more adverse prognostic factors according to IPI. Most PMLBCL patients (69%) had localized stage II-E. Local infiltration of adjacent tissues and organs was defined in all PMLBCL patients, bone marrow involvment was not observed. HDCT followed by autoHSCT was performed in 16 patients with DLBCL and 13 patients PMLBCL to consolidate the first partial/complete remission; 3 patients with relapsed DLBC and 6 patients with stable refractory diseases also received HDCT followed by autoHSCT. The source of hematopoietic progenitors was peripheral blood in all patients. In most cases (79%) mobilization and collection of HSC was performed after DHAP, Dexa-BEAM, mini-BEAM or ICE chemotherapy. The number of CD34+ cells in the autograft averaged $18.3x10^6$/kg ($3.4-98.1x10^6$/kg). Pre-transplantation conditioning was carried out according to BEAM protocol. After HDCT followed by autoHSCT 10 PMLBCL patients received radiation therapy on residual disease.
Results: Currently 31/38 (81,5%) patients with LBCL remain in first prolonged-complete/partial remission, including 16 DLBCL patients and 11 PMLBCL patients to whom HDCT followed by autoHSCT was performed to consolidate complete/partial remission. Prolonged remission after HDCT followed by autoHSCT is also achieved in 3 relapsed patients. First prolonged remission achieved the only one patient with refractory disease. Observation time varies from 3 to 78 months (mean 43 months). Early refractory relapses after HDCT followed by autoHSCT occurred in 4/5 patients with stable refractory disease. One patient died from other causes 30 months after autoHSCT in complete remission.In 3 PMLBCL cases recurrent disease was found after HDCT and autoHSCT.
Summary / Conclusion: Treatment of B-large cell lymphoma requires a differentiated approach. In the group with adverse prognostic factors patients achieving complete/partial remission should receive early intensification treatment including HDCT and autoHSCT. In patients with refractory disease

autoHSCT is not considered to be sufficiently effective. Treatment of these patients is advisable to carry out as part of research protocols in clinical trials.

B1694

FAVORABLE OUTCOMES OF REDUCED-INTENSITY CONDITIONING REGIMENS FOR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR CHILDREN WITH ACUTE LEUKEMIA

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Background: Reduced-intensity conditioning (RIC) has been successfully adopted for allogneneic HSCT for older patients or those with medical comorbidities, while experiences in children are limited.
Aims: This study was performed to evaluate the safety and efficacy of RIC for children with acute leukemia.
Methods: We retrospectively evaluated 17 patients aged ≤ 18 years with acute leukemia who received allogeneic HSCT with RIC from 2008 to 2012 in Asan Medical Center Children's Hospital. In our pediatric HSCT program, eligibility criteria for allogeneic HSCT with RIC included unresolved infection at HSCT, co-morbidity or poor performance status, a history of prior HSCT, and infants. Performance status was scored according to Karnofsky scale in patients aged 16 years and older, and Lansky scale in patients less than 16 years. Organ toxicity was evaluated according to Common Terminology Criteria for Adverse Events (CTCAE) v4.0.
Results: The median age at HSCT was 13 years (range, 0.7-16 years), and 3 were younger than 1 year old at HSCT. Indications for RIC were unresolved infection at HSCT (N = 10), performance scores < 90% (N = 3), infancy (N = 3), and a prior history of allogeneic HSCT (N = 1). The diagnoses were AML (N = 13) and ALL (N = 4). Disease status was first complete remission (CR) in 13, ≥ CR2 in3, and partial remission in 1. RIC regimens were TBI-based in 7 (fractionated TBI 800 cGy, fludarabine 150 mg/m^2, cyclophosphamide 120 mg/kg) or busulfan-based in 10 (intravenous busulfan 6.4 mg/kg with fludarabine 150 mg/m^2 and rabbit-ATG 7.5-9 mg/kg with or without cyclophosphamide). Donors were matched sibling donors in 6 and unrelated donors in 11. Grafts were mobilized-peripheral blood in 16, and cord blood in 1. Sixteen achieved neutrophil engraftment at a median of 13 days (range, 10-20 days), and platelet engraftment at a median of 19 days (range, 13-30 days), while one patient, who received a cord blood graft, failed engraftment and subsequently received rescue transplantation from a haploidentical donor. Median duration of absolute neutrophil count (ANC) < 0.1 × 10^9/L was 6 days (range, 2-21 days). Full donor chimerism was achieved in all but one who failed engraftment. No CTCAE grade III or higher toxicity was observed in the kidneys, lungs, heart, mucosa and nervous system, while grade III hepatic toxicity was observed in one patient, who experienced a moderate degree of hepatic sinusoidal obstruction syndrome. None experienced documented bacterial or fungal infection. Six patients experienced CMV reactivation, while no patients developed CMV disease. No TRM was observed. Cumulative incidence of acute GVHD at day 100 and chronic GVHD at 1 year were 37.5% and 36.5%, respectively. At a median follow-up of 25 months, the 2-year overall survival (OS) was 68.6% and the 2-year relapse incidence (RI) was 29.4%. OS and RI were not different between the patients with AML and ALL. Analyzing the outcomes of risk-based subgroups in AML, 2-yr OS were 33.3% in intermediate-risk group and 83.3% in poor-risk group. OS, RI, and GVHD incidence were not different between a busulfan- and TBI-based regimen, while days to neutrophil and platelet engraftment and duration of ANC < 0.1 × 10^9/L tended to be longer in a TBI-based regimen (*P*=0.124, 0.066, 0.001, respectively).
Summary / Conclusion: These results suggest that RIC is a feasible approach with low morbidity and TRM for children with acute leukemia. A long-term follow-up study in a larger population is warranted to evaluate the efficacy and long-term safety of RIC in children.

B1695

HEMATOPOIETIC STEM CELL TRANSPLANTATION IN MYELOFIBROSIS: A COMPARISON BETWEEN MYELOABLATIVE AND REDUCED INTENSITY CONDITIONING

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Background: Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) is indicated for patients with intermediate or high risk primary Myelofibrosis (MF), and also in patients with Policitemia Vera or Essential Thrombocythemia who have progressed to high risk MF. The results for allogeneic HSCT with myeloablative conditioning seem to be better for patients younger than 45 years, determining low risk to recidivate. However, Reduced Intensity Conditioning (RIC) for patients between 45 and 65 years-old has shown to be promising.
Aims: This study aims describing a series of patients diagnosed with myelofibrosis, transplanted in the Hospital de Clínicas (HC) from the Federal University of Paraná and Hospital Nossa Senhora das Graças (HNSG) (Curitiba, Brazil).
Methods: From 1984 to 2011, fourteen patients with MF were submitted to HSCT, eleven from the HC and three from HNSG. The median of age was 42 years (10-51). There were ten males and four females. In average, five blood transfusions were done per patient (0-61). Five male patients received the graft from a female donor, and two patients were submitted to non-related HSCT. The median of duration of the disease was 20 months (2-150). According to Dupriez Classification, all the patients were of intermediate and high risk. Five patients did the myeloablative conditioning (Busulfan plus Cyclophosphamide – n=4 – Cyclophosphamide plus Total Body Irradiation – n=1) while nine did the RIC (Fludarabine 150mg/m2 plus Melphalan 140mg/m2 –n=6– Fludarabine plus Melphalan plus Antithymocyte Globulin -n=2- Fludarabine 180mg/m2 plus Bulsufan 10mg/m2 plus Antithymocyte globulin 5mg/kg –n=1).
Results: In the myeloablative conditioning group (n=5), all the patients presented marrow engraftment; one recidivated MF later. Three patients presented grade II-IV acute graft versus host disease, and four evolved with severe extensive chronic graft versus host disease. Four patients expired, and the only survivor was 10 years-old. Median survivor was 479 days. In RIC group (n=9), engraftment didn't occur in one patient (which had splenomegaly of 18 cm at the time of transplantation), and another three had had the disease recidivated. The other seven patients remained alive, with median survival of 750 days (34-1872; P=0,000123).
Summary / Conclusion: In spite of the limited number of patients in this study, data suggest that RIC utilization can improve mortality in HSCT for myelofibrosis, even with greater risk to recidivate.

B1696

USE OF PROTON PUMP INHIBITORS IN HEMATOPOIETIC STEM CELL TRANSPLANTATION DOES NOT INCREASE THE FREQUENCY OF FEBRILE NEUTROPENIA

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Background: Despite the proton pump inhibitor (PPI) use is a risk factor for infections in heterogeneous groups of patients, the studies related to PPI use and febrile neutropenic episodes (FNE) in hematopoietic stem cell transplantation (HSCT) are limited.
Aims: Identifying whether PPI use is a risk factor for febrile neutropenia at HSCT.
Methods: One hundred fifty-three HSCTs' data from 145 patients who was underwent HSCT at the Ege University, Adult Hematology Transplant Center between 2005 and 2012 were analyzed retrospectively. We analyzed the relationship between PPI use and febrile neutropenic episodes, culture results, viral antigenemia and fungal infections. For this purpose, the described variables were calculated using the multivariate test (logistic regression). 111 HSCT were autologous and 42 were allogeneic. Median age was 49 years. The study subjects consisted of patients with multiple myeloma and primary amyloidosis (n=60), lymphomas (Hodgkin's or non-Hodgkin's [n=49]), acute leukemia's (lymphoblastic or myeloid [n=28]), and other diseases (n=8). The patients were composed of 83 (57.2%) males and 62 (42.7%) females. 8 males were retransplanted. 94 (61.4%) of the HSCTs was performed with central venous catheter. All of the HSCTs were performed with peripheral blood stem cell. Considering the duration of PPI use; 69 (54.8%) patients were used PPI with the beginning of the HSCT conditioning regimen until >+14 days of HSCT. Chronic PPI use was determined in 57 (45.2%) patients.
Results: One hundred thirty-one (85.6%) of the HSCTs was complicated with FNEs and in 22 (14.4%) HSCT FNEs was not observed. The median neutropenia period was 9.27 days. Blood cultures were positive in 25 (16.3%) samples. Ten (40%) of them were gram-negative and 15(60%) were gram-positive microorganisms. There was no statistically significant difference between gram-positive and gram-negative microorganism positivity with PPI use blood cultures. On multivariate analysis, PPI use was not significantly associated with FNEs (odds ratio [OR]2,10; 95% Confidence Interval [CI] 0,63 -6,94; P=0,225) and bacterial culture positivity (OR 0,99; 95% CI 0,35 -2,80; P=0,984).
Summary / Conclusion: Our study have been revealed that in the patient group which was consist of majorly with chronic PPI use does not propose any additional risk for FNE and bacterial culture positivity at transplanted patients.

B1697

LYMPHOCYTOSIS WITH CD3CD8 PREDOMINANCE ASSOCIATED WITH CHRONIC GRAFT-VS-HOST DISEASE

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Background: Chronic graft-vs-host disease (cGvHD) is the most important non-relapse complication that affects long-term survivals of allogeneic hematopoietic cell transplantation (HCT). Clinical symptoms often mimic certain autoimmune diseases and worsen the quality of life. Infectious complications related to GvHD and immunosuppression may be fatal.
Aims: Patients with acute GvHD (aGvHD) history have higher incidence rate of cGvHD but it is difficult to predict who will actually develop cGvHD.
Methods: We analyzed clinically and with the use of flow cytometry the cases

of living 16 patients who developed lymphocytosis >5 G/l after HCT performed between 2003-2012 due to hematological malignancies. Median age at HCT was 41 (range 23-60).The donor was sibling in 7 cases, 10/10 matched unrelated in 7 cases and unrelated with 1 locus mismatch in 2 cases. 14/16 patients developed aGvHD (3 in grade 3-4; 10 in grade 1-2). 14 patients had confirmed cGvHD diagnosis in their medical history. We classified 4 as mild, 2 as moderate and 8 cases as severe cGvHD, including 4 with sclerotic features.
Results: Lymphocytosis > 5 G/l first time appeared between 5-51 month after HCT (median 15 months) with maximal achieved value from5,15-10,66 G/l. It was not related to infection or EBV replication (excluded by PCR). In 5 (31%) cases increased lymphocyte count preceded cGvHD symptoms or cGvHD exacerbation, while in 6 (37.5%) was accompanying cGvHD symptoms. In 12/16 patients lymphocytosis maintained for median 10-month-period (range 3-65 months). 4 patients are still under surveillance because of lymphocytosis (lasting <= 4mc). Lymphocytes T predominance was confirmed in 12 (75%) cases. Inverted CD4/CD8 ratio was present in 14/16 analyzed patients. The ratio range varied from 1:2 to 1:25. LGL cells>10% in peripheral blood were present in 14/16 cases.
Summary / Conclusion: 1. Significant CD3CD8 lymphocytosis >5 G/l with the presence of NK cells may appear prior to or correspond with exacerbation of cGvHD clinical symptoms and therefore should be treated as important laboratory finding. This population of lymphocytes may contain alloreactive donor-derived cells with cytotoxic properties. 2. As a majority of patients in the analyzed group had a history of aGvHD that may lead to destruction of the thymus, it could explain the low output of CD3CD4 cells with extrathymic expansion of CD3CD8 cells leading to inverted CD4/CD8 ratio.

B1698
COMPARISON OF TOXICITY AND EFFECTS OF CONDITIONING SCHEME USING BUSULPHAN AND CYCLOPHOSPHAMIDE AS MODIFIED BUCY WITH CYBU IN PATIENTS TREATED WITH HEMATOPOIETIC CELL TRANSPLANTATION.
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Background: Conditioning treatment is important issue that affects course of hematopoietic stem cell transplantation, bothshort-term andlong-term results. One of the common ways of preparation for transplantation is chemotherapy based on busulphan and cyclophosphamide. Recently there is a EBMT recommendation to use rather the altering drug combination CyBu then original conditioning BuCy. The reasons for thischange are results of animal and then clinical studies, which demonstrated less hepatic toxicity including a decreased number of VOD incidents and early transplantation related mortality. Using busulphan as a first drug affects cyclophosphamide's metabolism and increases the level of its toxic metabolites by reducing the level of glutathione. In theory, the impact of toxic metabolites causes liver damage and high levels of proinflammatory cytokines: IL-2, TNF-alpha that could be of importance in the development of aGvHD. The other way to reduce the risk of VOD is the modification of BuCy conditioning with one day break between those two drugs and implementation of leucovorin supply after methotrexate dose, which we implemented in our unit in 2011.
Aims: The aim of our study was to compare the hepatotoxicity and its severity, the number and severity of VOD incidents and associated mortality, engraftment and aGvHD in cohort treated with modified, in a way described above, BuCy and the CyBu conditioning.
Methods: From May 2012 10 patients underwent transplantation with conditioning CyBu (60 mg/m^2 i.v. on days -7 to -6; 0,8mg/kg every 6 hours -5 to -2) . Data were compared with patient treated in 2011-2012 with BuCy (0,8mg/kg every 6 hours -8 to -5; 60 mg/m^2 i.v. on days -3 to -2) conditioning. In both groups immunosuppression therapy was similar consisted of cyclosporine (from -1) and methotrexate on day1,3,6, 11 (in BuCy cohort with intravenous lecovorin support) with thymoglobulin in case of MUD. The following parameters were estimated: liver toxicity (bilirubin, ALT level), the incidence and severity of VOD, aGvHD and engraftment.
Results: The characteristic of both groups (CyBu / BuCy): women 50% v 62% and men 50 % v 38% respectively, the medianage (and range) at the transplantation point was 36 (19-57) and 45 (31-62). Almost all patients were treated with myeloproliferative disease: AML 80% v 77%; CML 10% v 15% and because of MDS 10% v 8%. In each casewas the firstgraft. Donors were HLA – identical siblings (50% v 46%); matched (30 % v 31%) and unmatched (10% v 15%) unrelated donor; autologous transplantation was made in 10% and 8% of patients respectively. Stem cells source in CyBu cohort was PSC in 70% cases and BM in 30%, no BM was given in BuCy. Pretransplant EBMT risk score was similar in both groups – average 4. At the baseline liver function tests was similar with slightly increase of bilirubin in two patients in each group (median2,2 mg/dl). Also one patient at anyof the groups had HBV infection in history but with negative DNA HBV before transplantation. Values of bilirubin observed in period of transplantation showed a higher level in CyBu than in BuCy cohort [median2,74 (1-7,36) v1,74 (1-3,5) mg/dl] but alanine amino transferase were not statistically significantly higher in BuCy [median 87,5 (62-735) v 120 (50-656) U/l]. Reporteddeviationsoccurred earlier in BuCy cohort [average day 3] than in CyBu [average day 5] but resolved the same after 12 days. No VOD incidence was observed. One patient in CyBu cohort died before engraftment because of infections reasons. Times to myeloid engraftment were in BuCy cohort median 17 days (renge 13-24) vs CyBu cohort median 26 days (range 20-29). aGvHDappeared amongBuCycohort in 53% of cases with median range of severity 2 (1-4) compared to CyBu cohort in 20% with severity 2 and 1 in each cases.
Summary / Conclusion: Modified conditioning regiment BuCy with one day break between intravenous Bu and Cy with supportive care of leucovorin after methotrexate use could be more safe with less liver toxicity than CyBu conditioning but with more incidence of aGvHD.

B1699
T CELL REPLETE HAPLOIDENTICAL TRASPLANTATION. A FEASIBLE ALTERNATIVE WITH ENCOURAGING SURVIVAL FOR PATIENTS WITHOUT MATCHED SIBLING IN DEVELOPING COUNTRIES. 34 TRANSPLANTS IN ONE CENTER IN COLOMBIA
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Background: The use of matched unrelated donor or cord blood as a CD34 source are the usual alternatives for patients without a sibling matched donor, however their cost and the difficulty in their consecution are important barriers for being a real solution in countries with limited economic resources. In the other hand haploidentical transplant has the advantage of an easy, timely and not expensive form of procurement a donor
Aims: We would like to present our experience with haploidentical transplant using T cell replete peripheral blood as a CD34 source, a reduce intensity conditioning regimen and alemtuzumab or cyclophosphamide for prevention of GVHD, graft rejection and management of T cell alloreactivity
Methods: Regimen 1 :(29 transplants): Cyclophosphamide (cy) 500 mgs/m2, and fludarabine (flu) 30 mgs/m2 from d-5 to –2, five ptes received busulfan or thiotepa on d -6 and twenty TBI 200-400 cGy on d – 1. GVHD prophylaxis: alemtuzumab 0.2 mgs/kg/IV d-4 to cero, mycophenolato (MMF) and cyclosporine from D – 3 to + 45 and + 180 respectively
Regimen 2: (5 transplants): Busulfan 4-6 mgs/kg, cy 30 mgs/kg, flu 150 mgs/m2, TBI 400 cGy. GVHD prophylaxis: Ciclophosphamide 50 mg/kg d+3 and d+4, MMF and Cyclosporine from d+5 to + 90 and +180 respectively
Results: 26 ptes received 29 transplants with regimen 1(R1) (3 needed a second transplant after engraftment failure of the first one) and 5 were transplanted with regimen 2 (R2). Median age was 24 y (7-50), 10 had AML, 6 LLA, 6 Fanconi Anemia, 3 aplastic anemia, 2 myeloproliferative disorders, 2 CML and 2 other disease. The CD34 cells were obtained from peripheral blood and infused without manipulation, the median transplanted dose was 12 million/kg (8-20), the median time for neutrophil recovery was 11 days (9-14) for R1 and 14 (13-15) for R2, the platelet recovery was at day +12 (9-50) after R1 and d +14 after R2. The cumulative incidence of engraftment was 88% for R1 and 100% for R2, The incidence of aGVHD (G II-III) was 27% for R1 and it occurred in 2 out of 5 after R2, the incidence of cGVHD was 40%, all limited, after R1 and cero after R2. The median follow up for R1 is 540 days(100- 1.620,) the day 100 mortality is 17.2% and the actuarial 50 months survival is 50%. The median follow up for R2 is shorter, 5 months, range 3-10, all patients are alive and without recurrence of their disease
Summary / Conclusion: Haploidentical transplant using the protocols previously described is feasible, produce a fast and reliable engraftment, low incidence of aGVHD, low day 100 mortality and an encouraging survival. taking into account the low cost and easy form of procurement the donor, it is a very good alternative for patients without matched sibling in countries with limited economic resources.

B1700
THE ATG BASED MYELOABLATIVE CONDITIONING REGIMEN IS EFFECTIVE IN PATIENTS WITH RELAPSED OR HIGH-RISK T CELL LYMPHOMA
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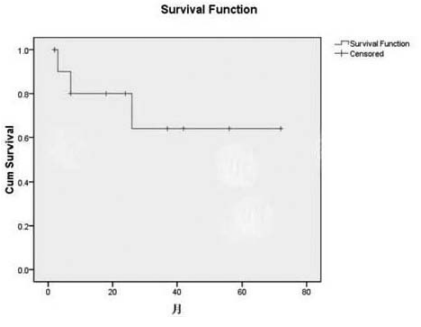
Aggressive T-cell lymphomas (ATCLs) represent 10% to 15% of non-Hodgkin's lymphomas (NHLs) in adults. Patients with relapsed or refractory disease are generally considered incurable with conventional therapies. ATG had been used in the conditioning regimen to reduce the incidence of Graft-versus-host disease □GvHD□for a long time especially in the matched unrelated donor HSCT. The early experiment result in our hospital showed that ATG inhibited the proliferation of lymphoid tumor cells in a dose-dependent manner especially in the T cell tumors.
Aims: We used the ATG as the part of the conditioning regimen in the all patients and to evaluate the long-term anti-leukemia effect, the safety and complication in the patients with relapsed or high-risk T cell lymphomas.
Methods: 12 patients(male6, female 6) were enrolled into this study. Median patient age at the time of transplantation was 27 years (range, 16–36 years).

At the time of transplant, 3 patients reached CR, 4 patients had a partial response (PR), 2 patients had relapsed disease and 3 patients had primary refractory disease. Donors were 10/10 HLA matched related (3), 10/10 matched unrelated (2) 8/10 matched unrelated (6) and mismatched related (1). The median number of CD34+ cells within the allografts was 9.31/kg body weight (BW) (range, 4.6–24.85/kg BW). All patients underwent myeloablative conditioning. Rabbit antithymocyte globulin (ATG 2.5 mg/kg×3-4 days) was used in all 12 patients. Eight patients underwent the conditioning regimen that included cyclophosphamide (120 mg/kg), VP 16 30-40mg/kg and total-body irradiation (10 Gy in five fractions). Conditioning regimens were cyclophosphamide and total-body irradiation (Cy–TBI) in 2 patients; VP16 and TBI in one patient; and CTX and fludarabine in one patient. GVHD prophylaxis was cyclosporine based, usually in combination with methotrexate. Quantitative chimerism analyzes were performed using short-tandem-repeat-based polymerase chain reaction techniques at regular intervals for every 4 weeks after transplantation in bone marrow at the first six months.

Results: All patients achieved a complete remission after allogeneic HSCT. At a median follow-up time of 21 months, nine(75%) patients are alive. OS at three years was 64% Three patients were died after transplant, two from relapse and one from treatment related complication. Acute GvHD grades I-II occurred in eight patients and grades III-IV in two patients. Eight out of ten patients with acute GvHD are currently alive and free of disease. Five of them developed chronic GvHD(4 limited, 1 extensive). One patients suffering from acute GvHD grade IV died due to treatment-related complications. One patient who suffering from acute GvHD grade I relapsed after decay of GvHD symptom. Eleven patients had suffered from CMV viremia in the first three months after transplant. And two of them gradually evolved into viral haemorrhagic cystitis. One patient suffered from aspergillus pneumonia while another two suffered from bacterial pneumonia. But, there was no patient dying from infectious complications.

Summary / Conclusion: Conditioning with ATG, TBI, CTX or VP16 is a feasible and effective alternative for patients with relapsed or high-risk T cell lymphomas.



B1701

HEMATOPOIETIC CELL TRANSPLANT CO-MORBIDITY INDEX IN DIFFUSE LARGE B-CELL LYMPHOMA: SINGLE EXPERIENCE

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoid malignancy in adults in western countries. The combination of anti-CD20 monoclonal antibody rituximab and CHOP chemotherapy every 14 or 21 days, is the standard treatment for DLBCL patients. The high-dose chemotherapy supported by autologous stem cell transplantation (HDC / ASCT) is recommended in young relapsed patients or in patients who have not achieved a complete remission (CR) after first-line chemotherapy.

The International Prognostic Index (IPI) end the revised version in the post-rituximab era (R-IPI) are the benchmarks of prognosis DLBCL. However, this system does not evaluate the comorbidities, that can influence the outcome of HDC.

Aims: The hematopoietic cell transplantation-specific comorbidity index (HCT-CI) was developed to assess the pretransplant comorbidities that may predict nonrelapsae mortality (NRM) and overall survival (OS) in patients undergoing allogeneic bone marrow transplantation.

Methods: We conducted an analysis on the prognostic role of HCT-CI on a group of DLBCL patients submitted to BEAM-conditioned ASCT for relapse or refractory disease to first line chemotherapy from 2001 to 2012. According to HCT-CI patients were classified as: low risk (no comorbidity), intermediate risk (1-2) and high risk (= or> 3). Furthermore, we considered as potential confounding prognostic factors both the pre-ASCT status and sex. OS and NRM were evaluated by Kaplan-Meier method.

Results: 57 patients were considered. The median age of the patient population was 53 years (range 20-71 years) and 31 (54 %) were male. HCT-CI was 0 in 17 (30 %), 1-2 in 31 (54%), and ≥ 3 in 9(16%). The pre-ASCT status was: progressive disease 16% , partial response 39%, and complete response in 45 %. The median follow-up time was 36 months (2-145 months). At 60 months the OS was 72 % and NRM was 6 %. The OS by HCT-CI was: low risk 90%, intermediate risk 59%, high risk 89% respectively (P<0.05). The OS was 72% both in men and women group. The OS according to pre-ASCT status was: 33% in progressive disease, 69 % in partial response and 90 % in complete response (P<0.05). The NRM by HCT-CI was: 0 % in low risk, 12 % in intermediate risk and 0% in high risk (P>0.05). The NRM was 8 % in men and 4% in women. The NRM by pre-ASCT status was: 11% in progressive disease, 5% in partial response and 3 % in complete response (P>0.05).

Summary / Conclusion: Our retrospective study confirms that pre-ASCT status has an important prognostic role. In this group aren't able showing a significant role of HCT-CI. This is possibly due to the low number of patients and the low number of events. An evaluation a larger number of patients is needed.

B1702

CAPILLARY LEAK SYNDROME IN HAEMATOLOGICAL PATIENTS: MANAGEMENT AND TREATMENT RETROSPECTIVE EVALUATION

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Background: Capillary leak syndrome (CLS) is an uncommon (< 10%) early complication of hematopoietic stem cell transplantation (HSCT) or intensive chemotherapy caused by the injury of the cytokines against the vascular endothelium, with loss of sodium, albumin and fluids into the interstitial space. The clinical features of CLS are: weight gain (>4% in 24h), generalized edema, hypotension and renal insufficiency of pre-renal origin. The CLS is related to a high risk of mortality due to frequent progression towards the multiorgan failure.

Aims: In order to treat early this syndrome, we designed a prospective protocol: all patients undergoing autologous or allogeneic HSCT or intensive chemotherapy for acute leukemia or bulky lymphoma.

Methods: From January 2004 to January 2013, 12 patients met the diagnostic criteria for CLS (HSCT: allogeneic n=2, autologous n=3; bulky NHL: n= 3; AML: n=3; AITL: n=1). All patients were monitored every 6 hours for weight, arterial pressure and fluid balance until 16 days after the end of intensive chemotherapy or pre-transplant conditioning. In presence of > 2% weight gain and/or 1000 cc positive fluid balance at 6 h, furosemide was given at dose of 0.4 mg/kg. After 1 hour, if patient didn't respond to furosemide, 100 cc of 18 % mannitolo and after 30 minutes 125 mg of furosemide were administered. In case of a persisting negative response, we began CLS therapy consisting of dopamine (4ug/kg/min), prednisolone (1 gm/kg) and furosemide (250 mg by continuous infusion) for 24 hours. The day after, if the response to therapy was positive,the pt continued the same therapy until the normalization of clinical parameters; in the other case we began the continuous hemofiltration until the normalization of clinical parameters.

Results: The 5 transplanted patients responded to the treatment with dopamine and prednisolone (PDN) with resolution of all symptoms within 24 hours. Two of 3 chronic myeloid leukemia (AML) patients and only one with angioimmunoblastic T-cell lymphoma (AITL) not responding to dopamine and PDN underwent hemofiltration within 24 hours with complete resolution of symptoms after a median time of 72 hours (range 48-96 h). One patient affected by LNH, currently hospitalized in the intensive care unit, seems respond to dopamine/prednisolone treatment and probably not need hemofiltration. Instead the other 3 patients (2 LNH and 1 AML), not responding to the first line therapy, for clinical and logistic reasons, underwent hemofiltration later than 24 h: all 3 patients progressed towards the MOF and died.

Summary / Conclusion: Altought limited to a small number of cases, from our experience we can draw the preliminary conclusion that our protocol seems to be effective 1) in monitoring patients at risk for developing CLS and 2) in permitting an early diagnosis and treatment of CLS. Furthermore, our study suggests that hemofiltration within 24 hours could be a crucial intervention for patients not responding to the first line therapy.

B1703

ZARZIO PLUS CHEMOTHERAPY AS PERIPHERAL BLOOD STEM CELL MOBILIZATION STRATEGY IN PATIENTS WITH HEMATOLOGICAL DISEASES CANDIDATED TO AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: Biosimilar granulocyte colony-stimulating factor (G-CSF) has been recently approved for mobilization of peripheral blood stem cell (PBSC).

Various recent reports describe a comparable efficacy as original G-CSF with a significant reduction of costs.

Aims: In this study we assessed safety and efficacy of Zarzio (Zarzio® Sandoz Biopharmaceuticals) in order to mobilize PBSC in patients (pts) with hematological malignancies and to ensure an optimal engrafment capability.
Methods: From June 2011 to December 2012, a cohort of 44 pts was studied. All pts received disease specific chemotherapy plus Zarzio at a dose of 5µg/kg body weight twice daily. Programmed CD34+ cells target dose was $4x10^6$/kg for pts with non-Hodgkin and Hodgkin Lymphomas, Leukemia and $6x10^6$/kg for Multiple Myeloma. Plerixafor was used in predicted poor mobilizer pts. Amicus Fenwal separator was used in all leukapheresis procedures.

Table 1 Patients' demographic characteristics

	N	%
N of patients	44	
Age	49,8	
Gender M/F	29/15	66/34
Diagnosis		
NHL/HD	22 /9	50 /20
MM	11	25
Acute Leukemia	2	4
Status of disease to mobilization*		
CR	17	38,6
PR	14	31,8
VGPR	5	11,4
NHL/HD	3 /3	6,8 /6,8
Previous stem cell transplantation	3	11,4
N° of previous chemotherapy regimen		
1 /2	27/7	61,4 /15,9
3	7	15,9
Mobilization chemotherapy regimen		
DHAP	20	45,5
HD-EDX	10	22,7
EDEV	9	20,5
Other	5	11,4
Mobilization failed	6	13,6
Mobilization failed after Zarzio plus Plerixafor	2	4,5
Mobilization achieved with Zarzio plus Plerixafor	7	15,9

Table 2 Patients' collection data

	median	range
Collection timing (days)	14	8 - 24
Pre-apheresis data		
CD34+/µL pool	38,3	10 - 303,5
WBC x10^6/ml	14,9	2,2 - 43,8
PLT x10^6/ml	28,8	11,8 - 348
HGB g/dL	10,5	7,9 - 13,9
Leukapheresis data (efficiency)		
CD34+(%)	79,2	33 - 99,8
MNC (%)	43,9	33,2 - 98,1
PLT depletion(%)	13,2	
Graft characteristics		
WBC x10^6/ml	119,8	42,1 - 212,9
MNC%	72,2	30,9 - 94,3
CD34+ x10^6/ml	2,7	0,3 - 13,8
CD34+ viability %	98,9	97,4 - 99,3

Results: Patients' demographic characteristics are listed in table 1. Median time of Zarzio administration was 8.2 days; 38 pts (86%) reached the target dose to perform ASCT with an average number of apheresis of 1.45. Collection data are in table 2. Poor mobilization was documented in 13 pts (29%), mainly of them received more than 3 chemotherapy regimens previously. Six pts failed mobilization, two of them even after the administration of Plerixafor. Among pts who have mobilized, 21 (55.3%) were submitted to high-dose chemotherapy followed by autologous (18) or allogenic (3) PBSC infusion. Median of $CD34x10^6$/kg infuse and viability were 4.3 $x10^6$/kg (0.8-6.2) and 75.3% (51.4-88.2), respectively. Engraftment occurred in all pts. The median time to absolute nucleated cells >500 and to platelets >20 000 was 12 days (10-23) and 14 (10-33) after CD34+ infusion, respectively.
Summary / Conclusion: Biosimilar G-CSF, such as Zarzio, may represent an effective strategy for PBSC mobilization and collection after chemotherapy in terms of CD34+ cell dose and timing of collection. A study of comparison will be necessary to establish advantages in terms of cost.

B1704
FREQUENCY OF CHRONIC GRAFT VERSUS HOST DISEASE AND THE FACTORS FOR DEVELOPMENT OF CHRONIC GRAFT VERSUS HOST DISEASE
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Background: Graft Versus Host Disease (GvHD) is a most common complication after allogeneic haematopoietic stem cell transplantation (AHSCT). Chronic GvHD (cGvHD) deteriorates quality of life.
Aims: To investigate frequency and factors related with development cGvHD.
Methods: A total of 100 patients who underwent AHCST in University of Erciyes, Faculty of Medicine, Haematopoietic Stem Cell Transplantation Unit were investigated, retrospectively. All transplantation procedures were performed from fullmatch donor and Seattle regimen was used for GvHD prevention. The frequency of cGvHD and factors related with development of cGvHD including recipient age, donor age, recipient gender, donor gender, recipient-donor gender match, blood group compatibility, amount of CD34+ given, conditioning regimen and prior acute GvHD (aGvHD) were investigated.
Results: The diagnosis of the patients were as follows: acute leukemia in 67 patients (69.1%), aplastic anemia in 7(7.2%), non-Hodgkin lymphoma in 6(6.2%), other diseases in 17(17.5%) (Hodgkin lyphoma, myelofibrosis, myelodysplastic syndrome, chronic myelogenous leukemia, chronic lymphocytic leukemia, paroxysmal nocturnal hemoglobinuria). 36 of patients were female (36%) and 64 patients were male (64%). 57 (58.8%) of patients were given non containing total body irradiation (TBI) conditioning regimen, 40(41.2%) of patients were given total body irradiation (TBI). The mean age of 100 patients was found 33.3 years (±11.04). The frequency of cGvHD was 26% (26 patient). No statistically significant difference was determined among development of cGvHD and patient age, diagnosis, recipient age, donor age, recipient gender, donor gender, recipient-donor gender match, blood group compatibility, amount of CD34+ given, conditioning regimen and prior aGvHD. The cGvHD percentage were 43.8% while female donor-female recipient, 11.8% while male donor-female recipient, 35% while female donor-male recipient, 20% male donor male recipient. It was seemed that the proportions were higher when donors were female. However the differences between these proportions were not found statistically significant (P=0,110).
Summary / Conclusion: Transplantation performed between female donor-female recipient and female donor-male recipient may increase risk of cGvHD development.

B1705
DEVELOPMENT OF A FAST PCR PROTOCOL ENABLING RAPID AND HIGH QUALITY STR BASED CHIMERISM ANALYSIS AFTER ALLO-HSCT
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Background: Chimerism analysis based on microsatellite (STR) genotyping is the standard method for donor cell engraftment monitoring after allogeneic hematopoietic stem cell transplant (allo-HSCT). Traditional STR-PCR amplification require approximately 3 to 3.5 hours, contributing a significant portion of the time required in chimerism analysis. So the PCR amplification time is an important rate-limiting aspect in clinical chimerism analysis and report. When using conventional Taq polymerase, the PCR products often incomplete adenylated due to DNA quality is not good enough or other reasons, thus sometimes interfere with the STR genotyping and accurately calculation of chimeric proportion.
Aims: We aimed to develop and validate a fast PCR protocol that enables rapid and high quality STR genotyping, and to shorten the chimeric analysis and clinical report time.
Methods: Peripheral blood, bone marrow and fingernail samples were collected from healthy donors and patients. Simulate mixed chimerism samples with different proportion of donor and recipient cells (1:2,1:4,1:8,1:16, 1:32,1:64,1:128) were prepared by white blood cell count and fold dilution. The AB Identifiler Kit including primer set and STR allelic ladders for 15 STR loci was used. Amplitaq Gold polymerase provided within the Identifiler kit and standard PCR cycling conditions were performed as control. Three newly bio-engineer modified fast polymerase MyTaq, Phusion and Q5 were used with each corresponding rapid cycling conditions. The amplification products were capillary electrophorese detected by using AB3500XL, and STR genotyping and chimerism as analyzed by using GeneMapper IDX software.
Results: All the four kinds of polymerase can be used for STR-PCR and genotyping. For the analysis of simulate mixed chimerism samples, the observation value were all in good consistency with expectations values for all of the four polymerase. . The STR-PCR cycling time for MyTaq, Phusion and Q5 were about 30 minutes, considerably less than 3 hours when using Amplitaq Gold polymerase. For the 3 kinds of fast polymerases, the Q5 polymerase showed the best balanced amplification efficiency for all of the 15 STR loci. The amplification product of Phusion and Q5 polymerases don't have the non-template addition "A" tail due to lack of adenylate activity, thus theoretically completely eliminated the problem of incomplete adenylation. But the corresponding allelic ladders without "A" tail needed for STR genotyping, which must be specially made. The MyTaq polymerase has extremely efficient of adenylate activity and showed complete adenylation in all of 8 crude DNA samples extracted from fingernail, actually fully resolved the problem of incomplete adenylation when compared with Amplitaq Gold polymerase.
Summary / Conclusion: We developed a fast STR-PCR protocol using MyTaq polymerase that enables rapid and high quality STR genotyping, decreased the STR-PCR time from 3 hours to 30 minutes and resolved the problem of incomplete adenylation. It could significantly shorten the chimeric analysis and clinical reports time.

B1706
THE EXPERIENCE OF PEDIATRIC ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: RESULTS FROM A SINGLE CENTER AT MIDDLE ANATOLIA, TURKEY
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Background: HLA matching plays a major role in determining the outcome of the hematopoetic stem cell transplantation (HSCT). An optimal donor for an allogeneic HSCT is genotypically HLA identical sibling of the patient. If the patient has no siblings, an extended family typing may identify HLA phenotypically matched relatives. Such donors usually share on haplotype with the patient and another one is matched or partially matched with the patient.
Aims: We would like to share our allogeneic HSCT experince at Pediatric

HSCT unit of Erciyes University.
Methods: Clinical and laboratary finding of our cohort is revisited
Results: During the last 2 years 35 allogeneic HSCT were performed in our clinic. Out of 35 patients, 10 with aplastic anemia (3 of them Fanconi), 6 were with thalassemia, 5 with AML, 4 with ALL, 2 with mucopolysaccaridosis type-I, 2 with familial hemophagocytic lymphohistiocytosis, 2 with CML, 2 with primary immune deficiency (1 ADA deficiency, 1 CD4 lymphopenia associated with NHL), 1 with Griscelli syndrome, 1 with relapsed Hodgkin's disease, respectively.Three of 35 HSCT were sibling umbilical cord blood transplantation, 3 haploidenitical transplantation (1 from mother, 2 from father), 1 unrelated donor with 1 mismatch allel of HLA-A. Related donors were one mother, one aunt, three fathers, and 22 siblings respectively. However, each of the two sibling donors had 1 mismatch allel of HLA-DR. All of patients except one transplanted from unrelated donor had myeloid engraftment at the median days of 18 (range: 12-35) and platelet engraftment at the median days of 32 (range: 20-51). In 4 patients mixed chimerism was detected in early period of HSCT but was soon followed by full donor chimerism. The patient with relapsed ALL and graft failure underwent alfa-beta T cell depleted haploidentical HSCT from father two months after the first unrelated HSCT. Although a patient with Griscelli syndrome died because of pneumonia, all the others survived.
Summary / Conclusion: In conclusion, we observed that allogeneic HSCT is a good substitution for relapsed hemato-oncological malignancies. Alfa-beta T cell depleted haploidentical can be a suitable source for patients with high risks.

B1707

THE FEASIBILITY OF ALLOGENEIC STEM-CELL TRANSPLANTATION IN THERAPY RELATED MYELOID NEOPLASMS: A RETROSPECTIVE ANALYSIS OF A SINGLE CENTRE EXPERIENCE

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Background: Therapy-related myeloid neoplasms (t-MN) include, according to the 2008 WHO classification, acute myeloid leukemia (t-AML), myelodisplastic syndrome (t-MDS) and myelodysplastic/myeloproliferative neoplasms occurring after chemo- and/or radiotherapy administered to treat a previous neoplasm or a non-neoplastic disorder. t-MN display a 5-year overall survival (OS) of less than 10%, worse than the *de novo* counterpart. The only curative option in suitable patients (pts) is allogeneic stem cell transplantation (aSCT). Literature data demonstrate an emerging role for hypomethylating agents instead of chemotherapy as a bridge to aSCT in *de novo* MDS and low-blast count AML, but scanty data is available for t-MN.
Aims: Our retrospective study has the purpose to analyse the feasibility of aSCT in t-MN, which are characterized by unfavourable clinical and biological prognostic features and to investigate the role of induction therapy before aSCT.
Methods: Our analysis includes 18 t-MN pts (11 females, 7 males) of a median age of 52.5 years (range 29-64), observed at our Hematology Department, who underwent aSCT between September 1999 and June 2012. All pts were treated for a primary neoplasm with chemo- and/or radiotherapy (median 2 lines of treatment). Primary neoplasms consisted of 7 hematologic malignancies (2 B-CLL/SLL, 1 low-grade NHL, 3 DLBCL, 1 HL) and 11 solid tumors, mainly breast cancer (8/11). The median time from the first treatment to t-MN was 33 months (range, 12-144 months). Eight pts had a t-AML, 9 t-MDS and 1 Ph+ t-ALL. Karyotype was available for 15/18 patients: 5 had a normal karyotype, 2 abnormalities of chromosome7, 2 complex karyotypes, 1 monosomy16/+13, 1 del(11)(q14;q23), 1 t(9;22), 1 t(9;16), 1 del(20) and 1 was hypodiploid. Median hematopoietic cell transplantation-comorbidity index (HCT-CI) was 3 (range 3-5), 7/18 pts had an HCT-CI >3. Fourteen pts received an induction treatment before aSCT, consisting of chemotherapy (10 pts) , 5-azacitidine (3 pts), or both (1 pt). Six pts (33.3%) were in complete remission (CR) at the time of aSCT. The conditioning regimen was myeloablative (MA) in 27.7% of pts (BuCy2). GVHD prophylaxis included cyclosporine, Methotrexate and MMF. All 10 pts receiving matched unrelated donor (MUD) aSCT were treated with additional immunosuppressive therapy.
Results: Three pts died within one month after aSCT (16.6%). Early deaths were attributable to infectious complications and acute GVHD. In 15 pts surviving at least 100 days after aSCT the response rate was 80%. Two pts relapsed at 6 and 12 months after aSCT, respectively, and both died with active disease, similar to two pts with refractory disease. Five pts died due to infectious complications (4/15) and GVHD (1/15) later than 100 days post-aSCT, respectively. At a median follow-up of 20.5 months from the diagnosis (range 9-149), 6 pts are alive in cCR (33.3 %). When analysing OS only status at transplant (responders, refractory and untreated) resulted significant (P=0.03), while other factors, as cytogenetic risk, conditioning regimen, source of stem cell, type of primary neoplasia, number of previous lines of therapy, disease status at aSCT did not play a significant role.
Summary / Conclusion: Our study confirm that aSCT is the only curative option in t-MN, although a better patient selection may reduce early transplant mortality. Of note, our data show that 60% of pts who underwent transplant in CR or were previously untreated for t-MN are alive and in CR at median of 102 months (range, 16-132 months).

B1708

G-CSF BIOSIMILAR (ZARZIO®) MOBILIZATION FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background: Recently, a new G-CSF biosimilar (Bio-filgastrim, Zarzio®, Sandoz) has been approved by the European Medicines Agency for autologous PBSC mobilization. However, this authorization was based on comparable safety and efficacy to the reference product (Neupogen®) in the management of chemotherapy-induced neutropenia, with no data available regarding PBSC mobilization in patients with Hematological Malignancies.
Aims: Since 2011, Zarzio® has been approved in our institution for all authorized indications including PBSC mobilization in autologous SCT. A total of 36 patients mobilized with bio-filgastrim were included in this retrospective study, and were compared with the previous 36 patients mobilized with reference filgastrim.
Methods: Neupogen® was given at a dose of 10 µg/kg/day while Zarzio® was given at a dose of 5 or 10 µg /kg/day (depending on the CD34 target). Both were given during 5 or 6 days with the first apheresis scheduled for day 5. The target CD34+ cell dose was 2 x 10^6/kg recipient body weight in Lymphomas and 4 x 10^6/Kg in Myelomas.
Results: Median age was 57 years (range 19 - 72). The median duration of G-CSF treatment was 5 days (5-6) and the median number of apheresis was 1 (1-3) in both groups equally. Zarzio® seemed to have been well tolerated with no long-term adverse events reported in our series. Side effects included mild bone/muscle pain in both groups. There were no significant differences in mobilization outcomes between groups receiving the bio-filgastrim or reference filgastrim. The median number of circulating CD34+/L at day 5 was 41.4x10^9 (range 3.2 - 256.5) and 19.6 x 10^9 (range 1.8 - 119.1) respectively (P=0.01). The median number of CD34+/kg was 4.64 x 10^6 (2.04 -13.42) and 3.38 x 10^6 (1.04 -10.98) in both groups (P=ns). All 36 patients (100%) in the bio-filgastrim group achieved the target CD34 cell dose compared to 28/36 (78%) patients in the control group (P<0.001). To date, 26 patients mobilized with bio-filgastrim and 27 with reference filgastrim, have undergone autologous SCT. All patients engrafted within a median of 13 days (10 - 19) for the bio-filgastrim group and 11 days (10-17) for the control group. Platelet engraftment was at 19 and 18 days respectively.
Summary / Conclusion: In our group of patients, our findings suggest that Zarzio® is well tolerated and safe when compared with Neupogen® for PBSC mobilization before autologous SCT in patients with Hematological Malignancies. However, further studies are required to assess longer-term safety of G-CSF biosimilars.

B1709

G-SCF-ASSOCIATED ADVERSE EVENTS IN PATIENTS WITH MULTIPLE MYELOMA DURING STEM CELL MOBILIZATION

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Background: High-dose chemotherapy followed by autologous stem cell transplantation is the standard care for multiple myeloma (MM) patients fit enough to undergo the procedure. Granulocyte colony-stimulating factor (G-CSF) alone or combined with cyclophosphomide is commonly used to mobilize blood progenitor stem cells to support high-dose therapy with melphalan.
Aims: To define G-CSF-associated adverse events in pts with MM during stem cell mobilization. Also we present here a 60 y.o. male patient with MM who developed a rare complication - acute exacerbation of gout during stem cell mobilization.
Methods: We have analyzed adverse events during stem cell mobilization in 198 pts with MM treated in our center from November 2000 to February 2013. There were 105 male, and 93 female, median age 52,5 (range 26-68). In mobilization procedure pts received CY 4-6 g/m2 followed by daily administration of G-CSF 5 mcg/kg or G-CSF 10 mcg/kg alone.
Results: The most common G-SCF-associated adverse events were bone pain (46%), myalgia (32%), low-grade pyrexia (24%) and vascular events (8,3%). One pt developed ischemic stroke on the third day of G-CSF administration due to thrombosis of medial cerebral artery. 4 pts (2%) had a previous history of gout and only 1 pt developed acute exacerbation of gout during stem cell mobilization. A 60 y.o. man was diagnosed with MM Bence-Jones lambda in June 2012. At the time of diagnosis blood chemistry analysis revealed increased level of uric acid (up to 495 mkmol/l). The pt was treated with 7 cycles of bortezomib+cyclophosphomide+dexamethasone (VCD) and after induction therapy achieved very good partial response. After the 1 VCD cycle the first episode of gouty arthritis had appeared. Pain and swelling developed at the 1st left metatarsophalangeal joint. The pt received allopurinol and non-steroidal anti-inflammatory drugs with success. During stem cell mobilization marked swelling of both feet developed on the second day of G-CSF administration. On examination his feet were red, swollen and sharply painful. He had had high-

grade pyrexia for 5 days. Deep vein thrombosis and sepsis were excluded. X-rays of feet revealed minor osteoarthritic changes at the 1st metatarsophalangeal joint of both feet but no chondrocalcinosis. During this period increased levels of uric acid had never been registered. Therapy with non-steroidal anti-inflammatory drugs and allopurinol was prescribed again. General relief of symptoms of gouty arthritis was registered on day 10 of the therapy. Stem cells were successfully collected and in February 2013 autologous stem cell transplantation was performed.
Summary / Conclusion: Acute gout can manifest in context of normal uric acid levels, precipitated by several factors such as dehydration, diet, trauma and drugs. Gout should enter the differential of acute joint pain in pts receiving G-CSF for stem cell mobilization.

B1710
PEG-FILGRASTIM AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION: A SINGLE-CENTRE EXPERIENCE
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Background: High – dose chemotherapy followed by autologous peripheral blood stem cell transplantation (auto-PBSCT) is considered the standard of care for patients affected by multiple myeloma or relapsing Hodgkin (HL) or Non Hodgkin lymphoma (NHL) with chemosensitive disease. Severe pancytopenia and subsequent febrile neutropenia are well recognized complications of autologous-PBSCT: therefore recombinant hematopoietic colony-stimulating factors (CSF) are currently used to accelerate cellular engraftment after stem cell reinfusion.
Aims: Peg-filgrastim (Peg-G-CSF) is a long-acting pegylated form of filgrastim, that is commonly used after myelosuppressive chemotherapy as single subcutaneous injection, with comparable effect to non Peg-G-CSF. Moreover, several studies have shown activity of Peg-filgrastim on neutrophil recover both after conventional chemotherapy and after auto-PBSCT. For these reasons we performed a retrospective analysis of safety and efficacy of Peg-GCSF in patients who received auto-PBSCT for NHL in our institution from April 2004 to January 2013.
Methods: Fifty-five patients (25 M; 30 F), with a median age of 45 years (r.21–65), were treated with Auto-PBSCT for relapsed NHL. Conditioning schemes used were: BEAM (BCNU 300 mg/m^2 day-6, Ara-C 400 mg/m^2 day -6→-3, VP-16 200 mg/m^2 day -6→-3, Melphalan 140 mg/m^2 day -2) in 28 patients, TEAM (Thiotepa 10 mg/Kg day-7) in 4 patients, FEAM (Fotemustine 150 mg/m2 day -8, -7) in 4 patients and BTM (BCNU 300 mg/m^2 day-6, ThioTepa 150 mg/m^2 day-5→-3, Melphalan 140 mg/m^2 day-2) in 19 patients. All patients received Peg-G-CSF as a single subcutaneous injection at day +1 after stem cell reinfusion. Antimicrobial prophylaxis consisted of levofloxacin 500 mg once daily and fluconazole 400 mg once daily from the first day of chemotherapy; antiviral prophylaxis consisted of acyclovir 5 mg/kg from day +1 and a single infusion of immunoglobulin at a dose of 500 mg/kg on day+1. Neutropenic fever was defined as a temperature >38° on 2 consecutive readings or >38,5° on a single reading. Empirical antibiotic therapy, red blood cell and platelet transfusions were given according to our institutional guidelines. The recovery of platelets (PLTs) and neutrophils (ANC) was considered if superior to 1x10^3/mmc and 20x10^3/mmc, respectively,on two consecutive determinations.
Results: The median time to ANC recovery was 10 days (5–13) and to PLTs engrafment was 13,5 days (4–48); 2 units was the median number of PLT or blood cell transfusions per patient (r.1-10 and 1–5, respectively). Neutropenic fever was observed in 20 patients, and bloodstream infection was documented in 5 of them (3 Gram+/ 2 Gram-). The median number of days of fever was2,5 (r.1–7), with a median time of intravenous antibiotic therapy of 10,5 days (3-21). Two patients died within 100 days from Auto-PBSCT (1 for pulmonary embolism and 1 for blood sepsis due to E. Coli). Oral mucositis was observed in 6 patients, while gastrointestinal mucositis was observed in 24 patients. The median time of hospitalization was of 18 days (r.15-30).
Summary / Conclusion: In our hands, Peg-G-CSF was safe and effective in the management of post auto-PBSCT neutropenia. We did not observe increased incidence of infectious episodes or delayed ANC recovery compared to literature references. However, prospective randomized trials comparing pegylated vs non pegylated G-CSF are needed to finally asses which form of G-CSF should be used in this particular setting of patients.

B1711
AML WITH MYELODYSPLASIA RELATED CHANGES: IMPACT OF CYTOGENETIC ABNORMALITIES ON TRANSPLANT OUTCOME
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Background: Acute myeloid leukaemia with myelodysplatic features (MRC-AML) is a 2008 WHO classification new category which includes both acute myeloid leukemias with morphological features of myelodysplasia and those with a prior history of myelodysplastic syndrome. Compared to other AML types, MRC-AML has a lower rate of complete remission and a poor prognosis. Association with high risk cytogenetic abnormalities seems more significantly related to poor outcome than myelodysplastic features. Role of cytogenetic abnormalities on transplant outcome is not established for MCR-AML patients.
Aims: We report a single centre retrospective analysis of 17 patients who received a stem cell transplant for MRC-AML from 2000 to 2012.
Methods: Male/Female ratio: 13/4. Median age at transplant: 43 years. Type of transplant: autologous: 4/17(24%), allogeneic: 13/17(76%). Donor: sibling: 53,8% (7/13), matched unrelated: 46,2% (6/13) Cytogenetic data: normal: 7 (41%), abnormal: 8 (47%), not available: 2 (12%). Abnormalities: del 5q (3), t(2;3) (1), t(3;11) (1), del Y (1), complex karyotype (1), t(6;19) (1). Patients with del5, del7, t (6;9), complex kariotype, t (2;3) and t (3;11) were assigned to the High Risk group (HR) whereas Standard Risk group (SR) included normal kariotype and del Y. Both groups were comparable except in the type of transplant: all HR patients received alloSCT, meanwhile half SR patients received an alloSCT and the other 50% an autoSCT.
Results: Patients without available cytogenetic analysis were excluded. HR group: 7 (46.6%); SR group: 8 (53.3%). Median follow up from SCT: 8 months (2-96). OS at Day 100 was 70.5% for HR and 62.5% for SR (P=0, 71). Median PFS was 9 months. To date, only 35% remain alive. In HR 2 of 7 patients are alive, 1 with relapsed disease and other in complete remission, both with a follow up of 5 months. In the SR group 4 of 8 patients are alive and with no evidence of disease progression: 2 received allogeneic SCT (follow up of 16 and 7 months) and 2 received an autologous SCT (follow up of 71 and 9 months). Global relapse rate was 13%.
Summary / Conclusion: Cytogenetic abnormalities are one of the most important prognostic factors in MRC-AML. Our review suggests that cytogenetic risk is crucial for a transplant satisfactory result. Outcome in HR cytogenetic patients, even treated with alloSCT, remains dismal. Autologous transplant could be a therapeutic approach in selected MRC-AML with favorable cytogenetic.

B1712
G-CSF BIOSIMILAR (ZARZIO®) STEM CELL MOBILIZATION IN SIBLING HEALTTHY DONORS
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Background: G-CSF mobilized peripheral blood stem cells (PBPC) have became the major source of hematopoietic stem cells for transplantation. Recently, a new G-CSF biosimilar (Bio-filgastrim, Zarzio®, Sandoz) has been approved by the European Medicines Agency for autologous and allogeneic PBSC mobilization. However, this authorization was based on comparable safety and efficacy to the reference filgastrim, (Neupogen®, Amgen) in the management of chemotherapy-induced neutropenia, with no data available regarding PBPC mobilization in healthy donors.
Aims: We present our experience with 9 HLA-identical sibling donors mobilized with Bio-filgastrim that were compared with the previous 9 donors mobilized with filgastrim. Both drugs were given at a dose of 5µg/kg/day for 4 or 5 days with the first apheresis performed on day 5. The target CD34+ cell dose was 5 x 106/kg recipient body weight.
Methods: Both drugs were given at a dose of 5µg/kg/day for 4 or 5 days with the first apheresis performed on day 5. The target CD34+ cell dose was 5 x 106/kg recipient body weight.
Results: Donor median age was 48 years (range 17 - 77). Median duration of G-CSF treatment was 5 days (5-6) and the median number of apheresis was 1 (1-2) in both groups. Side effects included mild bone/muscle pain all patients with no differences between the 2 groups. No immediate serious adverse effects were detected in either group. Bio-filgastrim seemed to have been well tolerated with no long-term adverse events reported.
There were no significant differences in mobilization outcomes between groups receiving the biosimilar or reference filgastrim. The median number of circulating CD34+ cells at day 5 was 69.09 x 10^9/L (24- 114) and 75.2 x 10^9/L (42.3-146.4) respectively. No mobilization failure was observed in either group. The median number of CD34+ cells/kg harvested was 6.8 x 10^6 (4-9.2) and 8.5 x 10^6 (6-14.3) respectively (P=0.12). Seven patients have received PBSC mobilized with bio-filgastrim with no significant side-effects during the infusion. All patients engrafted at a median of 23 days (18 - 28) for the bio-filgastrim group compared with 19 days (12-28) for the reference filgastrim group (P=0.11). Platelets engraftment occurred at 26 and 24 days respectively.
Summary / Conclusion: Our findings suggest that Zarzio® is well tolerated and safe as compared with Neupogen® for PBSC mobilization of HLA-Identical siblings before allogeneic SCT. Given the similar effectiveness and their reduced costs, G-CSF biosimilars may represent a cost-effective alternative for PBSC mobilization. However, further studies are required to assess longer-term safety of G-CSF biosimilars.

B1713

CLINICAL AND LABORATORIAL CHARACTERISTICS PREMOBILIZATION, DURING THE HARVEST AND IN RELATION WITH THE AUTOLOGOUS STEM CELL TRANSPLANTATION OF ALL PATIENTS MOBILIZED USING PLERIXAFOR IN OUR CENTRE

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Background: Plerixafor (AMD3100, Mozobil®) is a CXCR4 antagonist, receptor in the hematopoietic stem cells and its block releases them into the peripheral blood circulation. Plerixafor is the last drug in the list of movilizing agents and has been approved for steady-state mobilization with G-CSF for patients with high risk of mobilization failure.
Aims: Analyze the clinical and laboratorial characteristics before the mobilization and during the autologous hematopoietic stem cell transplantation (autoHSCT), and those in relation with the harvest in all patients mobilized using Plerixafor in our hospital.
Methods: Observational and retrospective study of the patients have been mobilized with Plerixafor in our centre (n=14). We have revised the medical notes of all these patients assessing these parameters: underlying hematological disease, premobilization factors associated with risk of mobilization failure (previous failure, advanced age, bone marrow infiltration by tumor cells, previous chemotherapy with Fludarabine, Melphalan or Lenalidomide, extensive radiotherapy to bone marrow sites and thrombocytopenia), time drug was administered, number of doses, adverse effects, total CD34 cells collected and amount after using Plerixafor, CD34 cells collected by apheresis session after G-CSF alone and after Plerixafor, complications during the product infusion in patients undergoing autoHSCT, time to platelet engraftment and discharge day. Mobilization regimen with G-CSF alone was 20 /kg daily. Plerixafor was administered at the standard dose of 0.24 mg/kg at 12±2 h before apheresis and concurrently with G-CSF.
Results: The underlying hematological disease was NHL (n=3), HD (n=4) and MM (n=7). Eleven (78%) showed premobilization factors associated with risk of mobilization failure. The total number of apheresis cycles after Plerixafor was 17 because three patients needed two apheresis cycles to collect the CD34 /kg goal. The time when this drug was administered was pre-emptive treatment in first apheresis (n=6), rescue during first apheresis (n=3), pre-emptive in second apheresis (n=7), and rescue during second apheresis (n=1). The average number of Plerixafor doses per apheresis cycle was 1.7 (range: 1-3). Only two administrations (11%) produced adverse effects and both were slight. The median CD34/kg collected in all apheresis sessions, including mobilization with G-CSF alone, was 1.9 (0.3-8.1). The median amount of CD34 cells per apheresis cycle collected after Plerixafor was 94% (47-100). Using G-CSF alone the median of CD34 per apheresis session was 0.3 (0.05-0.7) and after Plerixafor use 0.8 (0.3-8.1). One patient (7%) didn't get enough product to go to autoHSCT. In patients (n=12) undergoing autoHSCT nine (75%) suffered from complications during the product infusion, six (66%) were slight and three (33%) mild. The median time to platelet engraftment was 13 days after infusion (10-29), and the median time to the discharge was 17 days (14-31). One patient died during the autoHSCT and so these data are not avalaible.
Summary / Conclusion: Plerixafor is an effective and safety drug to mobilize patients with high risk of mobilization failure or after previous mobilization failure with any regimen. The amount of CD34 cells collected in this kind of patients after Plerixafor undergo the same volume leukapheresis seems to be higher than G-CSF alone. In spite of the clinical characteristics of these patients the engraftment and the discharge time doesn't seem to be later after autoHSCT compared to that patients mobilized with G-CSF alone althought no current data are available.

B1714

INVESTIGATION ON THE VARIATION OF PERIPHERAL BLOOD CD34+ CELLS DURING THE HEMATOPOIETIC STEM CELL MOBILIZATION AND ITS INFLUENCE ON THE COLLECTION: DATA ANALYSIS IN A CHINESE POPULATION

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Background: Effective mobilization and collection of hematopoietic stem cells were the key factors in peripheral blood hematopoietic stem cell transplantation. The number of $CD34^+$ cells number as well as mononuclear cells was the usual indicator used to predict the acquisition effect and to seize the right time of collection. But there were few reports about the variation of $CD34^+$ cells number during the course of the mobilization in Chinese population.
Aims: To investigate the variation of peripheral blood $CD34^+$ cells during the hematopoietic stem cell mobilization in Chinese population, and to determine its influence on the collecting timing and acquisition effect.
Methods: 27 cases of peripheral blood hematopoietic stem cell mobilization and collection from 2011 April to 2011 December were analyzed, including 13 autologous cases mobilized with chemotherapy combined with granulocyte colony-stimulating factor (G-CSF) 10 $\mu g \cdot kg^{-1} \cdot d^{-1}$, and 14 cases of healthy donor mobilized with only G-SCF 7.5 $\mu g \cdot kg^{-1} \cdot d^{-1}$. Peripheral blood $CD34^+$ cells count was monitored, and its correlation with the yield of mononuclear cells (MNCs) and $CD34^+$ cells were analyzed. Informed consent was obtained before the start of the mobilization.
Results: $6.59\pm2.26\times10^8$/kg/recipients body weight MNCs and $4.38\pm2.47\times106$/kg/recipient body weight $CD34^+$ cells were obtained in healthy donor, $6.58\pm3.72\times10^8$/kg MNCs and $3.98\pm3.06\times10^6$/kg $CD34^+$ cells were obtained in autologous cases, there was only 1 failure in autologous cases. The peak of peripheral blood $CD34^+$ cells in autologous cases was appeared in day 4 after the treatment of G-CSF, and in healthy donors the count of peripheral blood CD34+ cells on fifth day was still in ascendant phase. The percentage and absolute value of peripheral blood CD34+ cells were the only indicators positive correlated with the $CD34^+$ cells/kg in the collection products. The cases ratio of $CD34^+$ cells$\geq2\times10^6$/kg in the products of single collection was up to 76%(16/21) in the cases with peripheral blood $CD34^+$ cells absolute value greater than 20/μL.
Summary / Conclusion: The peripheral blood $CD34^+$ cells count was good monitoring indicators in hematopoietic stem cell mobilization and collection, $CD34^+$ cell absolute value≥20/μl could be used as collection threshold.

B1715

LONG-TERM DISEASE-FREE SURVIVAL IN MANTLE CELL LYMPHOMA (MCL) PATIENTS FOLLOWING REDUCED INTENSITY CONDITIONING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (RIC ALLO-HSCT)

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Background: MCL represents an aggressive and difficult to cure disease. Current practice for patients with chemosensitive MCL includes high-dose therapy followed by autologous hematopoietic rescue (autologous HSCT). However, this treatment modality does not offer long-term survival since the majority of patients will experience a, very poor prognosis, relapse. Allogeneic HSCT appears to be the only curative approach for MCL, however, this outcome is being compromised by the greater risk of non-relapse mortality.
Aims: Given the limited evidence existing for the role of RIC allo-HSCT as consolidation therapy for MCL patients, we retrospectively analyzed the outcome of RIC-HSCTs performed at our center, during 2001-2011, in 8 MCL patients. RIC-HSCT was administered either as consolidation therapy in selected patients in first complete remission (CR1) or in chemosensitive relapse, aiming to provide the benefit of the reported, although still conflicting, graft versus MCL (GvMCL) effect.
Methods: All patients (n=8) were males who presented with advanced disease stage at diagnosis (stage IV, 8/8 / bone marrow involvement, 7/8). The median age at diagnosis was 47.5 years (37-63). They all received multi-agent chemotherapy as induction therapy and achieved complete remission (CR1). They were transplanted at a median time from diagnosis 19.5 months (9-41), in CR1(4/8) or CR2 (4/8). Two of the patients transplanted in CR2 had previously received autologous HSCT as consolidation therapy at CR1 and relapsed, 11 and 18 months post auto-HSCT. The median age at allo transplant was 48.5 years (40-64) and the median HCT comorbidity index (HCT-CI) 1 (0-1). All patients received reduced intensity conditioning (fludarabine and cyclophosphamide) and they were grafted with peripheral blood stem cells (8/8) from sibling (6/8) or matched unrelated donors (2/8) (MUD). Patients who had sibling donors received cyclosporine + mycophenolate mofetil (MMF) as anti-graft versus host disease (GvHD) prophylaxis, whereas MUD recipients received tacrolimus + MMF.
Results: With a median follow up of 74.5 months (15-122) after allo-HSCT, none of these patients relapsed and 7/8 are alive (disease free survival and overall survival 87.5%). The patient who died succumbed to, refractory to many lines of therapy, chronic GvHD, 15 months post transplant (TRM 12.5%). Two patients developed steroid-responsive acute GvHD (2/8), whereas all of them developed extensive chronic GvHD (100%). In 4/7 alive patients, chronic GvHD resolved after therapy, but three patients are still under immunosuppressive therapy.
Summary / Conclusion: Despite the limited number of patients in this study, our results suggest that RIC-HSCT is a potentially suitable treatment modality for selected MCL patients with chemosensitive disease. Especially in young patients in CR1 and with low HCT-CI, RIC-HCT could become the preferred postremission consolidation therapy, since a strong GvMCL effect is implicated and the survival rates seem to override the rates of non relapse mortality. Safer conclusions for the efficacy of RIC allo-HSCT in the treatment of MCL, however, can be only drawn by large prospective randomized trials.

B1716

FOTEMUSTINE BASED PLATFORM AS CONDITIONING REGIMEN IN RELAPSED NON HODGKIN'S LYMPHOMA PATIENTS ELIGIBLE FOR AUTOLOGOUS STEM CELL TRANSPLANT

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Background: High dose chemotherapy followed by autologous stem cell transplant (ASCT) represents currently the standard of treatment for patients with relapsed/refractory and chemosensitive non Hodgkin's lymphoma (NHL).
Aims: The most used conditioning regimen in NHL is the carmustine containing protocol BEAM. Chemotherapy toxicity is associated with early and late onset complications. Fotemustine is a third generation chloroethyl-nitrosurea that in previous studies showed a better toxicity profile and good results in terms of survival compared to carmustine.
Methods: In our study, we used a fotemustine based regimen as conditioning pre ASCT in 6 patients with relapsed NHL. 3 patients were affected by aggressive NHL and 3 patients were affected by follicular lymphoma. Salvage chemotherapy was R-DHAP in 5 patients and R-IEV in 1 patient. After salvage treatment 4 patients were in complete remission (CR) and 2 patients were in partial remission (PR). All patients received FEAM regimen according to the following schedule: fotemustine 150 mg/m2 on days -7, -6, etoposide 200 mg/m2 and aracytin 400 mg/m2 on days -5, -4, -3, -2 and melphalan 140 mg/m2 on day-1. In all patients CD34+ cells source was peripheral blood and the median number of infused cells per patient was 2.75 x 10^6 CD34+/Kg (range 2-5.5).
Results: Median time to neutrophil recovery (>500 x 10^9/l) was of 8.5 days (range 5-11), median platelets recovery (>20.000 x 10^9/l) was of 9 days (range 4-20).All patients required platelets support and 2 patients also received RBCs infusions. 3 out of 6 patients experienced G3/G4 mucositis. Neutropenic fever occurred in 5 patients due to bacterial sepsis. Median follow-up was 10.5 months (range 4-13). Transplant related mortality at 100 days was 0%. Currently, 5 patients are in CR and 1 patient is in PR.
Summary / Conclusion: In our experience, fotemustine containing regimen showed an acceptable acute toxicity profile. In all patients, hemopoietic engraftment occured within the expected timing. Considering the short follow-up of our study, FEAM efficacy in terms of long term outcome and survival will require a longer observation to be evaluated.

B1717

EFFICACY AND TOXICITY OF BUMEL CONDITIONING REGIMEN IN AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA

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Background: Autologous stem cell transplantation (SCT) is still the standard treatment for patients with multiple myeloma (MM) who are candidates for high dose chemotherapy regimens. The classic conditioning regimen is Melphalan 200 mg/$m^{2.}$ The combination of Melphalan and oral Busulphan leads to increased disease-free survival with the counterpart of a greater toxicity, being sinusoidal obstruction syndrome (SOS) the most limiting complication. Intravenous Busulphan appears to maintain good responses whilst decreasing the toxicity associated with the oral formulation.
Aims: To evaluate the security of Busulphan-Melphalan (BUMEL) intravenous combination as a conditioning regimen for autologous stem cell transplantation (ASCT) in patients with multiple myeloma.
Methods: Conditioning regimen: BUMEL (Melphalan 140 mg/m^2 day -2 plus Busulphan 3.2 mg/Kg once daily days -5, -4 and -3). Patients: n=10. Average age: 56 years (range 39-66). First ASCT: 1; second ASCT: 9. Status pre-ASCT: complete remission (CR): 1 (10%), very good partial response (VGPR): 2 (20%), partial response (RP): 6 (60%), EE: 1 (10%). All of them received pegilated G-CSF on day +5. Stem cells were obtained after G-CSF mobilization. Average CD34+ cells: 2.24x10^6/Kg (range: 1.9-3.5).
Results: Neutrophil and platelet engraftment time were 11 (range: 10-13) and 13(range: 10-15) days respectively, with nil engraftment failures. TRM was 0% and OS at 375 days was 100%. The regimen had good tolerability (table 1). The only grade 3-4 toxicity found was mucositis which affected 3 patients (43%). Other important complications included 2 proven IFI (29%). Average length of hospitalization was 25 days (range: 16-44). Disease status evaluation at day +90 in 8 patients showed: 2 CRs (pre-ASCT status was PR and VGPR), 4 CR and 2 PR.
Summary / Conclusions: BUMEL conditioning regimen with unique daily dose of intravenous Busulphan was a safe option, even for patients submitted to a second ASCT. Our experience agrees with the published literature data revealing an effective and poor toxicity regimen. However, overall survival evaluation of BUMEL in prospective studies is still pending.

B1718

SALVAGE HEMATOPOIETIC STEM CELL TRANSPLANTATION WITHOUT CONDITIONING REGIMEN FOLLOWED BY CYCLOPHOSPHAMIDE AND MESENCHYMAL STEM CELLS (MSC) AS GRAFT-VERSUS-HOST DISEASE (GVHD) PROPHYLAXIS

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Background: Sometimes in clinical practice we have to break the canonical laws of HSCT regarding conditioning regimens, immunosupression protocols and inclusion criteria (f.e., absence of full blow infections). Transplant related mortality is strictly associated with these rules. But nowadays, new options appeared (such as cyclophosphamide (CY) as single agent for GVHD prophylaxis: Luznik et al., 2010) allowing us to be more flexible and confident in critical decisions.
Aims: We report here a 51-years-old man in 1st molecular CR of acute myeloid leukemia (AML) with t(8;21) who developed extremely severe and prolonged bone marrow aplasia (WBC<0.05x10^9/L more than 25 days), confirmed by threphanobiopsy, after consolidation with intermediate dose of Ara-C (1g/m^2 bid 1-3 d) and Mitoxantrone (10 mg/m^2 3-5 d) During that period he developed poorly controlled invasive aspergillosis (treated with voriconazole and caspofungin), Pseudomonas aeruginosa's sepsis.
Despite severe infections as salvage-therapy, he was transplanted from his HLA-identical sibling donor on day 30 after consolidation course. Bone marrow was infused in intensive care unit where the patient was transferred due to altered consciousness. There was no conditioning regimen and standard immunosuppression during post transplant period, except CY given at dose 50 mg/kg on day +3 after HSCT.
Results: WBC recovered at day +19. On day +20, 1*10^6/kg of MSC were infused for acute GVHD prophylaxis. At day +30 85% of donor chimerism was detected in the bone marrow. His infection complications slowly regressed and at day + 30 the patient had no fever.
At day +48 after BMT the patient developed aGVHD with skin and liver involvement (stage 2). After initiation of cyclosporine A (CSA) all aGVHD signs fully regressed. At day + 90 the patient was discharged from the hospital with full donor chimerism.
Summary / Conclusion: So, our case demonstrates, that 1) HSCT without conditioning regimen is possible if the patient has prolonged and profound aplasia after chemotherapy; 2) only WBC recovery due to HSCT made it possible to control infection; 3) CY and MSC as sole GVHD prophylaxis can be very effective in patients in whom standard immunosuppression is contraindicated.

B1719

CORD BLOOD TRANSPLANTATION FOLLOWING HAPLOIDENTICAL DONOR LYMPHOCYTE INFUSION IN A CASE OF REFRACTORY T-CELL ACUTE LYMPHOCYTIC LEUKEMIA; A NEW CONCEPT

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Background: Although a potent graft-versus-leukemia (GVL) effect is expected to work in haploidentical stem cell transplantation, fatal alloreactive complications such as graft-versus-host disease (GVHD) remain major obstacles in such patients. In contrast, relapse is a major concern in patients who receive cord blood transplantation (CBT).
Aims: To improve the outcome in chemo-refractory pediatric leukemia, we developed a new method incorporating haploidentical donor lymphocyte infusion (DLI) prior to CBT. The study was approved by the institution review board of Nihon University Itabashi Hospital.Written informed consent was obtained for parents of the patient in accordance with the Declaration of Helsinki.
Methods: The patient was a 5-year-old boy with T-cell acute lymphocytic leukemia who relapsed just before commencing maintenance therapy. He did not respond to various types of chemotherapy, and thus, a potent GVL effect was deemed necessary. Our new concept in the current study is to cause a short-term pure GVL to eradicate the leukemic cells using haploidentical lymphocytes, and then replace them with less alloreactive cord blood. The first conditioning step consisted of fludarabine (30 mg/m^2/day, day -15 to -12), cytarabine (2 g/m^2/day, day -15 to -12) and cyclophosphamide (60 mg/kg/day, day -11). At day -9, the patient received unmanipulated DLI from a HLA 3 loci mismatched related donor (father). The infused CD3+ T-cell number was 2.7 x 10^7/kg. At days -2 and -1, the patient received the second conditioning with total body irradiation (3 Gy/day) and dexamethasone to eliminate the haploidentical donor lymphocytes and prevent rejection of the stem cell graft. At day 0, the patient underwent HLA 1 locus (DR) mismatched CBT. The infused cell number was 6.5 x 10^7/kg. Cyclosporine A (CsA) with short-term methotrexate was used for GVHD prophylaxis.
Results: At 3 days following DLI, he developed a high-grade fever, severe diarrhea and skin eruption, symptoms similar to acute GVHD. Thereafter, his general status worsened and he developed disseminated intravascular coagulation. At day -2, haploidentical donor lymphocytes were present in the bone marrow (donor type: 12.2%, recipient type 87.8%) and peripheral blood (donor type: 23.5%, recipient type 76.5%). After the second conditioning, his general status and laboratory data immediately improved. Since no alloreactive signs such as acute GVHD were found after CBT, CsA was tapered off at day 17. Donor-type neutrophil engraftment was observed at day 22; however, a leukemic blast emerged at the same time. Thereafter, the leukemic blast count increased rapidly and the patient died at day 56.
Summary / Conclusion: Our method was similar to the 2-step technique pre-

viously reported by Grosso D et al. Since the patient achieved donor-type engraftment and showed no post-transplant complications, the method was deemed feasible. Nevertheless, the anti-leukemic effect was insufficient to eradicate the malignant clone in this patient. This short-term GVL effect (from days -9 to -3) might therefore have been too short to kill the residual blasts, but since the haploidentical lymphocytes induced severe GVHD-like organ damage, elongation of the incubation time seemed dangerous. The efficacy of this new approach requires detailed evaluation in more patients.

B1720

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN RELAPSED OR REFRACTORY AGGRESSIVE NON HODGKIN LYMPHOMA WITH ACTIVE DISEASE

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Background: According to the current guidelines an autologous hematopoietic stem cell transplant (HSCT) is considered the best choice as salvage for aggressive non Hodgking lymphomas relapsed or refractory (r/r) to standard treatment. Allogeneic approach seems to be harmful in fact it's proposed as a second line treatment, due to presence of GvHD and a heavier transplant related mortality (TRM) now its role is limited as a third line for the restricted number of patients still eligible after a long history of aggressive chemotherapy. Nevertheless Autologous HSCT requires a useful stem cell harvest and is precluded for the poor mobilizers, accounting for the 25% of the candidates; moreover though it can rescues 45% of chemo-sensitive patients its effectiveness falls to 10-20 % for the refractory ones. The disease status at transplant is the most relevant factor influencing the disease free and overall survival: patients with active disease usually die early (within 6 months) due to progression or relapse. For these r/r patients an early allogeneic HSCT could be the better choice.
Aims: We report a small series of patients (n=9) with r/r aggressive non Hodgkin lymphoma that undergo allogenic HSCT with active disease.
Methods: Our series included 4 diffuse large B cell (DBLCL), one of them was HIV+, 2 peripheral blood T cell (PTCL), 1 mediastinal lymphoblastic B cell,1 mantle cell (MCL), 1 T Angioimmunoblastic. All the patients were refractory or relapsed after standard treatment and have a relevant disease burden assessed by clinical or histological features and CT-PETscan, 4 patients previously undergone autologous-SCT. Five patients received a reduced intensity conditioning course (Tiothepa-Flu-Cy with or without rituximab) and the other 3 received a myeloablative course (BuCy or BuFlu or Tiothepa-BuCy). Patients were allografted from HLA identical donors related (n=5) or unrelated (n=2) or haploidentical related donor (n=1).
Results: All patient experienced III-IV degree of mucositis and 1 patient had a major infectious episode, nevertheless all reached a good engraftment and were in complete remission (CR) at the hospital discharge. The mediastinal lymphoma patient relapse early after the transplant and died due to relapsed disease. Two patient (1 PTCL and the HIV+ DBLCL) relapsed early too but were rescued with tapering of immunosuppressive treatment, without chemotherapy nor DLI infusion.After a median follow up of 18 months (range 2-33 months) 6 patients are alive in CR, 2 patients died, in CR, due to hemorrhagic cystitis and cerebral hemorrhage. Only one patient developed a chronic GvHD with sclerodermic features but limitated. The ORR is 100% (9/9 CR).
Summary / Conclusion: We didn't find any difference in toxicity among patients that previous undergone or not autologous SCT. Despite the short follow-up and the small number, the clinical course of our patients enlighten a major role of the immunological pathway to induce and maintain the disease remission by a graft versus lymphoma effect. It suggests that allogeneic transplant is a suitable and feasible choice as second-line treatment in relapsed or refractory lymphoma, instead of autologous HSCT.

Hematopoiesis, stem cells and microenvironment

B1721

COMBINED EFFECTS OF MESENCHYMAL STROMAL CELLS (MSC) AND STIMULATORY CYTOKINES ON THE *ex vivo* GROWTH OF UMBILICAL CORD BLOOD HEMATOPOIETIC PROGENITOR CELLS (UCB-HPC).

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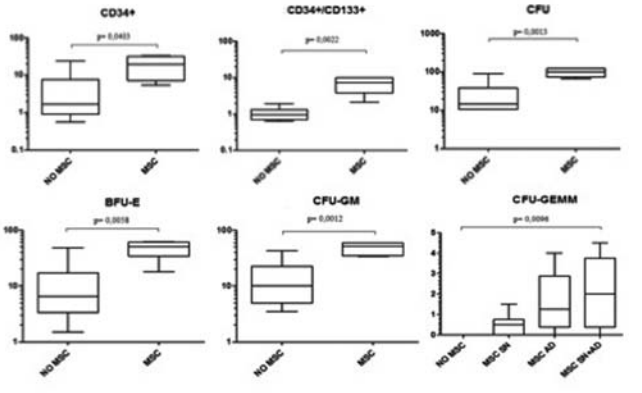
Background: Mesenchymal stromal cells (MSC) are a component of the hematopoietic niche. Due to their ability to support UCB-HPC expansion, MSC can be used for *ex vivo* experiments, in order to enhance the engraftment of adult patients who underwent UCB allogeneic stem cell transplantation (UCB-allo-SCT).
Aims: To set-up MSC and UCB-HPC co-culture experiments in order to better support UCB-allo-SCT in adult patients.
Methods: We have analyzed six previously frozen units of UCB. Cells were cultured in a serum free medium containing three different cytokines: granulocyte colony-stimulating factor (G-CSF), stem cell factor (SCF) and thrombopoietin (TPO), either with or without a layer of human bone marrow MSC. After 10 days we have evaluated the expansion rate of harvested cells grown with MSC both in suspension and directly adherent to the mesenchymal layer, and also of those grown without MSC. We have studied the immunophenotype of each population with a flow cytometry panel of CD45-FITC, CD34-PE, CD133-APC and 7-AAD. Furthermore the clonogenic capacity of these populations has been evaluated after a further 14 days culture in methylcellulose.



Labels:
NO MSC: colture of UCB-HPC in presence of cytokines only
MSC: co-colture of MSC+UCB-HPC in presence of cytokines
MSC SN: supernatant fraction of the co-colture MSC+UCB-HPC
MSC AD: adherent fraction of the co-colture MSC+UCB-HPC
MSC SN+AD: superanatant and adherent fraction of the co-colture MSC+UCB-HPC

Results: Ten days after seeding, a2,27-fold increase (range 0,80-15,93) in CD34+ cells and a1,04-fold increase (range 0,22-8,75) in CD34+/CD133+ cells have been obtained by cells cultured in absence of MSC. On the other hand, in cells co-cultured with MSC we have obtained a 17,14-fold increase (range7,77-33,26) in CD34+ cells and a4,87-fold increase (range1,94-9,86) in CD34+/CD133+ cells. Clonogenic assay confirms our data: we have registered a higher number of colony forming units (CFU) in UCB cells co-cultured with MSC, in comparison with cells grown without the MSC layer (respectively median of 100,3, range 65-123, and median of 14,8, range 10,5-91; P=0,0013). These data are confirmed in each kind of CFU subpopulation: BFU-E, CFU-GM and CFU-GEMM. No immature CFU have been detected in the cells grown without MSC, in comparison with the large number registered in the cells grown in presence of the MSC layer (P=0,0096). Moreover we have noticed that the number of CFU-GEMM was higher in the population of cells grown adherent to the MSC layer, in comparison with the number of CFU-GEMM registered in the population of cells grown in the supernatant fraction of the co-culture.
Summary / Conclusion: Our study strongly suggests that MSC act in a synergic manner on UCB-HPC expansion, thereby the co-culture of UCB-HPC with MSC and specific cytokines could represent a good approach to overcome the problem of the limited number of UCB-HPC in adult UCB-allo-SCT.

Work supported by project "Regione Lombardia-Piano Regionale Sangue", "Lions Bassa Bresciana" association and "Banca Credito Cooperativo di Pompiano e Franciacorta" donation)

B1722

INDIVIDUAL PECULARITIES OF MULTIPOTENT MESENCHYMAL STROMAL CELLS DERIVED FROM BONE MARROW OF HEALTHY DONORS

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Background: Multipotent mesenchymal stromal cells (MMSCs) are widely used for immunomodulation in the treatment of autoimmune diseases and graft versus host disease. The effect of such treatment varies significantly. The efficiency of the MMSCs could depend on the individual features of these cells.
Aims: The aim of the study was to investigate the individual characteristics of healthy donors' MMSCs.
Methods: Bone marrow samples were collected from healthy donors during the aspiration for allogeneic hematopoietic stem cell transplantation after informed consent. MMSCs from 50 donors (24 females and 26 males, age 13 – 59, median 32.5 years) were cultured in MEM alfa media supplemented with 10% fetal calf serum. Cumulative MMSCs production was counted after 5 passages. Colony forming unit fibroblast (CFU-F) concentration in bone marrow samples was analyzed in 14 days as additional characteristic of stromal cells. Gene expression level in 1st passage MMSCs was estimated by real-time PCR. The relative expression levels were calculated using the $\Delta\Delta C_t$ meth

Gene	Relative expression level (Mean ± standard error)				
	All	Male	Female	Younger than 32	Older than 32
BMP-4	1.1 ± 0.2	0.9 ± 0.7	1.3 ± 0.0	1.1 ± 0.3	1.0 ± 0.3
FGFR1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
FGFR2	1.7 ± 0.3	1.9 ± 0.4	1.4 ± 0.2	2.2 ± 0.3	1.2 ± 0.3
SPP1	0.8 ± 0.3	0.8 ± 0.6	0.8 ± 0.3	1.0 ± 0.6	0.6 ± 0.3
PPARg	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.6 ± 0.1
IL6	3.9 ± 0.8	3.9 ± 1.0	3.9 ± 1.5	4.8 ± 1.5	3.2 ± 1.0
CFH	0.9 ± 0.2	0.8 ± 0.3	1.1 ± 0.4	1.2 ± 0.4	0.7 ± 0.3
IDO1	2.1 ± 0.6	2.2 ± 0.9	1.9 ± 0.9	1.7 ± 0.7	2.3 ± 1.0
PTGES	2.2 ± 0.2	2.4 ± 0.2	1.8 ± 0.3	2.4 ± 0.3	2.1 ± 0.3
CSF1	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	1.0 ± 0.2	0.7 ± 0.1
PDGFRα	1.0 ± 0.1	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2

Results: There were no significant differences between concentration of CFU-F and cumulative MMSCs production in male and female donors. The age of MMSCs donor appeared to be very important for CFU-F concentration and MMSCs cumulative production. The concentration of CFU-F was 2.5 fold higher (P=0.02) and MMSCs production was increased 4 fold (P=0.01) in bone marrow of donors younger than 32 years. The expression level of FGFR2 was about 2 fold higher (P=0.02) in MMSCs of young donors (Table). It suggests the importance of this gene for growth characteristics of MMSCs. The expression level of other factors participating in cell growth genes (BMP4, FGFR1, PDGFRa) did not depend on donors' age and sex. As MMSCs population is heterogenic the genes marking the maturation rate were studied in MMSCs not induced to differentiation. The marker of adipose differentiation (PPARg) was 2 fold lower in young donors (P=0.02) without associations with sex. Osteogenic differentiation marker (SPP1) was higher in young donors, especially in males, but the difference was not significant. The expression level of immunomodulating genes varied individually but independently of sex and age.
Summary / Conclusion: MMSCs population is heterogeneous. There are significant individual variations in growth parameters and genes' expression in MMSCs of different peoples. Some of these characteristics could be important for therapeutic action of these cells.

B1723

OSTEOGENIC MESENCHYMAL STEM CELLS ARE ABLE TO PROMOTE THE PRESERVATION OF CD34+CD38- POPULATION OF HEMATOPOIETIC STEM CELLS

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Background: Hematopoietic progenitor/stem cell (HPSC) transplantation is the treatment of choice for a variety of malignant and nonmalignant diseases. To improve the therapeutic outcomes of transplantation one potential strategy is *ex vivo* expansion of HPSC.
in vivo HPSC are located in the specialized bone marrow (BM) niche that is supposed to support HPSC proliferation, differentiation and maintain their quiescence. Despite numerous studies, mechanisms of cell–cell interaction between HPSC and niche microenvironment remain unclear. For this reason, no methodology for successful expansion of HPSCs that could support efficient hematopoiesis has yet been established.
Aims: To estimate the impact of stromal feeder layer on the functional, phenotypic, and clonogenic parameters of HPSC at different oxygen tension conditions.
Methods: We investigated the interaction of CD34+ HPSC (obtained from healthy donor BM mononuclear cells and enriched with CD34+ fraction) with mesenchymal stem cells (MSC) without induced differentiation (ndMSC) or MSC stimulated into osteogenic differentiation (osteoMSC) in cytokine – based coculture system (IL-3 50ng/ml, SCF 150ng/ml, Flt-3L 150ng/ml). In view of critical role of oxygen in stem cell quiescence, coculture proceeded upon hypoxic (pO_2=5%) and normal oxygen conditions. HPSC parameters such as expansion, phenotypic and clonogenic characteristics were evaluated separately for suspensive and adhesive fraction of HPSC after5, 10 and18 days of coculture. The most significant immunophenotypic characteristic for both fractions was the presence of $CD34^+CD38^-$ primitive population and its preservation during long term coculture.
Results: Total multiplication of HPSC cocultured with osteoMSC versus ndMSC was 2.6±0.5 vs 1.12±0 fold after 5 days; 40.2±6.4 vs 33.6±9.6 fold after 10 days; 147.8±58.1 vs 153.2±20.2 fold after 18 days.The expansion of primitive $CD34^+CD38^-$ fraction in HPSC during co-culture with osteoMSC was on 5th day 2.4±0.5 fold, 10th day 31.2±7.0 fold, 18th day 22.5±14.8 fold vs coculture with ndMSC-4.5±2.5, 40.6±19.5, 22.9±17.4 fold, accordingly. Expanded suspensive and adhesive fractions were evaluated for their ability to preserve primitive population. The maximum increment of $CD34^+CD38^-$cells in suspensive fraction was observed on 10th day coculture and did not depend on differentiation of MSC, while osteoMSC demonstrated better preservation $CD34^+CD38^-$ population in adhesive fraction (Figure 1.). CFU-frequency evaluated on 10000 seeded HPSC on 5th day coculture with ndMSC in suspensive fraction was 875±219 colonies, in adhesive fraction-661±366 colonies versus 1053±283 colonies (in a.o. erythroid colonies) and 190±0 after coculture with osteoMSC, accordingly. Total expansion of HPSC cocultured with ndMSC under atmospheric oxygen conditions was higher than at hypoxic conditions and was 157.2±27.0 vs 67.6±16.0 fold on 18th day (P<0.05, n=3). Preservation of total CD34+CD38- fraction upon hypoxic conditions and normal oxygen tension was comparable and was better in suspensive fraction in both cases. After 5 days of coculture, HPSC grown under hypoxic and normoxic conditions were able to give CFU-GM and CFU-GEMM in equal ratios. Upon hypoxia adhesive fraction of HPSC was induced to give predominantly erythroid colonies on long term coculture.

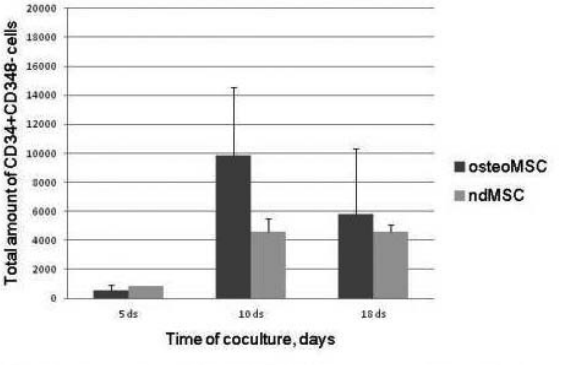

Fig.1. Dynamic of $CD34^+CD38^-$ increment in adhesive fraction during coculture with ndMSC and osteoMSC

Summary / Conclusion: OsteoMSC demonstrated better preservation $CD34^+CD38^-$ population in adhesive fraction. Low oxygen tension favored to increase erythroid progenitors from adhesion fraction of HPSC.Further investigations required.

B1724

BONE MARROW ENDOTHELIAL CELLS, PROGENITOR AND MATURE, IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA, SOLID TUMORS AND IMMUNE CYTOPENIAS

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Background: The role of endothelial progenitors (EPC) and mature cells (EC) in angiogenesis of malignant and blood diseases has been the focus of many recent studies.
Aims: The quantitation and immunophenotypic characterization of EPC and EC subpopulations in the bone marrow (BM) of children with acute lymphoblastic leukemia (ALL), solid tumors (ST) and immune cytopenias.
Methods: BM cells from children with ALL at diagnosis (ALLd, n=15), day 15 (ALL15d, n=12), day 33 of treatment when remission is achieved (ALL33d, n=11), at consolidation (ALLct, n=22), at the end of treatment (ALLet, n=21), solid tumors without BM involvement at diagnosis (ST, n=11) and under treatment (STth, n=11) and immune cytopenias (n=10) were studied. The putative

antigenic phenotypes of EPC and EC were assessed using a 4-color flow cytometry in the CD45negative cell subpopulation.
Results: The highest levels of EPC subpopulation immunophenotypically characterised by CD45negCD133$^+$CD34$^+$VEGFR-2$^+$ were estimated at diagnosis of ALL
with statistically significant differences compared with ALL33d (0.0869±0.0571 vs 0.01±0.0067, P=0.023), ALLet (0.0869±0.0571 vs 0.0185±0.0130, P=0.024) and cytopenias (0.0869±0.0571 vs 0.0051±0.0033, P=0.039). As far as ST is concerned, the highest levels of the triple combination of EPCs were determined at diagnosis (0.0176±0.0069). The levels of CD45negCD133+VEGFR-2+EPCs were also higher in ALLd (0.1144±0.0398) with statistically significant differences compared with ALL33d (0.1144±0.0398 vs 0.0473±0.0339, P=0.031) and ST (0.1144±0.0398 vs 0.028±0.0066, P=0.043). In addition, the highest levels of CD45negCD34+VEGFR-2+EPCs were determined in ALLd (2.6197±2.5522) with statistically significant differences compared with ALL33d (2.6197±2.5522vs 0.0436±0.0277, P=0.031), and ST (2.6197±2.5522 vs 0.0227±0.0065, P=0.032). For both the above EPCs subpopulations, there were found to be higher in ST under treatment compared to diagnosis.The highest levels of intermediate maturity combinations such as CD45negCD146+CD34+ were observed in ALLd with statistically significant differences compared with ALL33d (0.216±0.0569 vs 0.0460±0.0177, P=0.014) and the CD45negCD146$^+$CD34$^+$VEGFR-2$^+$ was also found to be highest at diagnosis of ALL (0.0288±0.0199) compared to low levels at ALL33d (0.0037±0.0035). The more mature EC subpopulation CD45negCD31$^+$CD34$^+$VEGFR-2$^+$ was estimated to be highest in the group of ALL15d (0.0263±0.0104) and ST under treatment (0.024±0.0186) followed by ALL at diagnosis (0.0152±0.0064). Statistical analysis revealed significant differences between ALL15d vs ALL33d (P=0.011) ALL15d vs ALLct (P=0.016) ALL15d vs ST (P=0.042).The levels of CD45negCD31+VEGFR-2+subpopulation were also higher in ALL15d with statistically significant differences compared with ALL33d (0.1542±0.0480 vs 0.0642±0.0293, P=0.037). This subpopulation was also found to be high in immune cytopenias (0.1589±0.0737).
Summary / Conclusion: At diagnosis of ALL the levels of both progenitor and more mature endothelial cells seem to be highest. Induction of remission is associated with the lowest levels of EPC and EC subpopulations. At solid tumors the progenitor endothelial cells' subpopulations were found to be more prominent while in immune cytopenias the levels of more mature ECs were found to be higher than EPCs one. The precise role of these findings warrants further investigation.

B1725

NEUTRALIZATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR-C INDUCES THE EXPRESSION OF FMS-LIKE TYROSINE KINASE 3 IN INTERLEUKIN 1-BETA-STIMULATED ADULT HUMAN DERMAL FIBROBLASTS

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Background: We previously reported that human interleukin 1-beta (IL-1-b) stimulated bone-marrow stromal myofibroblasts to express CD34 molecule. We also reported that when adult human dermal fibroblasts (HDFs) were cultured with IL-1-b and erythropoietin (EPO), hematopoiesis-related molecules were induced to express (17th EHA). And, when HDFs were cultured in knockout (k/o) DMEM and IL-1-b, lymphatic duct neogenesis-related genes expressed, and vascular endothelial growth factor (VEGF)-C was produced significantly (74th JSH, and 54th ASH).
Aims: To understand better the hematopoiesis and lymphangiogenesis, we observed effects of anti-VEGF-C-neutralizing antibody (Ab) when HDFs were cultured with IL-1-b.
Methods: HDF were purchased, and cultured in k/o DMEM with 20% KSR and recombinant human IL-1-b for two weeks with or without anti-human VEGF-C Ab. The morphological changes and the expressions of hematopoietic and lymphangiopoietic molecules were analyzed time-dependently. Then, HDFs were further cultured in DMEM/F12 with hematopoietic cytokines, and were characterized biologically.
Results: When HDFs were cultured with human IL-1-b for 2 weeks, the cellular morphology changed to the filamentous appearance. RT-PCR analyses revealed that the culturing cells expressed Prox-1, VEGF receptor type-3, Smad7, and PAI1; however, when anti-human VEGF-C Ab was added to the cultures, these lymphduct-neogenesis genes were down-regulated, and fms-like tyrosine kinase (FLT) 3 was expressed significantly. When HDFs were further cultured in DMEM/F12 with human stem cell factor, IL-6, FLT3 ligand, EPO, and granulocyte colony-stimulating factor, CD45 was induced to express, and these cells formed hematopoietic colonies.
Summary / Conclusion: When HDFs were cultured in k/o DMEM, a growth-condition for an embryonic stem cell, and with IL-1-b, several molecules relating to a lymphangiogenesis expressed, and they were down-regulated when VEGF-C neutralizing Ab was added to the cultures. By the addition of VEGF-C Ab FLT3 expressed in HDFs, and, when HDF were further cultured with hematopoietic cytokines, hematopoietic markers including CD45 were induced to express significantly. Currently we are validating the precise molecular mechanism on the expression of FLT3 in IL-1-b-stimulated HDFs in the presence of VEGF-C Ab using a microarray analysis, and also characterize the generated cells *in vivo* using an immunodeficiency murine transplantation-model.

B1726

THE RELATIONSHIP BETWEEN OXIDANT INJURY AND INSULINE RESISTANCE IN PATIENTS WITH FANCONI APLASTIC ANEMIA

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Background: Fanconi aplastic anemia (FAA) is an autosomal recessive chromosome instability disorder characterised by bone marrow failure. DNA repair mechanism is impaired in FAA and FA cells are intolerant of oxidative stress. Reactive oxygen species (ROS) could damage DNA and inability to repair of FAA hematopoeitic stem/progenitor cells (HCPCs). Diabetes is a common comorbidity associated with FAA. ROS have been proposed to play a causative role in insulin resistance (IR). Another cause of type 2 diabetes in FAA is elevated serum ferritin level. Increased serum ferritin concentration causes IR.
Aims: To investigate the relationship between oxidative injury, IR and iron load in patients with FAA. In addition, to indicate the risk factors to play a role in developing of IR in FAA patients.
Methods: Twenty-three patients (11 girls, 12 boys) ages between 4-19 years-old with FAA and 10 controls who were healthy ages between 4-17 years-old were included in the multicentre study. Samples for determination of complet blood count (CBC), alanin aminotransferase (ALT), aspartat aminotransferase (AST), gamma glutamiltransaminase (GGT), C-reactive protein (CRP), fasting blood glucose and simultaneous insulin, hepcidin, ferritin, 8-Oh deoxyguanosine (8-OHdG), protein cabonyl groups, malondialdehyde (MDA) and HOMO-IR were drawn in the patients and controls. The serum ferritin levels were performed monthly in the patients with high serum ferritin level and receiving iron chelating therapy. All tests were repeated when the serum ferritin level was decreased by at least 25% according to a previously determined serum ferritin level. Chromosomal breakages were investigated by incubation with diepoxybutane (DEB) of the peripheral lymphocytes of all patients, in-vitro. The patients with a positive DEB test were received as FAA, those with a negative DEB test were received as non-FAA. Statistical analyses were done with 'SPSS 13 (Statistical Package for Social Screnu) for Windows©' program.
Results: DEB test was positive in 16/23 patients.Bone marrow transplantation was done in seven patients.Three cases who were transfusion-dependent were receiving a chelating agent (Deferisirox) for iron overload. IR was present in 12 cases. 8-OH dG and MDA levels in FAA patients were significantly higher than those in controls (P=0.001 and P=0.002, respectively). There were no statistically significant differences between the serum hepcidine and the protein carbonyl levels in FAA and controls, separately (P>0.05). Serum ferritin level and IR were higher in FAA patients than controls (P=0.000, P=0.002). IR was significantly higher in patients with DEB test positive (10/16 patients) than those in patients with DEB test negative (2/7 patients) (P=0.03).There were not significant differences between serum ferritin, hepcidine, MDA, protein carbonyls, CRP and 8-OH dG levels in DEB tests positive and negative patients, separately (P>0.05). Five FA patients had transfusion-dependent and they were received packed red blood cell transfusions every 3-6 weeks. Four for five patients had IR and serum ferritin levels of the patients were 435-6702 ng/ml, serum ALT levels were 21-110 IU and serum GGT levels were 29-31 IU. There were significant differences between 8-OHdG, ferritin and MDA levels in patients with IR and without IR, separately (P=0.009, P=0.001 and P=0.013, respectively). There was a good correlation at the rate of 56.8 % between IR and ferritin level (P=0.05). The mean serum ferritin levels were 168.8±27.9 ng/ml in patients without IR and 1586,6±2436,7 ng/ml in patients with IR. There were no a good correlation between IR and hepcidine level and between ferritin and hepcidine levels (P=0.48 and P=0.95, respectively). There were not statistically significant correlation between serum ferritin and serum hepcidine, protein carbonyl, 8-0H dG, MDA levels, IR in 21 patients having serum ferritin levels lower than 1000 ng/ml, separately (P>0.05).
Summary / Conclusion: Lipid peroxidation and DNA oxidation due to increased ROS have been developed in FAA. FAA patients are under the risk of IR and diabetes mellitus followed by oxidative injury. There is a good correlation between IR and serum ferritin level in FAA. Body iron load and serum ferritin level play an important role to develop IR and diabetes mellitus in FAA in addition to oxidative stress.

B1727

NEW ASPECTS OF NORMAL BONE MARROW VALUES FOR MEGAKARYOPOIESIS FROM 110 HEALTHY BONE MARROW DONORS AND NORMAL BONE MARROW VALUES FROM 236 HEALTHY BONE MARROW DONORS

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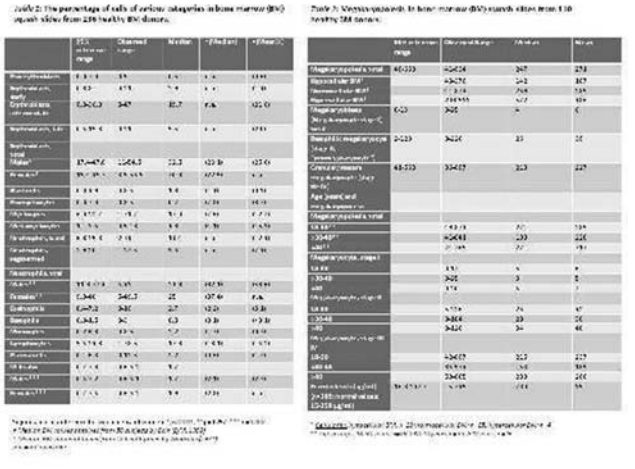
Background: In 1974, M.M. Wintrobe described a differential count of bone marrow (BM) aspirates from 12 healthy men. Twenty years later, Barbara Bain assessed the percentage of cells in BM films of 50 healthy subjects. Until today most laboratories rely on these normal BM values obtained from only small numbers of healthy subjects. Bain also described a varying number of megakaryocytes (6-77, mean 31) on wedge-spread BM films. Kenneth Kaushansky describes a prevalence of 20% megakaryoblasts, 25% basophilic megakaryocytes and 55% mature megakaryocytes within a normal BM (Wintrobe´s Clinical Hematology, 12th edition).
Aims: We gathered BM squash slides from over 400 healthy BM donors harvested over a 4 year period at our institution to investigate normal hematopoiesis.
Methods: BM squash slides from 236 healthy unrelated BM donors were examined independently by three experienced morphologists for normal BM values and 110 slides for megakaryopoiesis.. All volunteers underwent predonation health check-up about four weeks prior to the harvest. During the BM harvest, samples for this study were taken from the first aspiration.
BM cellularity, megakaryocyte numbers and morphology were assessed by examination at a 20fold magnification. Morphology of myeloid and erythroid cells was assessed using a x100 objective and cells being counted in the trails behind the particles. Each morphologist had to do a 200 differential cell count. Megakaryopoiesis was assessed for total counts and differentiated between megakaryoblasts, basophilic megakaryocytes and mature megakaryocytes. BM preparation and assessment was performed according to WHO, ICSH guidelines and the ELN recommendations (*Zini et al., 2010*).
Results: Normal values obtained from a 600 cell count are described in *T*able 1 together with the median values described by Bain and Wintrobe, respectively. Peripheral blood counts were in the normal range.



Compared to results described by Bain and Wintrobe, relevant differences can only be seen for the promyelocyte count. In contrast, significant differences between male and female donors were seen with regard to the M:E ratio and the percentage of erythroblasts , as formerly described by Bain, but not for the percentage of neutrophils. Megakaryopoiesis showed a high variety depending on cellularity with a mean of 271 for total megakaryopoiesis (Table 2). The percentage of megakaryoblasts accounted for 2%, promegakaryocytes 14% and mature megakaryocytes 84%, respectively. In donors between the age of 30-40 years, megakaryopoiesis appears to be less compared to younger or older ones.
Summary / Conclusion: According to our data, most of the formerly described normal BM values by Bain and Wintrobe were confirmed by a much larger cohort. The total median megakaryocyte counts seen in our study differ significantly from those described by Bain which might be explained by the fact that megakaryopoiesis was evaluated in BM squash slides and the larger cohort in which megakaryopoiesis was evaluated. According to our data, the prevalence of different maturation states also differ significantly compared to the data of Kaushansky, which might be due to the fact that megakaryopoiesis was evaluated primarily on trephine biopsies.

B1728

EXPERESSION AND REGULATION OF ERYTHROID DIFFERENTIATION RELATIVE FACTOR (EDRF) IN ERYTHROD DIFFERENTIATION

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Background: Erythroid differentiation relative factor (EDRF), also called Human α-hemoglobin stabilizing protein (AHSP), was an erythropoiesis related factor expressed only in erythroid cells. It played important roles in the production of Hemoglobin A by stabilizing free alpha-globin. But how it was regulated in erythropoiesis was still not very clear.
Aims: To investigate the expression of EDRF during erythropoiesis and to explore the mechanism of its regulation.
Methods: The expression of EDRF in mice fetus liver tissue and in erytholeukemia cell line HB22.2 which was deficient in erythroid differentiation was detected by Northern blot method, the DNA transmutation was detected by Southern blot method. The change of EDRF expression during erythoid differentiation was observed by using erythroleukemia cell line HB60-5 which could be induced to differentiation to mature erythrocytes by changing culture condition with different combination of several cytokines. And the regulation of EDRF expression by erythroid transcription factor GATA-1, NF-E2 and Fli-1 was detected with transgenic method.
Results: EDRF was high expressed in normal erythroid tissue, however the high expression of EDRF was greatly suppressed accompanied with the block of erythroid differentiation in erythroleukemia cells, and the abnormal of EDRF was not caused by DNA aberration. The expression of EDRF was gradually evaluated during the erythroid differentiation of HB60-5 cells. The transfection of NF-E2 into HB22.2 cells could restore the expression of EDRF and the transfection of GATA-1 into HB60-5 cells could promote EDRF expression, however, the transfection of Fli-1 into HB60-5 cells suppressed EDRF expression.
Summary / Conclusion: EDRF was an erythroid specific factor. It was related with erythoid differentiation and was highly expressed in normal Hematopoietic tissue. The regulation of EDRF maybe controlled by varies erythroid transcription factor such as GATA-1, NF-E2 and Fli-1.

B1729

PRESENCE OF ANEUPLOIDY CLONES IN CULTURED MESENCHYMAL STEM CELLS AND CHARACTERISTICS OF ANEUPLOIDY IN BONE MARROW CELLS WITH HEMATOLOGIC MALIGNANCIES

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Background: Emergence of aneuploidy during in vitro culture of cells is occasionally observed, but the significance of aneuploidy is not elucidated yet. We quantitatively assessed the aneuploidy of mesenchymal stem cells using multiple fluorescent in situ hybridization (FISH) probe panels and investigated the pattern of aneuploidy in stem cells.
Aims: The aim of this study was to comparison of the pattern of aneuploidy in stem cells with those of aneuploidy found in bone marrow cells from patients with hematologic malignancies, to have a guidance for assessing the significance of aneuploidy clones.
Methods: For the quantitative detection of aneuploid clones, we performed interphase FISH on 13 kinds of mesenchymal stem cells and 3 kinds of mesenchymal stem cell lines. Among hematologic diseases, we analyzed 77 patients with hematologic/non-hematologic diseases showing numerical chromosomal abnormalities.
Results: Interphase FISH showed variable proportions of cells with tetrasomy (0.5% to 10.5%) among various mesenchymal stem cells and the patterns of tetrasomy were asymmetric. However, numerical chromosomal abnormalities was absent in all of the 3 kinds of mesenchymal stem cell-lines. Diagnoses of 77 patients with hematologic diseases included, 22 acute lymphoblastic leukemia, 18 acute myeloid leukemia, 25 mature lymphoid neoplasms including 14 myeloma cases, 5 myelodysplastic syndrome (MDS), and 7 cases with anemia without definite evidence of hematologic malignancies. Of 77 cases, 48 cases showed < 10% of tetraploidy clones (5 acute leukemia, 5 myeloma, and 3 with mature lymphoid neoplasm). Higher tetraploidy clones and variability between percentage of tetraploidy clones were related to dysplastic features in MDS. Meanwhile, BM with erythroid hyperplasia and no definite malignancy usually showed <10% of tetraploidy clones with symmetric tetraploidy pattern.
Summary / Conclusion: Present study showed variable cell populations of tetraploidy among mesenchymal stem cells with asymmetric pattern. In contrast, patients without evidence of hematologic malignancy showed presence of tetraploidy with symmetric pattern. Presence of tetraploidy clones might mean the presence of senescent cell population or actually transforming cells into tumor. Evaluation of tetraploidy with its pattern might help in differentiation of active cellular hyperplasia and malignancies.

B1730

THE URGENT NEED OF PRIMARY PREVENTION OF STROKE IN CHILDREN WITH SICKLE CELL DISEASE IN SAUDI ARABIA USING TRANSCRANIAL DOPPLER

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Background: Stroke is a devastating clinical complication with increase morbidity among young patient with sickle cell disease (SCD). Limited data is available regarding stroke incidence in pediatric SCD in Saudi Arabia.
Aims: To determine the prevalence of stroke in pediatric SCD patient, and the recommendation to implement screening by Transcranial Doppler.
Methods: A retrospective study on pediatric patients with SCD treated at King Abdul Aziz University Hospital (KAUH), between January 2006 and December 2012. Stroke were diagnosed based on clinical and diagnostic radiology.
Results: A total of 203 patients with SCD were identified. Twenty eight patients (13.8%) were diagnosed with stroke. Fifteen patients (53.6%) were males and 13 patients (46.4%) were females, age range from 1-18 years (mean 8.46 years). Seventeen patients (61%) diagnosed with stroke were younger than 7 years. The clinical presentations of those diagnosed with stroke; 10 patients (35%) with hemiparesis, 6 patients (21%) were asymptomatic, 5 patients (17%) with headache, 4 patients (14%) with convulsions, 2 patients (7.1%) were irritable and 1 patient (3.5%) with meningeal signs
Summary / Conclusion: Children with SCD are at increase risk of stroke as early as infancy. Stroke prevention in SCD is needed as a routine before the age of 1 year.

Signalling, transcription and apoptosis

B1731

CHOLESTEROL SYNTHESIS INHIBITION INDUCES LEUKEMIA-SPECIFIC CYTOPROTECTIVE AUTOPHAGY VIA SUPPRESSION OF THE AKT/MTOR SIGNALING PATHWAY

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Background: High dose statins exhibit anti-leukemic properties *in vitro*. This is due to the suppression of the mevalonate pathway which is responsible for the synthesis of cholesterol and prenyl moieties required for the farnesylation and geranylgeranylation cell signaling proteins. We have shown that statin treatments at physiologically attainable and tolerable doses also have specific anti-leukemic activities. Low dose statin treatments induce autophagy in leukemic cells but not normal human lymphocytes, this is dependent upon the inhibition of cholesterol synthesis, not inhibition of protein farnesylation or geranylgeranylation. Inhibition of autophagy in leukemic cells reveals a remarkable apoptosis-inducing effects of low, non-cytotoxic doses of statins. This effect is mimicked by the inhibition of cholesterol synthesis, but not by the inhibition of prenylation, and is not observed in normal human lymphocytes. However, the mechanisms of statin and cholesterol synthesis inhibitor-induced autophagy are currently unknown.
Aims: Our aim was to examine the involvement of the Akt/AMPK/mTOR pathways in statin and cholesterol synthesis inhibitor-induced cytoprotective autophagy in leukemic cells.
Methods: The human leukemic cell lines REH, K562 and JVM-2, as well as PBMCs isolated from leukemic patients and healthy volunteers were used. Cells were treated with lovastatin (LS), simvastatin (SS) and atorvastatin (AS), the cholesterol synthesis inhibitor Ro-48-8071, and FTI-277 or GGTI-2133. Cell viability was determined by acid phosphatase assay. Apoptotic responses were analyzed by Western blotting for active caspase-3, and by flow cytometry using annexin V-FITC and propidium iodide for phosphatidylserine externalization and DNA fragmentation. Activation of Akt, AMPK, S6K, and mTOR were determined by Western blot analysis for the phosphorylated protein forms. Autophagy was determined by electron microscopy and Western blotting for the appearance of the LC3II. Inhibition of autophagy was performed by use of bafilomycin A1 and by RNA interference knockdown of the beclin-1 and LC3II proteins.
Results: Treatment with low dose LS, SS, and AS all reduced leukemic cell viabilities without the induction of cytotoxicity. Statins and Ro-48-8071 induced an autophagic response in a time dependent manner in leukemic cells patients, which peaked at 72h post treatment. Ro-48-8071 induced LC3II protein accumulation with faster kinetics than statins. Western blotting for activated Akt, AMPK, mTOR and S6K proteins showed that statins and Ro-48-8071 inhibited Akt, mTOR, and S6K phosphorylation with kinetics overlapping those of the appearance of LC3II, while phospho-AMPK levels remained unchanged after all treatments. None of these effects were observed after FTI-277 or GGTI-2133 treatment or in normal human lymphocytes. Akt inhibition by perifosine mimicked the effects of statin and Ro-48-8071 treatment in leukemic cells, but not normal lymphocytes.
Summary / Conclusion: Our study confirms that mevalonate pathway inhibition by low dose statins induces cytoprotective autophagy dependent specifically on the inhibition of cholesterol synthesis. Autophagy induced by cholesterol synthesis inhibition seems to be dependent upon the inhibition of Akt activity and not AMPK activation. The inhibition of Akt activation by cholesterol synthesis inhibitors leads to the inhibition of mTOR, and the induction of autophagy in leukemic cells but not normal human lymphocytes. Our results lend further credence to the idea that selective cholesterol reducing therapies in combination with autophagic inhibition warrant further exploration as potential candidates for selective anti-leukemic therapy.

B1732

RNA IN RETICULOCYTES IS NOT A WASTE BUT A FINAL MESSENGER TO BECOME RBCS

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Background: Reticulocytes represent the last stage of erythropoiesis before full maturation to red blood cells (RBCs) and initially contain both RNA and micro-organelles. Even though some hemoglobin and membrane-bound proteins are additionally synthesized in reticulocytes, the remnant RNA is regarded as waste material, and its importance has not been demonstrated.
Aims: Here we explore whether the small amount of residual RNA is essential for reticulocytes to become erythrocytes.
Methods: Fresh human or mouse peripheral blood samples with high reticulocytes were incubated with RNase or RNase inhibitor for 24~48 hr. Then, sur-

vival of reticulocytes, maturation states, viability, cell morphology, and changes of mRNA expression were analyzed compared to the control groups. Also, fresh reticulocytes were incubated in a methionine-free media to inhibit new protein synthesis.
Results: When we abruptly removed remnant RNA, reticulocytes could not survive or mature into RBCs in human and mouse. However, reticulocytes treated with an RNase inhibitor were able to form normal donut shaped cells with more preserved mitochondria and plasma membrane consistency. Furthermore, when we blocked protein synthesis, reticulocytes could not survive. To elucidate the relevant signals, we analyzed differentially expressed RNAs in human reticulocytes compared to terminally matured erythroblasts cultured from cord blood CD34+ stem cells. We found that several exocytosis, metabolism, and transduction related signals are relatively more expressed in reticulocytes.
Summary / Conclusion: Taken together, these results suggest that RNA in reticulocytes must be translated into neo-protein, which might then help to preserve mitochondria and maintain cell membrane consistency until maturation to erythrocytes. These results enhance the previously poor understanding of final erythropoiesis and low survivability of *in vitro* generated reticulocytes.

B1733
APOPTOSIS AND CXCR4 EXPRESSION IN CHILDHOOD LEUKEMIA AND SOLID TUMORS
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Background: The chemokine receptor CXCR4 is expressed on leukemic and hematopoietic stem cells and has been shown to play a crucial role in chemotaxis and homing.
Aims: The study of CXCR4 expression in marrow leukemic blasts and hematopoietic stem cells in solid tumors with and without marrow involvement, as well as the apoptosis of the CXCR4 population at diagnosis and remission in acute lymphoblastic leukemia.
Methods: BM cells from children with acute lymphoblastic leukemia at diagnosis (ALL, n=7) and on day 33 (ALLd33, n=6) of treatment when remission is achieved, as well as with solid tumors at diagnosis without (ST) and with BM involvement (STinv) (n=10 and n=4, respectively) were studied. Flow cytometry was performed to assess the expression of chemokine receptor CXCR4 in $CD34^+$ blasts or hematopoietic stem cells as well as the percentage of apoptotic subpopulations of these cells stained by PI/Annexin.
Results: The percentage of bone marrow leukemic blasts expressing CXCR4 at diagnosis of ALL was estimated to be 65.26% and only 5.61% of this population is apoptotic. On day 33 of treatment the apoptosis of $CD34^+$ marrow hematopoietic stem cells that express $CXCR4^+$ mounted up to 24.05% (P=0.035 compared to diagnosis). In solid tumors without BM involvement the percentage of $CD34^+$ marrow hematopoietic stem cells that express $CXCR4^+$ was calculated to be 60.01% whereas the same percentage for the group of ST with BM infiltration was 80.25%, a difference that was not found to be statistically significant. The apoptosis of $CD34^+$ marrow hematopoietic stem cells that express $CXCR4^+$ in the case of ST with BM infiltration was 3.93%, statistically significantly lower than the relevant percentage in ST without BM involvement (22.69%, P=0.014).
Summary / Conclusion: The above results point out that the apoptosis of $CD34^+$ marrow hematopoietic stem cells that express $CXCR4^+$ at remission is higher compared to diagnosis of ALL. The expression of CXCR4 on $CD34^+$ marrow hematopoietic stem cells in ST with BM infiltration seems to confer to resistant to apoptosis. These results warrant further investigation of the implication of CXCR4 in the mechanism of apoptosis.

B1734
ELEVATION OF CAMP LEVELS INHIBITS ARSENIC-INDUCED APOPTOSIS IN ACUTE PROMYELOCYTIC LEUKEMIA CELLS
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Background: Acute promyelocytic leukemia (APL) is the most frequently curable subtype of acute myeloid leukemia. Most patients are treated with all-trans retinoic acid (ATRA) and its combination with anthracycline-based chemotherapy. More recently, another natural compound, arsenic trioxide (As2O3), was integrated into APL treatment, showing high efficacy and tolerability in APL patients.
Aims: The aim of this study was to evaluate the effect of activation of cAMP signaling system on As2O3 -induced apoptosis in acute promyelocytic leukemia cells.
Methods: Cultured acute promyelocytic leukemia NB4 cells were exposed to As2O3 in the presence or absence of cAMP-increasing agent forskolin for 24h and then cells were subjected to apoptosis analysis by flow cytometry. Analysis of cell cycle was also performed by flow cytometry. Western blot method was used to analyze phosphorylation state of p53 protein, total p53, p21, cyclin-D1, and the levels of other proteins which were involved in arsenic-induced apoptosis.
Results: These results indicate that elevation of cAMP in acute promyelocytic leukemia cells profoundly inhibit the apoptotic response to As2O3. Analysis of the cell cycle distribution showed that NB4 cells significantly accumulated at the G0/G1 phase under forskolin treatment alone or when As2O3 and forskolin treatments were combined, but not in the presence of As2O3 alone. This inhibitory effect of cAMP was reversed by PKA inhibitor H89. Our results showed a dramatic increase of p21 without any change in p53 protein levels during forskolin treatment of NB4 cells. Increased cAMP levels also decreased arsenic-induced caspase-3 activation in acute promyelocytic leukemia cells.
Summary / Conclusion: As2O3 has dose-dependent dual effects on APL cells: triggering cell apoptosis at high concentration and inducing partial differentiation at low concentration. This study shows that elevation of cAMP levels in acute promyelocytic leukemia cells can inhibit apoptosis induced by high concentration (1-2 μM) of As2O3.

B1735
THE PROLIFERATION ARREST OF PRIMARY BONE MARROW TUMOR CELLS DURING CULTURE IS PRECEDED BY DOWNREGULATION OF MKI67 AND NUMEROUS MITOTIC AND TRANSCRIPTIONAL GENES
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Background: To identify the gene subset in bone marrow (BM) tumor cells which is controlled by the natural microenvironment we recorded recently the changes acquired in gene expression profile (GEP) after removal of the cells out of their niche. The results revealed prompt and sustained switch in the expression of numerous genes which started immediately following aspiration and persisted for at least 11 hours. It addition, most of the prominent changes were common to all types of tumors examined and the constitutive overexpression of master genes and tumor suppressor genes could account for the short survival of aspirated tumor cells *ex vivo*. Nevertheless, the almost complete regression of the above GEP changes under culture conditions that resumed cell-cell-matrix contact in vitro indicated that other factors are responsible for the proliferation arrest ensued.
Aims: To identify the factors responsible for the proliferation arrest of primary multiple myeloma (MM) and acute myeloid leukemia (AML) cells in culture.
Results: Despite the tremendous biological differences between MM and AML the post-aspiration switch in GEP regressed equally during culture and de novo changes appeared which were overlapping to large extent irrespective of the culture duration or the tumor type. The reproducible GEP changes appearing in culture compared with the expression recorded from the corresponding fresh samples included **Downregulated Genes:** Irrespective of the underlying tumor MM or AML, the aspirated BM cells showed significant downregulation of dozens of genes during culture (lasting between 1 to 29 days). However, the most interesting finding was the downregulation of a major subset of genes which had in common established roles in mitosis or during transcription. The combined downregulation of plenty of genes which are all function in the machinery of replication seems to represent the best explanation for the invariable proliferation arrest limiting culture of primary tumor cells.
Upregulated genes: The upregulated gene subset predominated by extracellular protein coding genes which included a variety of extracellular matrix (ECM) proteins (SPP1, FN1) and angiogenic factors (IL8, CCL2, CXCL5, FN1, MMP14, MT1G). Thus, the induction seemed to be oriented purposely towards angiogenesis and matrix enrichment.
Summary / Conclusion: In analysis of the various GEP changes evolved during culture the proliferation arrest could be best attributed to the excessive downregulation of mitotic and transcriptional genes. The latter insult in turn could be secondary to the loss of vital signals or direct contact with a feeder niche component not existing in the culture flasks. In this regard, the overexpressed extracellular genes pointed towards the angio-matrix tissue as the best candidate for being the missing feeder structure required for proliferation. The suggested relationships might therefore direct future antitumor treatments towards interruption of the feeder signals (which are not known yet) or to target the angiogenic/ECM recruitment factors (i.e., IL8, osteopontin, proteases) as alternatives to traditional cytotoxic drugs. In summary, our results encourage the future assessment of non-cytotoxic niche modifying agents, directed against IL8, osteopontin and certain proteases in various combinations as well as against amphiregulin and COX2 which are probably adding to tumor expansion.

B1736

SANGIVAMYCIN-INDUCED APOPTOTIC INDUCTION IN HL-60 CELL LINE

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Background: Sangivamycin (SGV) has shown a potent antiproliferative activity against a variety of human cancers. However, little is known about the mechanism of action underlying its antitumor activity in leukemia.
Aims: This study aimed to show the anti-leukemia effect of SGV on the HL-60 human acute myeloid cell line and to investigate the underlying molecular mechanisms governing these effects in terms of apoptosis.
Methods: To investigate the effects of Sangivamycin on cell cycle in HL-60 cells, cell viability were tested with MTT assay and we measured theDNA content of HL-60 cells using flow cytometry. In addition, TUNEL assay was used to examine apoptotic induction in treated cells, and the effects of Sangivamycin on the expression of apoptosis-associated proteins were examined by Western blot.
Results: This study showed that SGV inhibited the proliferation of HL-60 cells in a concentration- and time-dependent manner as measured by the MTT assay. Treatment of HL-60 cells with an IC50 of SGV resulted in morphological changes typical of apoptosis. Flow cytometric analysis of PI indicated that SGV-induced apoptosis in HL-60 cells occurs in a time-dependent manner. Tunnel assay was utilized to investigate the apoptosis induced by SGV. The pro-apoptotic activity of SGV was attributed to its ability to modulate, in a concerted manner, the expression of Bcl-2 and Bax proteins, which were down- and up-regulated, respectively. Caspase-9 and -3 were subsequently activated, however caspase-8 was not.
Summary / Conclusion: These results prove that SGV effectively induces programmed cell death and suggests that SGV-induced apoptosis in the HL-60 cells is mediated by the regulation of Bid cleavage and the activation of caspase-9 and -3.

Cytogenetics and molecular diagnostics

B1737

HIGH FREQUENCY OF NQO1 C609T VARIANT GERMLINE POLYMORPHISM IN MDS/AML WITH TRISOMY 8

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Background: Clues to the etiology of myeloid malignancies are expected to be gained through the study of genetic susceptibility in candidate genes. Models for MDS development suggest the role of cumulative genetic and toxic environmental factors in genetically predisposed individuals. The NAD(P)H:quinone oxidoreductase 1 (NQO1) enzyme detoxifies a number of endogenous and exogenous quinones, protecting cells against oxidative damage. The corresponding gene is subject to a single-nucleotide germline polymorphism ($C^{609}T$) resulting in a lowering of enzyme activity. Individuals homozygous for the mutant allele (T/T) completely lack NQO1 activity, whereas heterozygotes (C/T) present approximately threefold decreased enzyme activity.
Aims: Considering the critical role of the NQO1 enzyme in detoxification mechanisms implicated in MDS pathogenesis, the aim of the present study was to investigate whether the *NQO1* germline polymorphism influencing the NQO1 activity, confers susceptibility to MDS development and/or promotes certain chromosomal changes.
Methods: We conducted a case-control study to evaluate the *NQO1* genotype in a large series of 330 Greek patients with primary MDS (217 males, 113 females, median age 68 years) and 416 gender and age matched healthy individuals (270 males, 146 females, median age 66.5 years). Bone marrow specimens from patients and peripheral blood samples from healthy donors were used for DNA extraction and subsequent genotypic analysis. The *NQO1* gene status was also evaluated in relation to patients' characteristics and cytogenetic findings. Focusing on specific cytogenetic aberrations recurrently found in MDS/AML, we retrospectively analysed the *NQO1* genotypic distribution in a cohort of 566 patients with -5/del(5q), -7/del(7q), trisomy8, del(20q) and -Y. Among them, 199 cases showed -5/del(5q), 153 had -7/del(7q), 202 presented +8, 51 cases had del(20q) and 65 cases exhibited loss of the Y chromosome (-Y). The *NQO1* $C^{609}T$ SNP genotyping was performed by a Real-Time PCR method using Taqman technology.
Results: Our case-control study revealed no differences in the frequencies of the *NQO1* variant genotypes between patients and healthy donors. The higher incidences of variant *NQO1* genotypes observed in MDS patients with +8, prompted us to analyse the *NQO1* gene status in a large number of MDS/AML patients carrying certain specific abnormalities. Interestingly, the results showed that patients with either +8 or del(20q) exhibited a 1.8-fold increased risk of carrying at least one variant T allele, as compared to the allele frequencies of the control population (P=0.032, χ^2=10.526, df=4). Particularly, genotypic analysis revealed significantly increased frequencies of heterozygotes (C/T) among patients with del(20q) and homozygous variant individuals (T/T) among patients with +8, as compared to either healthy donors or other cytogenetic groups (P=0.001, χ^2=27.503, df=8).
Summary / Conclusion: Our case-control study, that represents the largest series of patients with primary MDS ever evaluated for the *NQO1* $C^{609}T$ gene polymorphism, showed no differences in the frequencies of the *NQO1* variant genotypes between patients and healthy donors. The result suggests that *NQO1* polymorphism does not correlate with susceptibility to MDS. The increased frequency of homozygous variant genotype (T/T) in patients with +8 might suggest a potentially causative role of lack of NQO1 protein in the pathogenesis of trisomy 8 in MDS/AML.

B1738

AN IMPROVED REAL-TIME QUANTATIVE PCR PROTOCOL FOR DETECTING VARIANT-TYPE PML-RARA FUSION TRANSCRIPTS

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Background: The variant-type PML-RARA (PML-RARA-V) transcript is typically refers to that the breakpoint of PML gene located in exon6 and sometimes accompanied by insertion of small pseudo exon sequences derived from RARA-intron2. The Europe Against Cancer Program (EAC) reported sets of reverse transcript quantitative PCR (RQ-PCR) protocols including PML-RARA-V type in 2003 and were widely referenced. But we find that the EAC2003 protocol couldn't efficiently detect 4 of the 11 PML-RARA-V transcripts identified in our laboratory.
Aims: To design a new RQ-PCR primer, in order to efficiently detect the PML-RARA-V fusion gene.
Methods: PML-RARA-V sequences were got by a longer fragment PML-RARA amplification and Sanger sequencing in 4 patients whom PML-RARA-V gene failed detected by using EAC-2003 primer set. Sequences were analyzed and new forward primer was designed and tested.

Results: Cases and PML-RARA-V sequences information are following: Case 1: The PML partial retained the first 110bp of exon6, with an 'AG' insertion; Case 2: Retained the first 112bp of PML-exon6, with a 63bp insertion; Case 3: Retained the first 115bp of PML-exon6, with a 9bp insertion, morphology and immunophenotype of the leukemia cells manifested as atypical APL with lack of granules and Auer rods; Case 4: Retained the first 172bp of PML-exon6, with a 36bp insertion, the leukemia cells showed normal chromosome karyotype but PML-RARA fusion signal can be detected by fluorescence in situ hybridization. Insertion fragments of the later 3 PML-RARA transcripts were all derived from RARA-intron2. All of the 4 patients were sensitive to all-trans retinoic acid and arsenic trioxide treatment. These 4 out of 11 PML-RARA-V transcripts were all failed to be detected by EAC2003 protocol due to the forward primer affected by their breakpoints. By combining use the newly designed forward primer 'GAGGGGAAGGAGGCAAGGTT' and EAC2003 forward primers, we could efficiently detect all of the 11 PML-RARA-V transcripts identified in our laboratory.
Summary / Conclusion: We propose combining use of a newly designed forward primer and EAC2003 forward primer to detect PML-RARA-V transcripts more efficiently.

B1739
DELETION OF 14Q IN COHORT OF 560 PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA
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Background: Deletion of 14q is rare but recurrent aberration in B-cell malignancies, especially in chronic lymphocytic leukaemia (CLL). A limited number of studies focused on characterization of patients with del(14q). Reindl *et al*. (2010) suggested that del(14q) is associated with short time to treatment. We analysed CLL patients from Czech republic by conventional cytogenetic analysis, fluorencence *in situ* hybridization (FISH) and molecular methods and characterized the cases with del(14) in detail.
Aims: The purpose of the current study was to focus on del(14q) in cohort of 560 CLL patients, to evaluate the incidence and to analyse additional cytogenetic abnormalities, *IGVH* mutation status and clinical outcomes.
Methods: In a period between 2008 to 2012, 560 cases with CLL were investigated. Conventional cytogenetics was performed on peripheral blood or bone marrow samples cultured in medium with CpG-oligonucleotides and IL-2 for 72 hours. I-FISH was performed on unstimulated cells for detection of *IGH@* rearrangement, + 12, del(11q), del(13q) and del(17p). The range of del(14q) was determined by multicolor banding. For analysis of the *IGVH* mutation status, monoclonal immunoglobulin rearrangements were amplified by PCR from cDNA. PCR products were purified in agarose gel and analysed by direct sequencing.
Results: A total of 519/560 (92,7 %) cases were succesfully stimulated for metaphase analysis. Del(14q) was detected in 12 cases (12/519,2,3 %) and it was mostly intersticial (11/12). It showed variable size but clustered at specific chromosomal bands. FISH revealed loss of 3´*IGH@* flanking sequences (8/12). In 5 cases del(14q) was found as sole abnormality, whereas additional aberrations were identified in 7 cases. Two cases had complex karyotype. *IGHV* status was unmutated in 9/11 cases.
Summary / Conclusion: Cytogenetic results are highly relevant in defining the prognosis of CLL patients. I-FISH is standard method for detection of important chromosomal aberrations. However, metaphase cytogenetic analysis reveals another abnormalities which may change the prognosis of patients defined by FISH. Our results are consistent with those of other studies. We confirmed the low occurence of del(14q), association with unmutated *IGVH* and the presence of additional aberrations together with del(14q).

B1741
MOLECULAR CHARACTERIZATION AND PRENATAL DIAGNOSIS OF FAMILIAL HEMOPHAGOCYTIC LYMOPHOHISTOCYTOSIS IN EGYPTIANS
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Background: Familial hemophagocytic lymphohistiocytosis (FHL) is a rare, autosomal recessively disease with a clinical manifestation during infancy in 70%–80% of the patients. Previous genetic studies in FHL revealed an extended genetic heterogeneity. The high rate of consanguinity in Egyptians makes this rare recessive disease relatively common.
Aims: To study molecular characteristics of the Egyptian patients with FHL and to find any potential genotype phenotype correlation
Methods: The study included 15 families presenting to the Medical Genetics Center for genetic counseling as one or several children had clinical and laboratory criteria consistent with FHL. Molecular diagnosis was done in 9 families by segregation analysis of polymorphic markers of *PRF1* (FHL2), *UNC13D* (FHL3), *STX11* (FHL4), and *STXBP2* (FHL5) genes. Sequencing of *PRF1* gene was performed to another 3 parents as their affected children were already deceased at presentation. Prenatal diagnosis was done for 2 mothers with previously detected mutations
Results: Mutations in FHL causing genes were detected in 5 patients (2 in the same family). This included homozygous Del218-224 mutation and compound heterozygous for G218A/C1304T mutation in the *PERF1* gene, homozygous 284delC mutation in the *STX11* gene and homozygous C1430T mutation in the *STXBP2* gene (one family). Prenatal diagnosis revealed normal fetus in *STX11* family while an affected fetus was detected in a family of *PRF1* gene.
Summary / Conclusion: The mutation spectrum of the FHL in the Egyptian children is expected to be heterogeneous due to the wide ethnic variation. The severity and the rapid progression of the disease was an obstacle in molecular diagnosis in many families as most families presented after death of one or several children. Molecular analysis should be further extended for carrier testing to define the actual frequency of this rare disease

B1742
ASSOCIATION OF GSTP1 POLYMORPHISM AND CHROMOSOME ABERRATIONS IN ACUTE MYELOID LEUKEMIA
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Background: The role of cytogenetics in determining the biologic basis of Acute Myeloid Leukaemia (AML) is widely recognized. Different cytogenetic abnormalities and normal karyotype have been described in AML, although aetiology of AML remains unknown. However, interactions between environmental exposure and genetic background postulated to be a possible cause of AML development. The GSTP1 gene implicated in the detoxification pathways of many genotoxic xeniobitics and the single-nucleotide polymorphism ($A^{313}G$) abolished GSTP1 enzymatic activity. Thus, homo-/hetero-zygotes (G/G, A/G) for the mutant allele present decreased enzymatic activity.
Aims: Our study aimed to investigate the potential implication of GSTP1 polymorphism in AML susceptibility and AML-specific chromosomal abnormalities.
Methods: Chromosome studies were performed on 184 AML patients. Patients were classified according to FAB classification. GSTP1 genotyping was performed by PCR-RFLP assay in all patients and 370 sex and age matched unrelated healthy controls.
Results: FAB classification was available in 102 patients with M2 (32.5%) as the most common subtype. Cytogenetic analysis was successful in 97.3% of patients. Abnormal karyotype was found in 62.6% of patients and among them, 32.1% were complex while 20.5% satisfied the requirements of the monosomal karyotype definition. The most common chromosome aberrations were +8 (21.4%), -7/del(7q) (20.5%), -5/del(5q) (13.4%), abnormalities of 11q23 (12.5%), t(15;17) (9.8%), t(8;21) (8.0%), -Y (8%), +22 (7.1%) and inv(16) (6.25%). The *GSTP1* genotypic distribution in patients and controls was significantly different($P<0.0001$). An increased frequency of heterozygotes A/G was observed in M2 (70.8%) and M4 (65%) subtypes. Interestingly, a higher frequency of homozygous mutant G/G in AML patients with normal karyotype was observed compared to the abnormal karyotype ($P=0.056$). Furthermore, a higher frequency of heterozygotes A/G was revealed in AML patients with -Y, -7/del(7q) and +22.
Summary / Conclusion: Our results suggest that the GSTP1 $A^{313}G$ polymorphism may be a predisposing factor for the development of AML-specific abnormalities. Moreover, homozygosity of the GSTP1 polymorphism seems to be associated with development of AML with normal karyotype.

B1743
EXTERNAL QUALITY ASSESSMENT OF KIT D816V MUTATION TESTING IN MASTOCYTOSIS
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Background: Mastocytosis is frequently associated with the somatic activating mutation D816V in the *KIT* gene; this results in constitutive activation of *KIT* tyrosine kinase activity, and provides resistance to tyrosine kinase inhibitors such as Imatinib. It is a minor criterion for classification of Systemic Mastocytosis (SM) according to the 2008 WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. The *KIT* D816V mutation is detectable in 95% of patients with SM but mutation load is often low, with previous studies showing a median 0.9% mutant versus wildtype. UKNEQAS LI, an international provider of External Quality Assessment (EQA)/Proficiency Testing (PT) services, has a commitment to improve laboratory testing quality through standardisation and education in haemato-oncology diagnostics.
Aims: To assess and improve the quality of diagnostic testing for *KIT* D816V mutation testing UKNEQAS LI have recently initiated a pilot scheme for *KIT*

D816V mutation.
Methods: Two vials of lyophilised cell-lines were distributed to 28 participants for *KIT* D816V mutation analysis. The samples were manufactured to contain a minority of *KIT* D816V mutated cells with a substantial background of unmutated cells, to mimic mutation levels typically seen at diagnosis in SM. Based on cell composition the level of mutation in the two samples was 1% and 2.5%, respectively, when analysing genomic DNA.
Results: 20/27 (74.1%) participants found a *KIT* D816V mutation in both samples issued. Six participants did not find a *KIT* D816V in either sample. One participant found a mutation in the 1% sample but not in the 2.5% sample and another participant found a mutation in the 2.5% sample but not in the 1% sample. This indicates a potential false negative rate of around 25% in clinical samples. Eleven participants used Sanger sequencing as part of their testing pathway. Interestingly, of the 8 participants who did not find a *KIT* D816V mutation in one or both of the samples, 7 used Sanger sequencing as part of their testing pathway, with 5 of these using it as their sole technique.
Summary / Conclusion: Direct Sanger sequencing has a sensitivity of 15-20% which is not adequate to detect low level *KIT* D816V mutations typically found in SM unless, perhaps, highly purified mast cells are analysed. Other more sensitive techniques such as nested PNA-PCR or real time PCR should be employed to provide optimal SM diagnosis and treatment.

B1744
SUBMICROSCOPIC DELETIONS OF 3'-CBFB GENE PORTIONS IN ACUTE MYELOID LEUKEMIA WITH INV(16): A COMPREHENSIVE DIAGNOSTIC APPROACH USING HIGH RESOLUTION MICROARRAY AND LONG DISTANCE-PCR
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Background: Submicroscopic deletions in leukemia occurs in rearrangements such as *BCR-ABL1*, while 3'-*CBFB* gene deletions in AML with inv(16) are rarely reported with the incidence ranging from 2% to 7.7% estimated by fluorescence in situ hybridization (FISH).
Aims: As almost all other reports were based on FISH analysis alone, we focused on the comprehensive molecular analysis of 3'-deletion of *CBFB* in AML with inv(16), obtained from bone marrow specimen of a male AML patient. Identifying the extent of submicroscopic deletion and involved genes could provide an understanding of presumably an inferior prognosis of such patients with submicroscopic deletion.
Methods: Workup included karyotyping, FISH, multiplex reverse transcriptase polymerase chain reaction (RT-PCR), long distance-PCR (LD-PCR), and high resolution microarray studies. A chromosomal microarray experiment using Cytoscan 750K array (Affymetrix, Santa Clara, CA) was conducted to confirm the presence of the submicroscopic deletion. To reconfirm the deleted regions, high resolution microarray was conducted using a customized Agilent Human aCGH Microarray 1x1M designed to include 698K probes of chromosome 16 (Agilent Technologies, Palo Alto, CA).
Results: Cytogenetic analysis resulted in the karyotype 46,XY,inv(16)(p13.1q22) in 19 out of 20 metaphases we analyzed. FISH analysis using Vysis LSI *CBFB* dual-color break-apart probe (Abbott Molecular/Vysis, Des Plaines, IL) also resulted in split signals in 94.5% of the analyzed cells. A notable finding was the loss of the green 3'-*CBFB* telomeric signal which indicated for a 3'-*CBFB* deletion, namely a submicroscopic deletion. The subsequent multiplex RT-PCR using Hemavision (DNA Technology, Aarhus, Denmark) revealed a *CBFB-MYH11* rearrangement. From LD-PCR analysis, *CBFB-MYH11* fusion gene was found to have been generated from a recombination event that occured within intron 5 of *CBFB* and intron 32 of *MYH11*. Microarray result was arr16p13.11(15,822,415-16,289,059)x1,16q22.1(67,122,027-68,312,149)x1 (Human Genome Build 37.2; http://genome.ucsc.edu). The deletion at 16p included the 5'-portion of the MYH11 gene and several genes located centromeric to *MYH11*. High resolution microarray resulted in arr 16p13.11(15,815,138-16,291,017)x1,16q22.1(67,123,219-68,307,465)x1 and gave a more detailed information of the deletion that occurred at 16p13.11 and 16q22.1 (Figure 1). Interestingly, the high resolution aCGH analysis revealed another deletion (exon 28-32) that occurred telomeric to the *MYH11* gene remnant at 16p13.11 identified and led to the deletion of the overlapping *NDE1* gene (exon 8).
Summary / Conclusion: Analysis of submicroscopic deletions in AML patients can be of great benefit for comprehension of patients' clinical courses and their corresponding genetics. Reports on submicroscopic *CBFB* deletions in AML patients with inv(16) are yet limited and require further studies to characterize the additional genetic lesions of these patients. The best strategy would be the use of commercially available SNP microarrays (Affymetrix, Santa Clara, CA) and customized aCGH arrays (Agilent Technologies, Palo Alto, CA) for chromosome 16 to systematically characterize more deletions in AML patients with inv(16). Deletion of the *NDE1* gene located centromeric to the *CBFB-MYH11* breakpoint was recently described to be disrupted in at least of 90% of inv(16)-positive AML cases. Since *CBFB-MYH11* fusion gene produces a large variety of alternatively spliced fusion transcripts (n~10), we recommend using the established genomic breakpoint analysis for the molecular characterization of inv(16) leukemia patients. In addition, genomic breakpoint analysis enables prompt diagnosis and confirmation of specific gene rearrangements in leukemia and even solid tumors. The combination of different molecular and array technologies will aid the understanding of cancer-based mechanism, and moreover, to translate this knowledge into clinical trials to improve cancer treatment in the future.

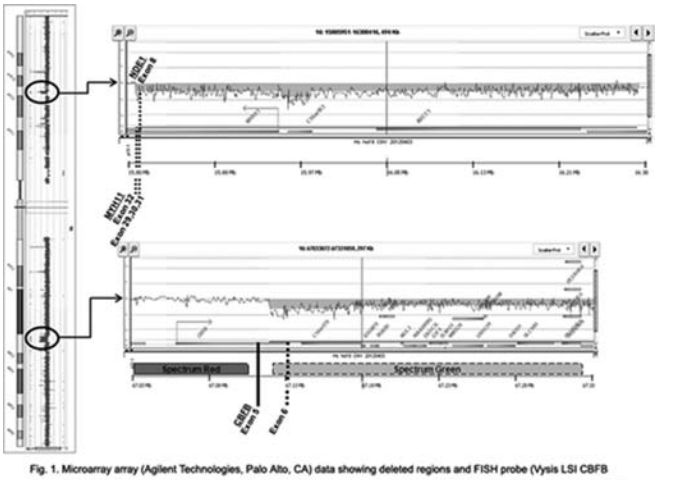

Fig. 1. Microarray array (Agilent Technologies, Palo Alto, CA) data showing deleted regions and FISH probe (Vysis LSI CBFB dual-color break-apart probe, Abbott Molecular/Vysis, Des Plaines, IL) binding sites on chromosome 16. At 16p, the 5'portion of MYH11 gene (exon 1-32) and overlapping 3'-portion of NDE1 gene (exon 8) were deleted. At 16q, region extending from intron 5 of CBFB to probe-binding site (3' CBFB green telomeric signal) was deleted.

B1745
MOLECULAR FACTORS ASSOCIATED WITH BONE INVOLVEMENT IN GAUCHER PATIENTS
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Background: Gaucher disease is one of the most common glycolipid storage disorders caused by mutations in the gene (GBA) which encodes human acid-β-glucosidase. Bone involvement is typical for Gaucher disease and varies from asymptomatic osteopenia to irreversible orthopedic defects. Bone marrow infiltration by Gaucher cells, impaired regulation of bone metabolism and ischemic osteonecroses are currently considered to be the main pathogenic mechanisms of bone involvement. However, the cause of exceptional heterogeneity of bone disease still remains unclear.
Aims: To study genetic factors possibly associated with severity of bone involvement in type I Gaucher patients: the most common GBA mutations and mutations causing a predisposition to thrombophilia.

Methods: The study group included 100 adult patients (36 males and 64 females aged from 18 to 79 years) with the diagnosis of type I Gaucher disease verified by enzyme assay. Patients (pts) were divided into 4 groups based on the severity of bone involvement: extremely severe (3 pts), severe (25 pts), moderate (58 pts) and mild (14 pts) bone involvement. The assessment of bone involvement was based on the severity of osteoporosis, presence of pathological fractures, avascular necroses of hips, osteonecroses, bone marrow infiltration assessed by MRI and X-ray examinations.
Allele-specific real time polymerase chain reaction enabling the identification of single nucleotide substitution in certain DNA segments was used to screen the pts for: 1) the most common GBA mutations (amino acid substitution N370S, G - insertion (84 GG), amino acid substitution L444P and inversion IVS2+1G>A); 2) mutations causing a predisposition to thrombophilia (G1691A Factor V Leiden, G20210A prothrombin, C677T MTHFR mutations and 4G/5G polymorphism of PAI-1).
Results: The most common mutation was N370S revealed in 93% of the total number of alleles, followed by L444P found in 22% of alleles. The method allowed to identify both mutant alleles in 39% of pts: the genotype N370S/N370S was revealed in 17% of pts, N370S/L444P – in 19%, N370S/IVS2+1 – in 2%, L444P/84GG – in 1% of pts. Only one mutant allele was identified in 57% of pts: the genotype N370S/Other mutation was found in 55%, L444P/Other mutation – in 2% of pts. None of the 4 analyzed mutations was found in 4% of pts. No correlation between the GBA genotype and sever-

ity of bone involvement was found in our group of patients.
We also searched for the markers of other hereditary diseases and predispositions among Gaucher patiens. Homozygous C677T MTHFR mutation was found in 7 (7%) of pts, heterozygous factor V Leiden mutation – in1, heterozygous G20210A prothrombin mutation – in 1; 4G/4G PAI-1 polymorphism – in 30 (30%) of pts. It should be noted that all 3 pts with extremely severe bone involvement carry molecular markers of thrombophilia: 1 pt – G20210A prothrombin mutation, 2 pts – 4G/4G PAI-1, however correlation with bone involvement is not statistically significant.
Summary / Conclusion: We found no association between the severity of bone involvement and GBA genotype. To assess correlation between thrombophilia markers and severity of bone involvement further research is needed.

B1747

CLINICAL SIGNIFICANCE OF PREVIOUSLY CRYPTIC COPY NUMBER ALTERATIONS AND LOSS OF HETEROZYGOSITY USING THE COMBINED ARRAY CGH AND SNP MICROARRAY ANALYSIS IN CHILDREN WITH AML AND MDS

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Background: Although cytogenetic analysis provides essential information for diagnosis and prognosis in patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), a significant proportion of patients lacks cytogenetic aberrations on metaphase cytogenetics (MC). The combined array comparative genomic hybridization and single-nucleotide polymorphism microarray (CGH+SNP microarray) platform can allow the simultaneous detection of copy number alterations (CNA) and copy neutral-loss of heterozygosity (CN-LOH), which cannot be detected by MC, in high-resolution without the need to run two separate microarray experiments. Whole-genome scanning studies using array CGH and SNP microarray are not sufficient in pediatric AML and MDS, and their clinical and biological implications are still to be investigated in pediatric population.
Aims: This study was performed to detect simultaneously cryptic CNA and CN-LOH by CGH+SNP microarray, and to characterize the clinical significance of the submicroscopic defects in childhood AML and MDS.
Methods: A total of 18 patients with AML (n = 15) or MDS (n = 3) younger than 18 years of age at diagnosis were included in this study. DNA extracted from frozen bone marrow (n=17) or peripheral blood (n=1) samples at diagnosis were used for CGH + SNP microarray analysis. LOH regions >5 Mb was regarded as significant. All samples were analyzed with Agilent CGH+SNP 180K kit microarrays (Agilent Technologies, Santa Clara, CA, USA). Data were analyzed using Agilent CytoGenomics 2.0.6.0 with an ADM-2 algorithm (threshold 8.0). The results were analyzed according to the genome build Hg19.
Results: CGH+SNP microarray revealed CNAs at 14 regions (7 gains, 7 losses) in 9 patients, while MC detected 11 regions (5 gains, 6 losses) in 8 patients. All genomic imbalances detected by MC were recognized by CGH, whereas 2 gains and 1 loss revealed by CGH were undetectable by MC. In addition, an accurate gain region, which was erroneously detected by MC, was corrected by CGH. With CGH+SNP microarray, LOHs larger than 5Mb were detected at 12 regions in 8/18 (44%), LOHs larger than 10 Mb were detected at 6 regions in 5/18 (28%), and LOHs, which were larger than 10 Mb and involved terminal regions or whole chromosome, were detected at 3 regions in 3/18 (17%). LOH affected the following chromosomal arms of 1p, 5q, 9p, 7q, 13q,6,15q, 18q, Xp, Xq, and recurrent LOH was observed at 1p33-p32 (n = 2). Of 5 patients with normal karyotype, CGH+SNP microarray revealed cryptic LOH with or without CNA in 3 patients. Of 13 patients with abnormal MC, CGH+SNP microarray detected additional cryptic CNAs (n = 2), and LOHs (n = 5) in 6 patients. In total, 9 patients were shown to have additional aberrations including CNAs (n = 3) and/or LOHs (n = 8). Certain segmental LOH or CNA regions included candidate genes responsible for leukemogenesis, such as *CDKN2C* (1p), *CDKN2A* (9p), *JAK2* (9p), *PAX5* (9p), *BEX1* (Xq), *ETV6* (12p), *IRF4* (6p), *DEK* (6p), and *FLT3* (13q). Of 15 patients with AML, 3 patients with terminal LOH larger than 10 Mb showed significantly inferior relapse-free survival rate (*P*=0.041), whereas interstitial LOH larger than 5Mb or those larger than 10 Mb did not influence the outcome.
Summary / Conclusion: This study showed that CGH+SNP microarray allowed simultaneous detection of previously cryptic CNA and LOH in the patients with abnormal karyotypes as well as normal karyotype. The results showed that CGH+SNP microarray can be used complimentarily with the conventional cytogenetics to delineate the genetic aberrations of childhood AML and MDS. In addition, segmental LOHs harboring oncogenes or tumor suppressors associated with leukemogenesis may imply pathobiological significance. To refine the prognostic impact and biologic implications of hidden genetic aberrations in childhood AML and MDS, a prospective study on larger pediatric population with matched DNA sample is warranted.

B1748

HYPOXIA INDUCIBLE GENES, ERYTHROPOIETIN AND VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN RENAL CELL CARCINOMA

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Background: More than 90% of kidney cancers are of epithelial cells origin, referred to as renal cell carcinoma (RCC). RCC can be familiar or sporadic, often associated with distinct genetic mutations with the most prominent being von Hippel-Lindau (VHL) gene mutations. The germ line mutation in the VHL gene is usually associated with familiar RCC and VHL syndrome. The VHL protein is a component of an E3 ubiquitin ligase complex that regulates hypoxia-inducible factor (HIF). If VHL is inactivated, then a defective VHL protein is produced and HIF is not degraded.
Aims: Accumulation of HIF induces over-expression of hypoxia related genes including erythropoietin (EPO), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), thus promoting angiogenesis, proliferation and tumorogenesis. The aim of this study was to examine hypoxia related genes in RCC.
Methods: We analyzed the tumor and surrounding healthy tissue in 10 patients who had radical nephrectomy because of RCC. We analyzed mRNA expression of VEGF and two VEGF receptors, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1, KDR) in tumors vs. surrounding healthy tissue by real time quantitative PCR. In addition, we determined EPO and EPO receptor (EPOR) mRNA expression in tumors compared surrounding tissue. DNA was isolated from blood and from tumor and surrounding healthy tissue for sequencing of VHL gene. Also, DNA methylation of VHL gene was analyzed in tumors.
Results: We found that VEGF was induced several folds in all examined tumors compared to healthy tissue.VEGF binds with high affinity to two receptors, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1, KDR),and we found that VEGFR-2 was induced several folds in tumors compared to healthy tissues, while VEGFR-1 was not induced. However, EPO expression was only detected in 4 out of 10 tumor samples, suggesting dependence of tumor localization in the kidney, since only peritubular fibroblasts produce EPO. Erythropoietin receptor (EPOR) was detected both in tumor and healthy tissues with no difference in expression.DNA was isolated from the blood of all examined patients before radical nephrectomy and was used for sequencing of the VHL gene, checking for sporadic and hereditary forms of RCC. Only one patient had a substitution of leucine to proline at position 25 (P25L) as a polymorphism of the VHL gene. Using sequencing, we demonstrated two different mutations in VHL gene, one was deletion of a nucleotide in codon 147 of exon 2 and second was deletion of 22 nucleotides in codon 99 of exon 1. Since sporadic RCC goes with inactivation of the VHL, either through hypermethylation or mutation, the other 7 tumor samples showed no increase in DNA methylation of VHL gene.
Summary / Conclusion: These results suggest that defective production of VHL protein and accumulation of HIF, EPO and VEGF partially explains the angiogenesis, proliferation and tumorogenesis of sporadic RCC.

B1749

PRODUCTION OF PLASMID CONTROL REAGENTS FOR MOLECULAR MONITORING OF ATYPICAL BCR-ABL1 FUSIONS

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Background: Approximately 97-98% of CML patients express e13a2 and/or e14a2 *BCR-ABL1* fusion transcripts resulting from translocation breakpoints that fall within the major breakpoint cluster region of *BCR* and a large region upstream of *ABL1* exon 2. Residual disease after therapy for these cases is typically monitored by qRT-PCR using a single primer/probe set. The remaining 2-3% of CML patients express atypical *BCR-ABL1* fusions, the majority of which are accounted for by five variants: e1a2, e6a2, e8a2, e13a3, e14a3, e19a2. These atypical transcripts cannot be monitored by qRT-PCR using standard methodologies and for most of these cases no molecular monitoring option is available.
Aims: To facilitate molecular monitoring for patients with atypical *BCR-ABL1* transcripts we sought to develop five plasmid constructs containing *BCR-ABL1* transcripts e1a2, e6a2, e8a2, e14a3, or e19a2 along with the three principal controls genes *ABL1*, *BCR* and *GUSB*. Construction of plasmids containing the *BCR-ABL1* variant and the control genes allows the same plasmid standard to

be used for both targets.
Methods: The e6a2, e14a3, and e19a2 transcripts were PCR amplified from patient blood samples to produce amplicons upstream of the *BCR* breakpoint to *ABL1* exon 10. E1a2 was amplified in a similar fashion from the SD1 cell line. The e8a2 transcript was also amplified from patient leukocytes from *BCR* exon 8 to *ABL1* exon 5 and included a 55bp inverted insertion between the two genes that was derived from ABL1 intron 1. Three transcripts (e6a2, e14a3, e19a2) were cloned into pCR2.1 (Invitrogen). e6a2 and e19a2 (HindIII/XbaI) fragments were subcloned into a previously constructed vector pUC18_GUSB_BCR (HindIII/XbaI) and e14a3 (HindIII) was subcloned into pUC18_ABL1_BCR_GUSB (HindIII). E8a2 and e1a2 were amplified using primers with SalI tags and subcloned into pUC18_BCR_GUSB (SalI). Plasmid sequences were verified by sequencing the cloned insert and then plasmids were linearised using HindIII (e6a2, e19a2), EcoRV (e1a2, e8a2) and SalI (e14a3). Each plasmid was quantified and the copy number determined. Ten-fold serial dilutions were prepared in 0.1xTE buffer containing 50µg/ml tRNA and analysed using real time PCR with transcript specific forward primers and the Europe Against Cancer reverse primer and probes ENR561 and ENP541 (e1a2, e6a2, e8a2, and e19a2) and ENR1063 and ENP1043 (e14a3).
Results: Copy numbers were assigned to each plasmid and their performance evaluated on replicate serial dilutions. Standard curves showed acceptable gradients: -3.33 (e1a2), -3.33 (e6a2), -3.36 (e8a2), -3.45 (e14a3), and -3.36 (e19a2) and all had an r^2 above 0.99.
Summary / Conclusion: We conclude that this set of 5 plasmids are suitable for calibrating residual disease in patients with atypical *BCR-ABL1* transcripts. Because of the rarity of these fusions, we suggest that molecular monitoring is performed in a very limited number of centres to facilitate cost effective, quality controlled analysis.

B1750
COMPARISON OF CONVENTIONAL CYTOGENETICS, MULTIPLEX RT-PCR, NPM AND FLT3 MUTATION FOR THE DETECTION OF GENETIC ABNORMALITIES IN ACUTE MYELOID LEUKEMIA
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Background: Acute myeloid leukemia (AML) is a clonal disorder resulting from genetic alterations. Although conventional cytogenetic analysis is a most common method to detect many genetic abnormalities, it requires time for dividing cells and 40 to 50% of AML show normal karyotype. For these limitations, new molecular methods such as multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) and gene mutations have been implemented in characterization of AML to determine diagnosis, prognosis and treatment.
Aims: We studied the clinical usefulness of each method as a routine diagnostic practice.
Methods: We collected data of 104 newly diagnosed AML patients between January 2010 and December 2012. Cytogenetic analysis was performed on unstimulated 24- and 48-hour cultures of bone marrow and multiplex RT-PCR was performed using Hemavision system (DNA Technology A/S, Aarhus, Denmark) to detect 28 different translocations. In addition, *FLT3*-ITD mutations and *NPM1* mutations were analyzed by PCR and sequencing.
Results: Of 104 bone marrow specimen, 57 (54.8%) had clonal abnormalities in cytogenetic analysis and 26 (25.0%) were positive for multiplex RT-PCR. There was no case with more than 1 translocation in multiplex RT-PCR. Among 26 multiplex RT-PCR positive cases, 24 cases had same translocations in cytogenetic analysis. But cytogenetic analysis missed translocations in 2 cases and false negative rate of cytogenetic analysis was 7.7% (2/26). One case with t(9;11) showed normal karyotype in cytogenetic analysis and 1 with t(10;11) had no abnormalities of chromosome 10 and 11 except i(17). Among 57 cases with cytogenetic abnormalities, 32 cases had genetic aberrations not targeted by multiplex RT-PCR but 1 with t(15;17) showed false negative for multiplex RT-PCR due to very low RNA quantity (9.8 ng/µL). So false negative rate of multiplex RT-PCR was 3.7% (1/27). The *NPM1* mutations were identified in 19 (18.3%) of the 104 AML and in 18 (40.0%) of the 45 AML showing normal karyotype and negative multiplex RT-PCR. And type A *NPM1* mutations were 15 (78.9%). The *FLT3*-ITD mutations were found in 6 (13.3%) of the 45 AML with normal karyotype and were a sole detected genetic abnormality in 4 cases.
Summary / Conclusion: In AML, genetic changes could be detected in 76.9% using cytogenetic and molecular analysis including gene mutations. Cytogenetic analysis has the extensive capacity to detect chromosomal abnormalities. Multiplex RT-PCR has an advantage in detecting cryptic translocations missed by cytogenetic analysis and gene mutations provide additional prognostic data. Therefore, combination of cytogenetic and molecular analysis should be used at the time of diagnosis routinely.

B1751
CLINICAL AND MOLECULAR FINDING IN MACEDONIAN FAMILY WITH NIJMEGEN BREAKAGE SYNDROM
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Background: Nijmegen breakage syndrome (NBS) is a rare autosomal recessive chromosomal instability disorder characterized by microcephaly, immunodeficiency, radiosensitivity and a very high predisposition to malignancy. The gene responsible for NBS, NBS1, is located on chromosome 8q21 and encodes a protein called nibrin. This protein is a component of the hMre11/hRad50 protein complex, suggesting defective DNA double strand break (DSB) repair or cell cycle checkpoint function in NBS.
Aims: In this report we describe two patients with the NBS in Macedonian family. To our knowledge this is the first family with NBS reported from Macedonia.
Methods: Clinical and laboratory examinations with regard to lymphoma and NBS. Mutation detection using genomic DNA was performed by PCR with specific primers for the human *NBS1* gene, followed by automated DNA sequence analysis
Results: Both children presented with microcephaly, syndaktylia and by the development of T cell non Hodgkin lymphoma at the age of 7 and 10 years, respectively. Molecular analysis showed homozygositye for 657del5 mutation in the NBS1 gen. The parents were heterozygotes and they had no knowledge of consanguineous relationship. The first child was treated with BFM-NHLprotocol and achieved a complete remission which lasted for 21 months. A subsequently, he developed a medullar relapse with hiperleukocytosis and died due to lethal CNS complications. The second child was treated according to AIOP-BFM ALL 2009 protocol. The remission was not achieved in this patient and she passed away after a very brief and severe episode of gram-negative sepsis and SIRS during bone marrow aplasia after the intensive chemotherapy block in the induction phase for bone marrow transplantation.
Summary / Conclusion: Because of increased sensitivity to radiation therapy and chemotherapy, the treatment of malignancies in patients with NBS can be difficult. The ability to diagnose disease at the molecular level allows prenatal diagnosis in families at risk to having a child with NBS which is warranted in these families.

B1752
A MULTIPLEX REAL TIME QUANTITATIVE PCR METHOD TO DETECT THE CO-EXPRESSION OF WT1 AND MDR1 GENE EXPRESSION IN ACUTE LEUKEMIA PATIENTS
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Background: The interaction between Wilms tumor gene 1 (*WT1*) and the promoter region of the multidrug resistance-1 (*MDR1*) gene has been previously reported but the clinical significance of the coexpression of *WT1* and *MDR1* in acute leukemia *(*AL*) is still largely unknown*. So correlative studies of *MDR1* and *WT1* expression in leukemia cells may ultimately contribute to a better understanding of the relationship of *WT1* and *MDR1* gene expression and elucidate the clinical significance.
Aims: To establish a multiplex real-time quantitative PCR (RQ-PCR) method to detect the co-expression of *WT1* and *MDR1* in acute leukemia patient.
Methods: Total RNA was extracted from k562 cell line and was revrerse transcribed to cDNA by the outer primers of *WT1* and *MDR1* respectively. The cDNA of *WT1* and *MDR1* were purified and digested by BamHI and BglII, and then the two fragments were ligated to form the recombinant fragment *WT1+MDR1*.The outer forward primer of *WT1* and outer reverse primer of *MDR1* were used to amplify the recombinant fragment *WT1+MDR1*.The PCR product was purified and connected with pMD18-T vector, and then transferred into Ecoil DH-5α. A new kind of *WT1-MDR1*-contained plasmid was gained from the positive colony. The recombinant plasmid was verified by digestion with restriction enzyme and PCR amplification. A multiplex real time quantitative PCR method was set up with FAM-labeled *MDR1* probe and VIC-labeled *WT1* gene in one reaction tube. The absolute copy numbers of these two genes were calculated according to their own standard curve. positive plasmid and 47 acute leukemia(AL) patients were tested by this method.
Results: The recombinant plasmid was confirmed by EcoR1 digestion and PCR amplification. The multiplex RQ-PCR technique can catch the sensitivity of *WT1* gene and *MDR1* to 10 copy/µl. The standard curve slope were 0.998 and 0.999. The expression results were the same tendency with route RQ-PCR method. The *WT1* and *MDR1* expression levels of *de novo* AL patients were significantly higher as compared to the controls P<0.001. The *WT1* and *MDR1* expression correlated to the clinical outcome, the AL patients with *WT1* and *MDR1* expression levels significantly decreased after chemotherapy and retained in a low range had a favorable outcome, while patients with high *WT1* and *MDR1* expression levels persistent or increased significantly predicted an adverse prognosis.
Summary / Conclusion: A multiplex real time quantitative PCR method to detect *WT1* and *MDR1* gene were constructed successfully, and it could be used to detect the expression levels of *WT1* and *MDR1* genes simultaneously. The expression of *WT1* and *MDR1* may provide useful information for AL patients prognosis.

B1753
UNUSUAL CONSTITUTIONAL ANOMALY T(11;12;20) IN A CASE OF MULTIPLE MYELOMA
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Background: It is accepted that constitutional structural chromosome aberrations are associated with cancer predisposition. The cytogenetic investigation of these anomalies could be of major importance for identification of the molecular events responsible for the initiation steps in oncogenesis. However, because of insufficient data, the main question, are constitutional chromosome aberrations a part of the multistep malignant process has not been as yet clarified.
Aims: . In this report we describe a new constitutional rearrangement in a case of multiple myeloma. The aim of our study is to investigate is there a link between the constitutional anomaly and the tumor clone.
Results: The patient a 71-year old female was presented with lower back pain, X-rays data of multiple loci of osteolysis in the pelvis, mild anemia (Hb 114 g/l), elevated serum monoclonal protein concentration (35g/l; IgA/kappa), kappa light chain in the urine and 30 percent plasma cells in the bone marrow (BM). Cytogenetic examination of phytohemagglutinin stimulated peripheral blood (PB) established 12 cells (50 cells analyzed) with the following three-way reciprocal translocation 46,XX,t(11;12;20)(p11.2;q21.32;q11.1). Fluorescent in situ hybridization (FISH) of PB with whole chromosome probes for 11 (WC11) and 20 (WC20) confirmed the translocation. Cytogenetic study of BM with G-banding method revealed pathological clone with different numerical and structural anomalies. The clonal aberrations are summarized in the following composite karyotype: 80-81,XX,+1,+1,+2,+2,+3,+3,+4,+4,+5,+5,+dic(5;?;9)(5pter->5p12::?::9p11-9qter),+6,+6,+8,+10,+10,+11,+11,+13,robt(14;15)(p11;p11),+15,+der(17)t(17;?)(p13;?),+18,+18,+19,+19,del(20)(p11.1p11.2),+21,+21,+22,+22,+3mar,dmin.[cp5]. The remaining 18 cells analyzed showed normal diploid karyotype 46,XX. Constitutional anomaly t(11;12;20) or some of the constitutional derivative chromosome №№11, 12, 20 are not presented in the malignant clone. Fish analysis of BM with WC11 and WC20 did not find constitutional rearrangements in chromosome 11, 12 and 20, but demonstrated that the deleted segment 20p11.1-11.2 is inserted in the dicentric chromosome between the parts of chromosome 5 and 9. Split assay with DNA specific probes for IgH region at 14q32 and DNA specific probes for 1q21/1p36, p53 showed normal FISH signals. DNA specific probe for 13q14 indicated clone with monosomy 13 – nuc ish(DLEUx1,13qterx1)[10/100]. In conclusion, the pathological clone in our case has not been originated from the cells with constitutional anomaly and the both are independent cytogenetic events.
Summary / Conclusion: In conclusion, the pathological clone in our case has not been originated from the cells with constitutional anomaly and the both are independent cytogenetic events.

Drug resistance and pharmacology

B1754
EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN 1 INCREASES DRUG-RESISTANCE THROUGH ENHANCED PKD
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Background: Epstein-Barr virus (EBV)-encoded latent membrane protein-1 (LMP1) is a transmembrane protein essential for EBV-induced immortalization and transformation of B cells. Protein kinase D (PKD, also called PKCmu) as a serine/threonine kinase processes important to cancer development, including cell growth, apoptosis, motility, and angiogenesis.
Aims: We sought to identify molecular targets of LMP1 and examined the effects of a specific LMP1 target on the chemo-susceptibility of EBV-infected B cells.
Methods: To investigate the effect LMP1 on B cell, we compared gene expression profile according to the LMP1 expression, using commercially available cDNA microarray. Additionally, we examined that LMP1 and PKD co-localize in cytoplasm by confocal microscopy and interact by immunoprecipitation. Cytotoxicity assay was determined whether LMP1-induced PKD drug resistance by promoting cell survival.
Results: LMP1 induces expression of endogenous PKD in B cell specific manner. LMP1 and PKD were co-localized interaction in cytoplasm. We verified that NF-*K*B binding is required for LMP1-induced enhancement of PKD expression. Furthermore, PKD was required for LMP1-induced B cell survival. First, PKD expression enhanced the survival of durg-treated BJAB cells. Second, inhibition of PKD expression by siRNA (siPKD) and GÖ6983 effectively suppressed LMP-1-induced cell survival. Third, PKD mutation and other PKC isoform decreased LMP1-induced drug resistance.
Summary / Conclusion: PKD is a downstream target cellular target of LMP1 via NF-kB and co-localized interaction in B cells. These finding suggest that PKD may be a candidate for new therapeutic interventions aimed at EBV-associated B cell lymphoma.

B1755
TRIPTOLIDE, A CHINESE HERBAL EXTRACT, SENSITIZES RESISTANT LEUKEMIA CELL LINES THROUGH DOWN-REGULATION OF HIF-1A AND NRF2
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Background: Emergence of cancer cells resistance might be responsible for relapse of leukemia while addition of another cytotoxic drug into the chemotherapy regimen commonly leads to more side effects. Thus, it is challenging to find a novel chemotherapy sensitizer which could be used in a relative lower dosage. Triptolide (TPL) is the extract of old Chinese medicine that has recently been indicated to show cytotoxicity to multiple cancers. However, its application is limited by its narrow therapeutic window. Previous studies have demonstrated that it could re-sensitize resistant cancer cells however the mechanism remains unclear. HIF-1α and Nrf2 are two transcriptional factors which was reported to be related to chemo-resistance.
Aims: To investigate the ability of TPL to re-sensitize HL60/A cell line to Adriamycin (ADM) and K562/G to Imatinib(IM) and related mechanism.
Methods: HL60/A and K562/G cells were subjected to different treatments and thereafter MTT assay, flow cytometry and Western blot or RT-PCR were used to determine IC_{50}, apoptotic status and expression of Nrf2, HIF-1α and their target genes.
Results: Cytotoxic effect of TPL to HL60/A and K562/G cell line were assayed using MTT assay after being exposed to a serial concentration of TPL for 48h. TPL demonstrated a high toxicity to HL60/A and K562/G with IC_{50}= 43.064±1.064 nM and 55.142±5.220 nM respectively. MTT assay showed that a relatively low dose of TPL (IC_{20}: 14nM for HL60/A and 25nM for K562/G) enhanced the cytotoxicity of ADM (IC_{50}:14.36±2.23 vs. 7.9±0.33μM, 1.82 fold; *P* =0.008) to HL60/A and IM (IC_{50}: 62.54±4.33 vs. 53.11±0.57μM, 1.18 fold; *P* =0.02) to K562/G. Results of combination index showed that TPL and anticancer agents had synergistic effects when fraction affected was below 70% (ADM) and 90% (IM). Moreover, apoptotic ratio of cells treated by ADM or IM together with TPL was significantly increased compared to cells treated by ADM or IM alone (ADM: 19.55±1.70% vs. 72.62±4.83, *P* =0.000; IM: 24.78±1.12 vs. 77.52±7.75, *P* =0.000). To further explore the mechanism, Nrf2 and HIF-1α expression was detected by western blotting and RT-PCR with down-stream genes being assessed after HL60/A and K562/G cells being exposed to indicated drugs (ADM 7μM plus TPL 14nM; IM 50μM plus TPL 25nM respectively). In combination with TPL, both ADM and IM down-regulates Nrf2 and HIF-1α expression in HL60/A and K562/G cells at both protein and mRNA levels with Down-stream genes of Nrf2 e.g NQO1, GSR and HO-1 as well as target genes of HIF-1α such as BNIP3, VEGF and CAIX also down-reg-

ulated at mRNA level.
Summary / Conclusion: In conclusion, our study demonstrated that TPL reverses chemo-resistance of HL60/A and K562/G in vitro through down-regulation of Nrf2 and HIF-1α. This work indicates a new role for TPL in cancer therapeutics.

B1756
CHANGES IN GENE EXPRESSION PROFILES IN RESPONSE TO APIGENIN IN IMATINIB SENSITIVE AND RESISTANT CHRONIC MYELOID LEUKEMIA CELLS
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Background: Chronic Myleoid Leukemia (CML) results from BCR-ABL gene which is the fusion product of a reciprocal translocation between chromosomes 9 and 22. Currently,tyrosine kinase inhibitors (TKIs) such as imatinib, nilotinib and dasatinib, are generally used for the treatment of CML. Although these inhibitors have significant therapeutic efficacy, the development of resistance is a major problem in terms of patient benefit. Many novel compounds are currently being investigated preclinically and clinically in terms of their therapeutic potentials to overcome imatinib resistance. Apigenin (4',5,7- trihydroxyflavone), a plant dietary flavonoid, has anticancer effects on various cancer types. However, there is no information about the precise mechanisms by which apigenin exerts its antileukemic effects.
Aims: The aim of this study is to obtain comprehensive gene expression profiles altered by apigenin in imatinib-sensitive and imatinib resistant, Philadelphia-positive K562 CML cells.
Methods: K562 and 3 micromolar imatinib resistant K562 cells (K562/IMA-3) cells were treated with 100 μM apigenin for 72 h. Then, total RNA was isolated and converted to cDNA by reverse transcription reaction. Gene expression profiles were determined using Human HT-12v4 beadchip (Illumina, whole genome expression beadchip). Quality control of raw data was performed with the Genome Studio software (Illumina). We performed network and pathway analysis using Ingenuity Pathway Analysis (IPA) software.
Results: Cellular development, hematological system function and hematopoesis were the highest rated Networks in K562 cells as compared to K562/IMA-3 cells. Moreover, hematological disease (p-value:2,78E-04) and cancer (p-value:3,34E-02) were the most statistically significant biofunctions in K562 cells treated with 100 μMapigenin as compared to K562/IMA-3 cells. We also found that the expression levels of 17 genes were statistically affected in both cancer and hematologic disease pathway (p value=.6,44E-03) after apigenin treatment in K562 cells and we determined that these genes are responsible for the development of chronic leukemia. Human serine/threonine protein kinase B-raf proto-oncogene, human non-receptor tyrosine kinase proto-oncogene (FYN), non-polymorphic integral membrane protein (CD74) and interleukin-8 genes are downregulated while programmed cell death-1-ligand-2 and mir-15 genes are upregulated in 100 μM apigenin-treated K562 sensitive cells as compared to untreated controls. ADAMTS5 matrix-metalloproteinase and tumor necrosis factor receptor-associated factor-5 and interleukin-15 receptor alpha genes are upregulated in 100 μM apigenin-treated K562/IMA-3 cells. Downregulated genes in K562/IMA-3 include FOXA2 (Fork-head box) and CYP4Z1 (a cytochorome P450 enzyme) as compared to untreated controls.
Summary / Conclusion: Our results demonstrated that apigenin affected the expression of a large number of genes that are related to the development of CML, cell survival and physiologic behaviors in both cell types. This may help to determine the molecular mechanisms by which apigenin exert pleiotropic effects on K562 and K562/IMA-3 cells. Furthemore, this information could be investigated for the development of chemopreventive and/or therapeutic strategies for CML treatment.

B1757
VARIATION IN LEVELS OF SERUM IMMUNOGLOBULINS AND ITS RELATIONSHIP WITH THE DEVELOPMENT OF SERIOUS INFECTIONS IN PATIENTS TREATED WITH RITUXIMAB
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Background: Rituximab is a chimeric monoclonal antibody directed toward CD20, a pan B-cell surface marker that has been proven effective in depleting normal and malignant B cells *in vivo*. Rituximab has demonstrated in combined with chemotherapy and stem cell transplantation, is an effective therapeutic option in the treatment of B lymphoproliferative disorders who expressing CD20. Recent studies have indicated that patients treated with Rituximab, have an increased risk of developing hypogammaglobulinemia, which can increase risk of infectious processes with clinical relevance. However this observation has notthe same significance for all patients, so there must be other factors that have influence in the development of hypogammaglobulinemia (hGM) and severe infections. In this sense, FCGR3A-158V/F polymorphism and its relationship with serum immunoglobulin levels after treatment with Rituximab has been reported as a possible risk factor for developing these complications.
Aims: We report initial data of a study in which we evaluate the impact on variations IG levels in patients treated with Rituximab and its influence on the development of clinically relevant infections.
In a second phase (ongoing) we will analyze the presence/absence of the FCGR3A-158V/F polymorphism in blood samples; from two groups of patients, with and without hypogammaglobulinemia.
Methods: Retrospective study conducted between, January 2008/Maye 2012, which includes all patients who received treatment with Rituximab in our hospital. We analyze demographic parameters (age, sex), hematologic pathology, IG values (pre and post treatment), defining the hypogammaglobulinemia according to the following cutoffs: IgG ≤750mg/dl, IgA ≤80mg/dl and IgM ≤45mg/dl, and its association with the presence of clinically relevant infections (such as reason for hospital admission) and analyzed in respect of type of infection, causing germ and final outcome. Statistical analysis was performed using SPSS v.15: Test of homogeneity for comparison two proportions (Z test for comparing).

	Patients	Infection	Site of infection	Germ	Exitus
hGM	n=41 (45%)	n=26 (63%)	Respiratory n=11 Bacteremia n=13 Others n=2	GNB n=19 CG+ n=4 Virus n=3	hGM with infection n=11 (66%) hGM without infection n=1 (6%)
IG-n	n=51 (55%)	n=15 (29%)	Respiratory n=2 Bacteremia n=8 Others n=4	GNB n=3 CG+ n=2 Others n=10	n=6 (33%)
Differences	---	**p<0,01**	---	---	**p<0,01**
Total	n=92	n=41 (44,5%)	---	---	n=18 (19,5%)

Results: Included 92patients [54%♂/♀ 46%], median age of 63 years (range:18-85). Pathologies distribution was: Chronic Lymphocytic Leukemia (CLL)(12%) Follicular Lymphoma (FL)(38%), Diffuse Large Cell Lymphoma (DLCL)(36%), Mantle Cell Lymphoma (MCL)(4%),Burkitt Lymphoma (BL)(4%), Immune Thrombocytopenic Purpura (ITP)(3%), and others (3%). According IG serum (IgG, IgM, IgA), pretreatment, only 15patients (16%), had values below the normal range at the expense of mainly IgG. Administered once treatment with Rituximab, 41patients (45%) also showed predominantly hGM IgG (47%), followed by IgM (44%) and IgA (9%). 41patients (45%) had an infectious event from the start of therapy until end. The analyzed data are shown in Table 1.
Summary / Conclusion: In our series, Rituximab has been associated with a high incidence in developing hGM, being charge or a greater number of infections (63%vs.29%)(p <0.01) vs. patients without alterations in IG basal levels. We found no differences in the site of infection or germ, compared to patients without hGM. For the number of deaths in our study, is higher in patients with infection associated hGM vs. IG patients in the normal range (61% vs.39%)(p <0.01) were statistically significant difference despite the small sample size. These results will be completed and checked in the future with the determination of FCGR3A-158V/F polymorphism. Second phase is ongoing, and results will be presented as soon as possible.

B1758
EFFECTS OF APIGENIN ON IMATININ-SENSITIVE AND RESISTANT CHRONIC MYELOID LEUKEMIA CELLS
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Background: Chronic myeloid leukemia (CML) is characterized by a reciprocal translocation between BCR and ABL genes. The resulting BCR-ABL1 oncogene encodes the chimeric BCR-ABL1 protein with constitutive kinase activity. In the clinic, tyrosine kinase inhibitors, especially imatinib, are used for CML therapy. Despite its therapeutic efficacy, not all patients benefit from imatinib because of the development of resistance. Many novel compounds are currently being investigated preclinically and clinically in terms of their therapeutic potentials to overcome imatinib resistance. Apigenin (4',5,7- trihydroxyflavone), a common plant dietary flavonoid, has been paid attention as an alternative anticancer compound.
Aims: The aim of this study is to examine antiproliferative, cytostatic and apop-

totic effects of apigenin on imatinib-sensitive and imatinib resistant K562 CML cells.
Methods: Growth-inhibitory effects of apigenin on K562 and 3 micromolar imatinib resistant K562 cells (K562/IMA-3) were determined by MTT cell proliferation assay. Apoptotic effects of apigenin were determined by changes in caspase-3 enzyme activity, loss of mitochondrial membrane potential (MMP), and Annexin-V staining by flow cytometry. Cytostatic effects of apigenin were examined using DNase-free RNase and propidium iodine by flow cytometry.
Results: There were significant decreases in proliferation of both K562 and K562/IMA-3 cells in a dose- and time dependent manner. IC50 values of apigenin were found to be 65- and 63 μM for K562/IMA-3 cells for 48- and 72 hours, respectively, while it was 16- and2,5 μM for K562 cells, respectively. Increasing concentrations of apigenin triggered apoptosis in both cell types. There were 160- and 320% increases in apoptotic K562 cell population in response to 50-, and 100 μM apigenin as compared to untreated cells, respectively. On the other hand, 50- and 100 μM apigenin caused 10- and 20% increases in apoptotic K562/IMA-3 cell population as compared to untreated cells. Apigenin caused the loss of MMP in both sensitive and resistant cells exposed to same concentrations of Apigenin. The loss of MMP were 30-, 70-, 110-, and 130% in K562 cells treated with 5-, 10-, 50-, and 100 μM apigenin, respectively. The same concentrations of Apigenin led to 18-, 19-, 30-, and 41% increases in loss of MMP in K562/IMA-3 cells, respectively. Moreover, there were increases in caspase-3 enzyme activity in response to Apigenin in a dose-dependent manner in both K562 and K562/IMA3 cells. Treatment of K562 cells with Apigenin resulted in small increases in the percentage of cells in the G2/M phase at 5 to 20 μM Apigenin but in response to 100 μM Apigenin, there was a significant increase. There was no significant effect of Apigenin on G2/M phase of K562/IMA-3 cell cycle. However, K562/IMA-3 cells arrested in S phase especially at 100 μM apigenin.
Summary / Conclusion: Our results, in agreement with each other, showed that apigenin has cytotoxic, apoptotic and cytostatic effects on both cell types in a time- and dose- dependent manner. However, K562/IMA-3 cells were still more resistant to apigenin than K562 cells. But comparing the imatinib resistance, K562/IMA-3 cells demonstrated less resistance to Apigenin.

B1759

A CLINICAL MICRODOSE STUDY WITH 14C-ELACYTARABINE IN HEALTHY MALE SUBJECTS TO GENERATE MASS BALANCE DATA TO SUPPORT REGULATORY SUBMISSION: AN INNOVATIVE MICROTRACER APPLICATION

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Background: Obtaining human mass balance data for a cytotoxic drug product has always been regarded as a challenge. While it is not feasible to perform a conventional mass balance study at clinical doses in healthy volunteers, it is difficult to subject severely ill patients to the rigours of prolonged residency in a clinical unit and to the comprehensive excreta collection and blood sampling that is required. Notwithstanding these challenges, regulatory authorities do ask pharmaceutical companies to describe the routes and rates of elimination for cytotoxic drug molecules as a minimum description of drug metabolism in the registration packages for new drug products.
Elacytarabine is a novel, lipid derivative of the anti-cancer drug cytarabine commonly used in the treatment of acute myeloid leukemia. The drug is in the final stages of a phase 3 study in relapsed or refractory AML. Elacytarabine is a stable ester of elaidic acid and cytarabine.
Aims: This paper describes an open label, single-dose study to assess the mass balance recovery of an intravenous microdose of ^{14}C-elacytarabine in healthy male subjects. The study requirement was driven by a specific request from the US Food and Drug Administration (FDA) to provide supporting information on the routes of elimination of total radioactivity and total recovery. The study synopsis was reviewed by the FDA before implementation.
Methods: The program of work from the radiosynthesis of the ^{14}C API through to the final study report was delivered as a synthesis-to-clinic package. The clinical study design comprised a single intravenous administration of 50μg ^{14}C-elacytarabine containing not more than 270nCi of ^{14}C to a single cohort of 6 healthy male subjects. The subjects remained resident in the clinical unit for 7 days post dose and returned for 24 hour visits on Days 10 and day 14. Blood, urine and faecal samples were collected through the residential periods and samples were submitted for total ^{14}C analysis by accelerator mass spectrometry.
Results: Average urinary mass balance recovery in excess of 85% (draft data) in the first 48 hours post-dose was achieved. The complete cumulative mass balance recovery will be presented together with the conclusions drawn about routes of elimination.
Summary / Conclusion: This study demonstrated the validity of a microdose approach to generate mass balance data to support regulatory submission for a drug where obtaining the data by traditional methods was not possible.

B1760

ROLE OF VKORC1 AND CYP4502C9 POLYMORHISM IN RESISTANCE TO WARFARIN THERAPY AMONG EGYPTIAN PATIENTS

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Background: Warfarin is still considered the principal oral anticoagulant. Its effect is subjected to a maltitude of factors among which are the VKORC1 and CYP4502C9 SNPs. Resistance to warfarin therapy is an emerging clinical problem especially that other oral anticoagulants do not cover completely the spectrum of anticoagulation indications. Pharmacogenetic testing has been approved for dosing calculation though its routine use was not recommended. The role of warfarin pharmacogenetics and pharmacogenomics had not been widely tested.
Aims: To screen Egyptian warfarin treated patients for various VKORC1 and CYP4502C9 polymorphisms
To stratify the warfarin effect regarding the genotype of the patient in order to spot any racial or ethnic variations between Egyptians and the previously published international data. To spot any relation between patient genotype and resistance to warfarin therapy.

	VKORC1	CYP4502C99		N	Minimum	Maximum	Mean	Std. Deviation
control	GG	1*/1*	dose	6	2	12	7.50	3.406
			INR	6	2	5	2.93	1.237
		1*/2*	dose	1	5	5	5.00	.
			INR	1	2	2	2.00	.
		1*/3*	dose	4	5	6	5.25	.500
			INR	4	2	4	3.32	.922
	GA	1*/1*	dose	9	3	10	5.78	2.587
			INR	9	2	5	2.68	.967
		1*/2*	dose	7	3	12	6.29	3.352
			INR	7	2	7	3.05	1.889
	AA	1*/2*	dose	1	6	6	6.00	.
			INR	1	2	2	2.00	.
case	GG	1*/1*	dose	12	16	20	18.67	1.303
			INR	12	1	1	1.08	.122
		1*/2*	dose	3	20	20	20.00	.000
			INR	3	1	2	1.23	.252
		1*/3*	dose	4	18	25	20.75	2.986
			INR	4	1	2	1.28	.222
	GA	1*/1*	dose	11	18	25	19.73	2.005
			INR	11	1	2	1.24	.150
		1*/2*	dose	1	30	30	30.00	.
			INR	1	1	1	1.00	.
		1*/3*	dose	4	18	20	19.00	1.155
			INR	4	1	1	1.25	.173
		3*/3*	dose	1	18	18	18.00	.
			INR	1	1	1	1.20	.

Methods: We performed a case-control study. Informed consent was obtained from each participant. We enrolled patients failing to reach a target INR of 2-3 after using large doses of warfarin (more than 15mg PO daily) as cases (n=36). Age and sex matched patients successfully reached target INR with usual doses were considered as controls (n=28). Both groups were screened for VKORC1 -1629G>A and CYP4502C9 variants after at least 8 weeks of therapy.
Inclusion Criteria:
- Informed consent
- Indication for longterm coagulation

Exclusion Criteria:
- Extremes of age (>70y and <18y)
- BMI >25
- Disturbed baseline AST, ALT, Bilirubin, Albumin, PT, APTT, CBC
- Drug intake other than warfarin and LMWH
- Current or history of malignancy

Results: On analyzing our results, we found more males among the control group (53.6% males compared to 46.4% females) though statistically insignificant (p value 0.44), while among cases females were more encountered (58.3% females compared to 41% males) which reached statistical significance (p value 0.02). On testing for VKORC1 mutations, we found 47.2% of cases with mutant allele, all heterozygous. On testing the control group for the same mutation, we found 60.7% of subjects (n=17) with mutant allele, 3.6% (n=1) was homozygous (AA) genotype, though not achieving statistical significance (p value = 0.27). On testing for mutations affecting CYP4502C9 allele, we found 36.1% (n=13) of cases with mutant alleles, 11.1% (n=4), 22.2% (n=8) and 2.8%(n=1) with 1*/2*, 1*/3* and 3*/3* respectively. Regarding controls, we detected mutations in 46.4% (n=13) of which, 32.1% (n=9) had 1*/2* and 14.3 (n=4) had 1*/3* alleles, though not achieving statistical significance (p value = 0.15). On correlating VKORC1 genotype with mean INR among controls, GG variants achieved a higher INR of 2.99 followed by genotype GA (INR=2.84)

and the least with genotype AA (INR=2) at a relatively constant warfarin dose (p value 0.05). On correlating CYP4502C9 genotype with mean INR among controls, the wild genotype 1*/1* was associated with the lower INR of 2.78, followed by the genotype 1*/2* (INR=2.82) the genotype 1*/3* (INR=3.32) with the same median warfarin dose of 5 mg per day (p value = 0.02). Same correlation was demonstrated among cases. When combined genotype possibilities were correlated to the mean INR we found the highest INR with the genotype GG/1*/3* (INR=3.32) and the lowest with AA/1*/2* (p value=0.014). We found that patients carrying at least one wild type allele needed higher doses of warfarin.

Summary / Conclusion: Our study confirmed the relation between warfarin pharmacogenetics and dosing although our patients needed higher warfarin doses than internationally reported one. We did not prove any relation between VKORC1 and CYP4502C9 SNPs and resistance to warfarin therapy.

B1761

EVALUATION OF CHEMICAL STABILITY OF ROMIPLOSTIM IN STERILE WATER

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Background: Romiplostim is an Fc-peptide fusion protein (peptibody) that signals and activates intracellular transcriptional pathways via the TPO receptor (also known as cMpl) to increase platelet production. Romiplostim is indicated for adult chronic immune (idiopathic) thrombocytopenic purpura (ITP) splenectomised patients who are refractory to other treatments (e.g. corticosteroids, immunoglobulins). Romiplostim can be considered as second line treatment for adult non-splenectomised patients where surgery is contra-indicated. The overall platelet response rate was noted in 88% of non-splenectomised and 79% of splenectomised patients (Kuter et al., Lancet 2008). Romiplostim is supplied as powder (Nplate, Amgen Dompè®) to be reconstituted with sterile water. After reconstitution, drug solution is considered stable, and may be consequently used within 24 hour when protected from light and kept in the original vial. The expected average annual expense per patient is about 47000€ .
Aims: to evaluate the chemical stability of Romiplostim after 24 hours
Methods: Spectrophotometric analysis was performed using a UV spectrophotometer Shimadzu RF-1501, in the range of wavelength between 200 and 800 nm. Chemical stability of romiplostim was evaluated immediately, after reconstitution of the powder with the correct volume of sterile water for injection, and then at day1,4, 7 and 10. After reconstitution, samples were kept in the dark at °4C.
Results: The results obtained do not show spectrophotometric changes of the samples over time.In particular a maximum peak of absorption at 280 nm was observed in all analyzed samples and no changes attributable to derivatives with different optical characteristics were observed.
Summary / Conclusion: Spectrophotometric analysis shows that Romiplostim is stable after reconstitution in sterile water for 10 days at 4°C. Romiplostim reutilization after reconstitution could allow cost reduction whereas the retail price of the bottle of 250µg is € 994,37. For example, a 70 kg patient receiving 3µg of product, draws 0.42 ml and threws 0.08 ml of romiplostim corresponding to € 159 that is € 7632 annual. From microbiological point of view further studies could better define the sterility of romiplostim solution over time.

B1762

SIROLIMUS EFFICIENCIES ON PEDIATRIC HEMATOLOGY PATIENTS

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Background: *Sirolimus (rapamycin)* is a macrolide immunosupresant which has a distinct mechanism of action by allostherically inhibition of the mammalian target of rapamycin (m TOR), a central controller of cell growth and proliferation.
Aims: To evaluate sirolimus effect on different childhood diseases.
Methods: In our Pediatric Hematology-Oncology Clinic we used sirolimus in fourteen patients aged 1-13 (group 1: arteriovenous malformation AVM, group 2: autoimmune lymphoproliferative syndrome ALPS and group 3: tuberosclerosis TS) after failing multiple other therapies.
Results: *Group 1:* Sirolimus is given to seven patients who have AVM resistant to corticosteroid, propranolol interferon and thalidomide treatments. Especially one of them showed marked improvement. This case had been diagnosed as blue rubber bleb syndrome by cutaneous lesions at birth and gastrointestinal hemorrhages after age1, who needed multiple blood transfusions, 1-2 times per week. His lesions and symptoms were resistant to corticosteroid, propranolol, interferon and thalidomide treatments. Four months after the beginning of the sirolimus treatment, blood transfusions were needed only 2 times and his cutaneous lesions reduced markedly. Similarly, marvoious response has been realized in other six patients' symptoms and cutaneous lesions. *Group 2:* Sirolimus is given to three patients two of whom are siblings, with ALPS at the dosage of 1.6 mg/m^2/day after failure of corticosteroids or due to adverse effects of it. One of them, has been using sirolimus regularly since2,5 years. The other , who was diagnosed and treated erroneously. as diffuse T cell lymphoma, got the true diagnosis after 2 years period when clinically relapsed. After sirolimus treatment, hepatosplenomegaly and lymphadenopaties were disappeared and his blood counts returned to normal. His sister with the same complainments were easily diagnosed as ALPS when she was 3 months old. *Group 3:* Four patients, two of whom are siblings, were diagnosed as tuberosclerosis with intracranial masses and got sirolimus treatment. After sirolimus, intracranial masses showed mild regression in two of them and had no new epileptic attacks. However; the other sibling couple although getting sirolimus treatment regularly since1,5 years had no clinical improve
Summary / Conclusion: In our Pediatric Hematology- Oncology Department, sirolimus efficiency and safety are assessed in 3 groups of patients (AVM, ALPS and TS). These patients showed significant improvement in their clinical status with no side effects. The siblings with tuberosclerosis and intracranial masses, who are still getting sirolimus, have not shown enough recovery and they still have convulsions. No side effects except indefinite discomfort in an infant with arteriovenous malformation have been distinguished. The oral form of the medicine made the dosage adjustment and easy usage available. We think that with the efficiency on different diseases, it's tolerable side effects and easy usage, sirolimus will have a place in childhood diseases treatment regimens in future.

B1763

ACCURACY OF PHARMACOGENETIC ALGORITHMS FOR THE PREDICTION OF ACENOCOUMAROL STABLE DOSES IN ANDALUSIAN POPULATION

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Background: There is high risk of bleeding and thrombus in initiation acenocoumarol treatment. Currently, initial dose of acenocoumarol is prescribed based only on clinical variables. Clinical and genetic variables have an influence of approximately 50-60% on the required dose to achieve stable anticoagulation. Several pharmacogenetic algorithms to improve the prediction of acenocoumarol stable dose in European and Spanish patients. The EU-PACT pharmacogenetic algorithm includes the variables: age, male sex, weight, height, amiodarone, CYP2C9 (rs1057910 and rs1799853) and VKORC1 (rs9923231) genotypes. The Spanish pharmacogenetic algorithm by Borobia et al. was developed in Madrid and includes additional clinical and genetic variables: inhibiting/inducting concomitant drugs, pathologies, smoking status, CYP4F2 (rs2108622) and ApoE (rs7412) genotypes to improve prediction of acenocoumarol stable dose (mg/sem).
Aims: Our aim was to evaluate the accuracy in the prediction of the dose of these two pharmacogenetic algorithms in 15 patients with acenocoumarol stable dosis achieved at Virgen de las Nieves University Hospital.
Methods: Fifteen atrial fibrillation patients with acenocoumarol stable doses treated at a third level hospital of Andalusian were recruited.
Two pharmacogenetic algorithms (EU-PACT and Borobia et al) were used to calculate the predicted dose for every patient, and this predicted dose was compared to the actual stable dose received using a reliability analysis.
Results: Concordance between both algorithms and actual stable doses was moderate-low. The intraclass correlation coefficient (ICC) for the comparison of the European pharmacogenetic algorithm with stable doses received was 0.524 (p-value: 0.088), and for the Spanish pharmacogenetic algorithm, the ICC was 0.268 (p-value: 0.283).
Summary / Conclusion: The accuracy in the prediction of the doses by these two pharmacogenetic algorithms is low, at least for the Andalusian population studied. Therefore, pharmacogenetic algorithms developed to predict acenocoumarol doses should be tested before applying in different populations. In some cases, it could be necessary to develop a predictive pharmacogenetic model adapted to specific populations.

Genomics and proteomics

B1764

FC GAMMA RECEPTOR POLYMORPHISMS IN PATIENTS WITH AUTOIMMUNE HEMOLYTIC ANAEMIA

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Background: Autoimmune hemolytic anemia (AIHA) is a second most common autoimmune blood disorders. AIHA may occur as primary (idiopathic) or secondary to other lymphoproliferative or immune disease. The etiology of AIHA remains unclear, but both genetic and environmental factors are thought to play role in the development of the disease. The aim of our study was to investigate a possible role of two polymorphisms in the Fc gamma receptor 2A and 3A (FCGR2A and FCGR3A) in the development of AIHA.FCGR2A is polymorphic and has two alleles, FCGR2A-H131 and FCGR2A-R131. This polymorphic variation of FCGR2A is due to a single base substitution of nucleotide adenine for guanine in position 494. The allele FCGR2A-H131 has a higher affinity for human IgG2, comparing to FCGR2A-R131. The gene for FCGR3A has also two polymorphic variant alleles: 158 valine (V158) and phenylalanine (F158) due to single base substitution of timidine to guanine at nucleotide position 559. This polymorphism influences ligand binding and FCGR3A-158V allele variant has higher affinity for Fc fragment of IgG1 and IgG3 than 158F variant. These Fc gamma receptor polymorphisms may influence antibody-mediated phagocytosis and antigen presentation activity.
Aims: The aim of our study was to investigate a possible association of these two single nucleotide polymorphisms in Fc gamma receptor genes (FCGR2A +494A/G) and (FCGR3A +559T/G) with autoimmune hemolytic anemia.
Methods: We have analyzed 70 adult patients with AIHA; 35 patients with idiopathic AIHA and 35 patients with secondary AIHA and chronic lymphocytic leukemia (CLL). Controls were 120 healthy individuals. DNA was isolated from peripheral blood mononuclear cells with standard phenol-chloroform extraction. Genotyping was performed by using PCR and RFLP methods. The distribution of genotypes and allele frequencies were compared between patients and controls using a chi-squared test or Fisher's exact test.
Results: Our results demonstrated significantly different genotype distribution for FCGR2A+494A/G in patients with AIHA (n=70; A/A=45, A/G=19, G/G=6) and controls (n=120; A/A=55, A/G=50, G/G=15), P=0.048. There was also significantly higher frequency of the high affinity FCGR2A-131H (+494A) allele in patients with AIHA comparing with controls (66.6% versus 77.8%; P=0.028). Statistical analysis of the genotype distribution for FCGR3A+559T/G showed significant difference between patients with AIHA (n=70; T/T=22, T/G=23, G/G=25) and controls (n=120; T/T=52, T/G=46, G/G=22), P=0.025. We also found significantly higher frequency of the high affinity FCGR3A-158V (+559G) allele in patients with AIHA comparing with control individuals (47.9% versus 37.5%; P=0.007).
Summary / Conclusion: Results of this study, suggest possible role of both FCGR2A and FCGR3A polymorphism in the etiology and development of autoimmune hemolytic anemia, but further larger prospective studies are necessary to confirm these results.

B1765

MOLECULAR CHARACTERIZATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY IN ABU DHABI DISTRICT, UNITED ARAB EMIRATES (UAE)

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Background: Glucose-6-Phosphate dehydrogenase (G6PD; E.C.1.1.1.49) is a key enzyme of the pentose monophosphate pathway, and its deficiency is the most common inherited enzymopathy and affects 400 million people worldwide with more than 140 genetic variants. Acute haemolytic anaemia and Jaundice are the main clinical symptoms, triggered by infection or ingestion of Fava beans or oxidative drugs. It is common among populations of the Arabian Peninsula, including UAE population. The objective of this study is to determine the prevalence of this enzymopathy and to clarify the molecular basis of G6PD deficiency among different age groups of individuals including newborns, children and adults in Mafraq Hospital in Abu Dhabi.
Aims: The aims of this study is to determaine the prevalence and molecular basis of G6PD deficincy within the UAN nationals and non- UAE nationals in Abu Dhabi District.
Methods: Blood samples were collected from subjects after approved by the Research Ethical Committees of Mafraq hospital. It was conducted within a period of three years, i.e. from January 2006 to January 2009. There were 15995 subjects involved in this study: 8984 males and 7011 females. From this there were 6302 UAE nationals: (2996 males and 3306 females) and 9693 were Non- UAE nationals (i.e. 5988 males and 3705 females). Five milliliters of peripheral blood samples were collected in EDTA from each individual to perform routine hematologic investigations and screening for G6PD was done using the florescent spot method followed by the quantitative G6PD assay.
Genomic DNA was extracted from peripheral blood leukocytes for 225 G6PD deficient subjects using Qiagen DNA extraction kit. PCR amplification of the G6PD gene was performed using specific primers. Analysis for the samples were then subjected to PCR-based methods using restriction enzyme digestion (RFLP), denaturing high performance liquid chromatography (DHPLC) and finally confirmation using automated gene sequencing.
Results: Among UAE nationals, the prevalence of G6PD deficiency was 466 (7.4%). From this 347 (11.6%) were males and 119 were females (3.6%). The prevalence of G6PD deficiency among non-nationals was 364 (3.8%). From this 301(5%) were males and 63 (1.7%) were females. The molecular basis of G6PD among nationals of UAE revealed the mutations Mediterranean 563C→T, Aures 143 T→C, Africa A–202G→A and Chatham 1003 G→A representing mutations frequencies 0.785, 0.118, 0.083 and 0,007 respectively. A novel mutation 1011T-G with predicated amino acid change of phe337leu was identified in exon 9 for one UAE national boy. For the non-nationals, genotyping showed the mutations Mediterranean 563C→T, Aures 143 T→C , A–202G→A and Union 1360 C–T representing frequencies 0.744, 0.122, 0.11and 0.024 respectively. PCR products digested with *Mbo*II restriction enzyme, analysis of the PCR product before and after digestion of *Mbo*II enzyme for the detection of Mediterranean mutation in G6PD gene on 2% agarose gel from left to right. The arrow shows restriction sites in normal and deficient patients. Lanes1, 10, and 12: normal samples; Lane 2: positive control, Lane 5: 100 bp ladder marker; Lanes3,4,7,8, 9, 11: G6PD Mediterranean mutation; Lane 6: PCR product, and Lane 13 free nuclease water (negative control).
Summary / Conclusion: The findings suggest that G6PD Mediterranean mutation is the most common mutation underlying G6PD deficiency in UAE which indicates the gene flow from the Indian subcontinent and other parts of the Mediterranean basin. All UAE chromosomes with the Mediterranean mutation had the polymorphic 1311T allele (*Bcl*I+). Such a Med+/*Bcl*I+ haplotype is typical for the Mediterranean region and the Middle East, while most cases from India and South East Asia are Med+/*Bcl*I−, thus suggesting independent origin of Mediterranean mutations on two different haplotype. The presence of G6PD A– is regarded as significant gene flow from sub-Saharan Africa. For the Aures mutation, it was documented as specific mutation for the central and southern part of the Arabian Peninsula thus our findings suggest that the genetic background of UAE nationals descended from authentic Arab tribes.

Novel therapeutics, targeted therapies and gene therapy

B1766

REGULATION OF HEPCIDIN TRANSCRIPTION BY K-7174

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Background: Hepcidin (HAMP) is the principal iron regulatory hormone, controlling the systemic absorption and remobilization of iron from intracellular stores. The expression of *HAMP* increased in patients with anemia of chronic disease. Previously, a synthesized compound K-7174 (Kowa Company Ltd., Tokyo, Japan) was identified through chemical screening as a novel inhibitor for the adhision of monocytes to cytokine-stimulated endothelial cells (Umetani et al. BBRC 2000). Interstingly, K-7174 restored anemia induced by inflammatory cytokines in mice (Imagawa et al. FASEB J 2003), implying that K-7174 might modulate hepcidin level.
Aims: To assess the impact of K-7174 on hepcidin expression based on human hematoma cell line and *in vivo* mice.
Methods: The HepG2 hematoma cell line was used for the analysis. The cells were treated with K-7174 at doses of 10 and 20 uM for 24 h.For transcription profiling, Human Oligo chip 25K (Toray) were used for K-7174-treated HepG2 cells. Western blot analysis was performed with antibody to GDF15 (abcam). GDF15 concentration in the K-7174-treated media was evaluated with ELISA (R&D systems). For *in vivo* analysis, ICR mice were injected intraperitoneally with PBS (control) or 30 mg/kg K-7174, respectively, on day 0-3 and 5-8, and the samples were taken on day 9. Serum hepcidin1 concentration was determined with LC-MS/MS method (MCProt Biotechnology, Kanazawa, Japan).
Results: We first demonstrated that K-7174 treatment in HepG2 cells significantly decreased HAMP expression in a dose- and time-dependent manner. Thus, we next conducted microarray analysis to reveal the molecular mechanism by which K-7174 inhibits the *HAMP* expression. Transcriptional profiling confirmed the downregulation of *HAMP* as well as erythropoietin. Surprisingly, we found that K-7174 strongly induced GDF15, a negative regulator of *HAMP* expression (Tanno et al. Nat Med 2007). Quantitative RT-PCR analysis, Western blot analysis as well as ELISA analysis confirmed the induction of GDF15 by K-7174 treatment, suggesting that K-7174-mediated induction of GDF15 participates in the inhibition of *HAMP* expression.
Next, we assessed if K-7174 inhibits hepcidin expression in mice. Quantitative RT-PCR analysis with liver sample from K-7174-treated mice demonstrated significant upregulation of *Gdf15* and downregulation of *Hamp* ($n = 8$, $P<0.05$). Furthermore, serum hepcidin concentration was also significantly decreased in K-7174-treated mice (Average: 138.1 and 110.4 ng/mL for K-7174-treated and control mice, respectively. $n = 8$, $P<0.05$).
Summary / Conclusion: K-7174 inhibits hepcidin expression partly by inducing GDF15. K-7174 might be considered as a potential therapeutic option to treat anemia of chronic disease.

B1767

GIVINOSTAT INDUCED APOPTOSIS IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Background: Givinostat (ITF2357) is a histone deacetylase inhibitor with potential anti-inflammatory, anti-angiogenic, and antineoplastic activities. Givinostat is in clinical trials for Hodgkin's lymphoma, chronic lymphocytic leukemias and myelomas, and has been granted orphan drug designation in the European Union for the treatment of systemic juvenile idiopathic arthritis and polycythemia vera. The effect of Givinostat on acute lymphoblastic leukemia (ALL), an incurable disease with resistance to therapy developing in the majority of patients, is still unknown.
Aims: We examined the antitumor effect of Givinostat, being able to inhibit class I and class II histone deacetylases (HDACs), on the proliferation and apoptosis of human leukemia cell lines K562, SUP-B15, CCRF-SB, and NALM.
Methods: SUP-B15 is a precursor B-lymphoblastic with both transcripts of t(9;22)(BCR-ABL) and isoform IK6 of IKZF1, while CCRF-SB and NALM didn't. K562 derived from acute erythroblastic leukemia with t(9;22)(BCR-ABL). Cell proliferation and viability, induction of apoptosis, immunohistochemical identification of cell death signalling molecules, and pharmacological inhibition were assayed to detect the apoptotic cell death pathways. Finally, genetic mutation status of these celllines, detected by Ampliseq Version2, was correlated to the effect of Givinostat.
Results: Givinostat inhibited proliferation and induced apoptosis in all B-lymphoblastic leukemia, in a dose-dependent manner, in the sequence of SUP-B15 (IC50=180 nM) > NALM1 (IC50= 250nM) > CCRF-SB (IC50= 0.5 uM). Givinostat had no effect on K562 (IC50>4 uM). Western blot analysis showed significant reduction of pBCR/ABL, pSTAT5, and pCRK-L in SUP-B15 but not in K562. The early and late phase of apoptotic fractions determined by propidium Iodide (PI) and annexin-V staining followed by flow cytometry, in SUP-B15 (35.75% and 72.4%), and NALM1 (27.67% and 61.10%), in the presence of 250 uM Givinostat, were significantly higher than those induced in CCRF-SB (3.5% and 47.61%) and K562 (10.4% and 56.2%), with 1 uM Givinostat for 24 and 48 hrs, respectively. Accompanied with the apoptosis in SUP-B15 and NALM1 were cleavages of procaspase proteins (Casp-3 and -7) and PARP. Elevation of p21 expressions was observed in SUP-B15 and NALM1. In contrast, elevation of miR-20b, the negative regulator of p21, was present in CCRF-SB when treated with Givinostat. The molecular profiling of these four cells lines includes the presence of BCR-ABL+ (p185) and BCR-ABL+ (p210) in SUP-B15 and K562; P72R (+/+) and P72R (+/-) of TP53 in SUP-B15 and CCRF-SB; p.Q135Pfsx12 of TP53(-/-) in K562; and IKZF1 isoform IK6+ in SUP-B15.
Summary / Conclusion: Our data suggest that Givinostat might be used as a potential and effective therapy for acute lymphoblastic leukemia. The presence of BCR/ABL, IK6 isoform, and other mutation types don't alter the proapoptotic effect of Givinostat in SUP-B15. An intact p53/p21 signal pathway might be a dependent factor for Givinostat-induced apoptosis.

B1768

4SC-202: A NOVEL EPIGENETIC MODULATOR TO TARGET CANCER STEM CELLS

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Background: 4SC-202 is an orally available inhibitor of HDAC1, 2 and 3 with a unique combination of anti-cancer activities actually under investigation in a phase I clinical trial in patients with hematological malignancies.
Aims: We wanted to investigate the MoA of this novel epigenetic modulator and show its tolerability and potency as an anti-cancer drug in a phase I clinical trial.
Methods: The potency of 4SC-202 was tested in *in vitro* and *in vivo* model systems for efficacy to target 1) the cancer stem cell phenotype (anchorage independent growth, spheroid formation, migration and invasion), 2) the Wnt signalling pathway (Affymetrix Chip analysis in tumor samples from an orthotopic xenograft model), 3) tumor growth (xenograft models), 4) angiogenesis (HUVEC tube formation assay), and 5) to modulate gene and protein expression (Chip analysis, qPCR and reversed phase protein arrays). Tolerability, safety and efficacy are under investigation in an ongoing clinical trial in patients with hematological malignancies.
Results: 4SC-202s pharmacology comprises epigenetic regulation, targeting of cancer stem cells and inhibition of angiogenesis. 4SC-202 has demonstrated effective modulation of Wnt-signaling in preclinical *in vitro* and *in vivo* models. Notably, epigenetically driven Wnt-modulation results in the inhibition of cancer stem cell related properties like anchorage independent growth, formation of spheroids and prevention of migration and invasion. Additionally, 4SC-202 is highly active on a number of preclinical hematological and solid tumor models. Currently, 4SC-202 is under clinical phase I evaluation in pretreated patients with hematological tumors (TOPAS study). A biomarker program including *ex vivo* determination of HDAC activity and protein acetylation as well as gene expression analysis is implemented.
Summary / Conclusion: 4SC-202 is a selective HDAC inhibitor with a unique combination of anti-cancer activities. In the ongoing phase I clinical trial, 4SC-202 shows good PK properties, good tolerability and signs of clinical efficacy. According to the MoA and preclinical results multiple development options arise with a focus on hematologic malignancies and WNT related solid tumors.

B1769

NECROX-7 AS A NOVEL HMGB1 BLOCKADE ATTENUATES GRAFT-VERSUS-HOST DISEASE BY AUGMENTING REGULATORY T CELLS AND INHIBITING DEVELOPMENT OF TH17 CELLS

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Background: Extracellular HMGB1 is the best characterized damage associated molecular pattern (DAMP) molecule, which functions as a potent innate immune mediator implicated in aGVHD.
Aims: In this study, we investigated the effects of a novel HMGB1 blockade, Necrox-7 on aGVHD model.
Methods: After allogeneic BMT, recipients (BALB/c) were given Necrox-7 injections in 2 day intervals for 2 weeks. All mice were monitored for survival and

clinical signs of aGVHD. Secretion and mRNA expression of HMGB1 were confirmed by HMGB1-specific ELISPOT assay and real-time PCR. Intracellular reactive oxidative stress (ROS) production was detected by flow cytometry. Th17 and Treg cells were analyzed by the measurement of cytokine expression.
Results: Administration of Necrox-7 significantly attenuated the aGVHD-related mortality and inhibited severe tissue damages in the intestine, liver and lung. In *ex vivo* analysis, these protective effects correlated with the observed decrease in HMGB1 expression and ROS in the PB. Furthermore, administration of Necrox-7 showed the suppression of donor T lymphocyte proliferation in MLR and reduced serum levels of the pro-inflammatory cytokines. Blockade of HMGB1 signaling augments CD4+CD25+Foxp3+ Tregs reconstitution and attenuates the severity of aGVHD whereas Th17 cells reduced in spleen.
Summary / Conclusion: These results suggest that HMGB1 could be a promising target for aGVHD treatment and a critical role for the Necrox-7 as novel blockade of ROS and HMGB1 in pro-inflammatory events contribute to prevent aGVHD.

B1770
MK-2206 SENSITIZES ACUTE MYELOID LEUKEMIA CELLS TO CYTARABINE VIA APOPTOSIS INDUCTION
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Background: Acute myeloid leukemia (AML), a heterogeneous hematopoietic malignancy, results from various genetic abnormalities affecting proliferation or differentiation. Genetic alterations in phosphoinositide 3-kinases (PI3Ks)/AKT cascade have been linked to various human cancers, including AML, accordingly targeting the critical nodule AKT becomes expected for effective therapy. The specific allosteric AKT inhibitor, MK-2206, was developed and proved survival advantage for patients with advanced solid tumor including thyroid cancer, metastatic head and neck squamous cell carcinoma and hepatocellular carcinoma cells by single or combined usage. Previously, we elucidated the molecular mechanism of MK-2206 on leukemia cells and found that MK-2206 resulted in G_1-phase arrest and apoptosis induction in leukemia cells. In addition, downregulation of myeloid cell leukemia-1 (Mcl-1) through glycogen synthase kinase 3β (GSK3β)-mediated and proteasome-dependent protein degradation by MK-2206 was noted to be associated with apoptosis induction. However, the therapeutic potential of AML remains unclear.
Aims: To elucidate the therapeutic efficacy and therapeutic potential of MK-2206 in leukemia cell lines.
Methods: Leukemia cell lines U937, OCI/AML3, MOLM-13, and MV-4-11 were used in this study. Apoptosis and cell cycle distribution were determined by flow cytometry analysis. Expression profiles of anti-apoptotic protein family were determined by western blotting. Drug combination effects of MK-2206 with cytarabine on leukemia cells were evaluated by cell proliferation assay and combination Index (CI) values were calculated by CompuSyn software.
Results: MK-2206 represented a significant cytotoxic effect on all these leukemia cells with IC_{50}s of 0.6 mM to 2.5 mM for 72 h treatment, but with minor effect on normal peripheral blood mononuclear cells (PBMCs) from two healthy volunteers with IC_{50}s of 18.8 mM and 19.5 mM. Drug combination analysis on leukemia cells demonstrated that MK-2206 could sensitize most leukemia cell lines except U937 to cytarabine treatment, a first-line strategy for *de novo* AML treatment. The CI values of the combinations in MV-4-11, MOLM-13 and OCI/AML3 were at ED_{50} of 0.59, 0.37 and 0.38, respectively. Consistent results were observed in the ED_{50} isobologram analysis. Further analysis revealed that this sensitization was associated with induction of the cleavage of PARP. In addition, we explored that MK-2206 at physiologically plasma concentration (200nmol/L) could significantly reduce the IC_{50} of cytarabine in leukemia cells.
Summary / Conclusion: We explore the therapeutic potential of MK-2206 and strengthen the efficacy of cytarabine treatment coupled with MK-2206 in myeloid leukemia.

B1771
TIME TO HEMATOLOGIC AND RENAL IMPROVEMENTS IN AHUS PATIENTS WITH PROGRESSING THROMBOTIC MICROANGIOPATHY AFTER COMMENCEMENT AND DURING CONTINUATION OF ECULIZUMAB FOR MORE THAN 2 YEARS
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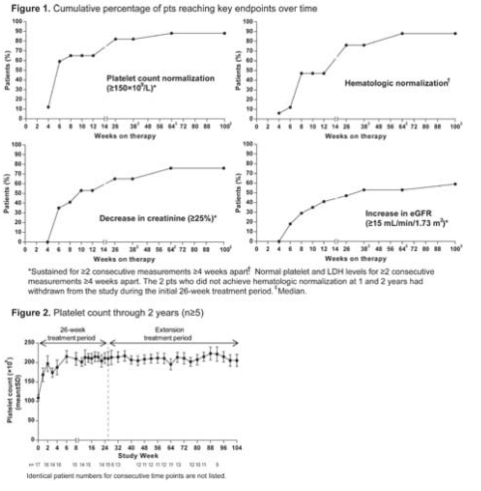
Background: Atypical hemolytic uremic syndrome (aHUS) is a life-threatening disease caused by inherited and/or acquired defects of complement system regulators, resulting in chronic, uncontrolled complement activation that causes platelet activation, thrombosis, hemolysis, and thrombotic microangiopathy (TMA). Despite plasma exchange/plasma infusion (PE/PI), patients (pts) may develop end-organ damage, frequently in association with anemia, thrombocytopenia, undetectable haptoglobin levels, and/or elevated lactate dehydrogenase (LDH) levels. Up to 65% of pts develop permanent renal damage or die in the first year of diagnosis. Eculizumab (Ecu), a humanized monoclonal antibody that is a terminal complement inhibitor, binds with high affinity to the human C5 complement protein and is the first approved treatment for aHUS in pediatric and adult pts.
Aims: Investigate the cumulative percentage of pts reaching key hematologic and renal endpoints over time in a phase 2 study of Ecu in aHUS pts with progressing TMA.
Methods: Pts age ≥12 years with clinical evidence of progressing TMA (platelet count <150×10^9/L and decreased >25%, despite ≥4 PE/PI sessions in the prior week) were enrolled and received Ecu. Pts provided informed consent and the study was approved by each center's institutional review board. Data are reported up to 104 weeks.



Results: The 17 pts (median age 28 years, 71% female, 41% with prior renal transplant) had a median time from diagnosis to screening of 10 months. Hematologic parameters (mean±standard deviation [SD]) at baseline were: platelets 109.0±32.1×10^9/L, LDH 323.0±138.2 U/L, hemoglobin (Hb) 89.1±14.0 g/L, and haptoglobin 0.5±0.4 g/L. All pts presented with estimated glomerular filtration rate [eGFR] <60 mL/min/1.73 m^2, and mean±SD serum creatinine was 351.5±214.9 µmol/L. The median time to the start of platelet count normalization (first platelet count ≥150×10^9/L) was 7 days (range 1–218). Nine of the 10 pts with abnormal LDH at baseline (90%) showed LDH improvement ≤ upper limit of normal (ULN). The time to first occurrence of LDH ≤ ULN was 14 days (range 0–56) after the first Ecu dose. The cumulative percentages reaching key hematologic and renal endpoints are shown in Figure 1. Also, 76% of pts had sustained improvement in Hb levels (≥20 g/L) at 64 weeks (median), and 71% of pts had improvement in chronic kidney disease stage (≥1 stage) at 100 weeks (median). Mean platelet count (Figure 2) and renal function (as measured by creatinine levels and eGFR) rapidly improved and was sustained through 2 years of Ecu treatment. Only 2 pts (12%) required PE/PI after starting Ecu (1 pt at 10 days after discontinuing treatment due to a protocol violation, the other at 84 days). Four of 5 pts (80%) receiving dialysis at baseline discontinued dialysis; the time of discontinuation ranged from 1 to 14 days after the first Ecu dose. There were no reported deaths during Ecu treatment (28.5 patient-years).
Summary / Conclusion: The majority of pts showed improvement in hematologic and renal parameters within several months of starting Ecu. Ongoing treatment with Ecu led to sustained improvement in hematologic outcomes in nearly all pts by 2 years, including inhibition of TMA, in association with ongoing improvements in renal function. As such, Ecu may decrease mortality in aHUS patients when compared to the natural disease progression. These results provide a framework for treatment expectations with Ecu and highlight the need for early and ongoing Ecu treatment in pts with aHUS and progressing TMA.

B1772
RHTRAIL AND SURVIVIN DOWNREGULATORS AS NEW THERAPEUTIC APPROACH IN ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: Acute lymphoblastic leukemia (ALL) represents a biologically and clinically heterogeneous group of hematological malignancies, which results from the abnormal proliferation and accumulation of immature lymphoid cells (blasts) within the bone marrow and lymphoid tissues. Although, chemotherapy is a hallmark treatment for ALL many patients become resistant to therapy or may relapse, requiring the development of alternative treatment strategies. Tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL/Apo-2L) is a type two membrane protein which selectively induces apoptosis in transformed or stressed cells but not in most normal cells. Nevertheless, in some cases treatment with TRAIL alone may not be sufficient for an effective response. Take into account, combined TRAIL therapies not only with conventional chemotherapeutic agents but also with survivin downregulators, namely gambogic acid (GA) and silibinin (SLB) have given encouraging results to restore TRAIL sensitivity.
Aims: In the present study, we aimed to explore the therapeutic efficacy of a recombinant human TRAIL (rhTRAIL) and survivin inhibitors, GA and SLB, alone and in combination with conventional chemotherapeutic agents, in ALL.
Methods: For this purpose we maintained in culture an ALL cell line established from a patient at disease presentation, the CEM cells, in absence and in presence of different concentrations of rhTRAIL, GA and SLB in monotherapy as well as in association with each other and with conventional drugs (Doxorubicin or Vincristine). Cell viability was assessed by the trypan blue assay and cell death by Optical Microscopy (May-Grunwald staining) and flow cytometry (FC) using the Annexin V/Propidium Iodide double staining. TRAIL, TRAIL-Receptors, survivin, Transferin receptor (TfR), activated caspase-3 and cytocrome *c* expression levels were also evaluated by FC using specific monoclonal antibodies. Mitochondrial membrane potential was assessed by FC using JC-1 probe.
Results: Our results show that, as single agent, rhTRAIL, GA and SLB induce antiproliferative and cytotoxic effects in a dose, time and administration type dependent manner. We observed an IC_{50}, at 72h of exposure, for rhTRAIL, of 500ng/mL. On the other hand, when CEM cells were treated with GA and SLB, the IC_{50} is reached at 24h and is approximately 400nM for GA and 100μM for SLB. Moreover, when we administrated these compounds at low concentration daily in monotherapy, as well as in a combination schedule, with each other or with conventional chemotherapy, we observed a synergist cytotoxic effect. The different therapeutic efficacy of rhTRAIL, GA and SLB, in CEM cells, may also be correlated with the major expression of TRAIL pro-apoptotic receptors than anti-apoptotic receptors, TfR and survivin basal expression levels, respectively. Furthermore, these compounds induce cell death mainly by apoptosis which may be related with membranar and mitochondrial apoptotic pathways activation.
Summary / Conclusion: Our findings suggest that rhTRAIL and survivin downregulators, in monotherapy or in combination, may constitute a new potential therapeutic approach in ALL treatment.

B1773
SCREENING AND DELINEATION OF MOLECULAR MECHANISMS OF ACTION OF NOVEL AGENTS FOR THE TREATMENT OF B-THALASSAEMIA

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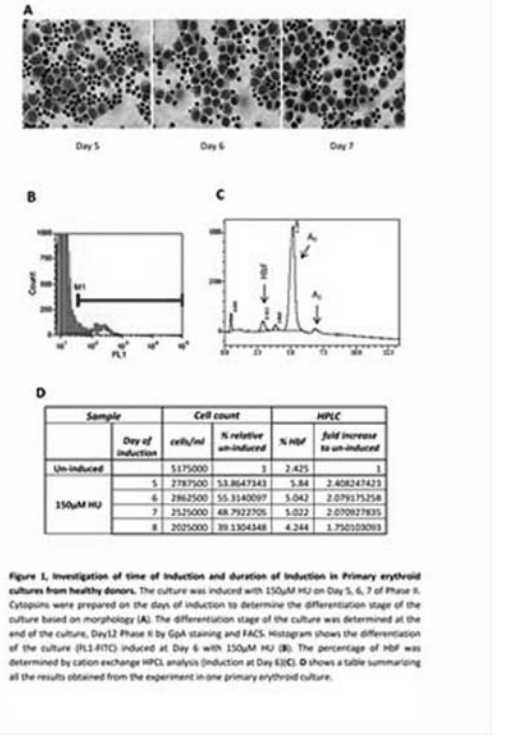
Background: Beta-thalassaemia, an inherited disorder of beta haemoglobin chain production, is a global health burden. The current management is regular blood transfusions with regular iron chelation therapy. However, this is not curative and has substantial morbidity related to iron overload. Pharmacological reactivation of gamma-globin genes for the production of foetal haemoglobin (HbF) is a very promising therapeutic approach for beta-thalassaemia, as foetal haemoglobin can substitute for the absent adult beta-haemoglobin. Although clinical trials have shown notable results for some agents, these were not very encouraging for large-scale use (Perrine, 2008) due to low efficacy and specificity and high toxicity (Gambari and Fibach, 2007). Furthermore, pharmacological attempts to find suitable HbF inducers are limited since none of the mechanisms of action of these agents in activating gamma-globin expression is fully understood. Understanding how the transcriptional network causing the switch from gamma to beta-globin works will provide a new mechanism-based approach for the reactivation of HbF synthesis in beta-thalassaemia.
Aims: Based on the literature and preliminary studies, four pre-identified HbF inducers; Lenalidomide, Angelicin, 5-aza-2'-seoxycytidine (decitabine) and Mithramycin were selected with the aim to investigate their HbF inducing activity *in vitro* on primary erythroid cultures from healthy donors and the potential delineation of the molecular mechanisms of action of the agent with the best activity. In the current study, all of the four agents will be screened on K562 cell line to select the two agents with the highest inducing activity. In addition, the optimum protocol for induction of primary erythroid cultures will be determined which will be then used for screening of the two selected agents on primary erythroid cultures for selection of the agent with the best inducing activity.
Methods: K562 cell line was induced with different concentrations of each agent for five days and the HbF induction activity was determine by benzidine staining. The two-phase method originally identified by Fibach (1998) was used for preparation of primary erythroid cell cultures from peripheral blood obtained from healthy individuals. Experiments were performed to identify the best time that the agent should be added to the culture on phase II as well as the duration of induction with the agent to be investigated. The level of induction of foetal haemoglobin was detected at the protein level by cation exchange HPLC as defined by Ou and Rognerud (1993), at the mRNA level by multiplex-Real time PCR. The level of differentiation was investigated by Glycophorin A staining followed by FACs analysis and morphologically by cytospins stained with methylene blue and eosin red dyes.
Results: The two agents identified with the highest HbF inducing activity following screening in the K562 cell line were Mithramycin and Decitabine. These agents will be screened on primary erythroid cultures for identification of the most active compound. Analysis of the culture induction profiles in primary erythroid cultures showed that for optimal induction, the agents should be added to the culture at the prepro- to pro-erythroblast stage. Collection of the culture samples for analysis should be carried out when the culture reaches a plateau stage and before showing any signs of cell death, with a minimum induction period of 5 days.
Summary / Conclusion: Following induction of K562 cell line with the four agents decitabine and mithramycin were selected as the two agents with highest HbF inducing activiy. Having decided on the experimental protocol for induction on primary erythroid cultures, the two selected agents will be screened for identification of the agent with the best inducing activity and least cytotoxicity *ex vivo*. The best agent will be used in future studies for delineation of the molecular mechanisms for potential use on target-based therapeutic approaches.



Sample		Cell count		HPLC	
	Day of induction	cells/ml	% relative un-induced	% HbF	fold increase to un-induced
Un-induced		5175000	1	2.425	1
150μM HU	5	2787500	53.8647343	5.84	2.408247423
	6	2862500	55.3140097	5.042	2.079175258
	7	2525000	48.7922705	5.022	2.070927835
	8	2025000	39.1304348	4.244	1.750103093

Figure 1, Investigation of time of induction and duration of induction in Primary erythroid cultures from healthy donors. The culture was induced with 150μM HU on Day 5, 6, 7 of Phase II. Cytospins were prepared on the days of induction to determine the differentiation stage of the culture based on morphology (A). The differentiation stage of the culture was determined at the end of the culture, Day12 Phase II by GpA staining and FACS. Histogram shows the differentiation of the culture (PL1-FITC) induced at Day 6 with 150μM HU (B). The percentage of HbF was determined by cation exchange HPCL analysis (induction at Day 6)(C). D shows a table summarizing all the results obtained from the experiment in one primary erythroid culture.

B1774
HISTORICAL EVIDENCE OF SYSTEMIC MULTIORGAN COMPLICATIONS IN ATYPICAL HEMOLYTIC UREMIC SYNDROME (AHUS) PATIENTS BEFORE ECULIZUMAB THERAPY EITHER IN PROSPECTIVE TRIALS OR IN REAL-WORLD USE

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Background: aHUS is a rare, systemic disease characterized by chronic, uncontrolled alternative complement pathway activation leading to thrombotic microangiopathy (TMA). Within 1 year of diagnosis, up to 65% of patients develop permanent renal damage or end-stage renal disease or die. Extra-renal involvement and overall rate of complications in the course of aHUS are poorly documented. Eculizumab is the first approved treatment for aHUS in pediatric and adult patients.
Aims: Identify and describe evidence of historical systemic complications in aHUS patients from three trials: two phase 2 clinical trials of eculizumab (002 and 003), and in a cohort of aHUS patients who were treated with eculizumab outside clinical trials.

Table. Baseline characteristics and historical incidence of complications by system

Baseline characteristics	Retrospective study (N=30)	Patients with progressing TMA[a] (Study 002) (N=17)	Patients with long duration of aHUS and CKD[b] (Study 003) (N=20)
Age, mean, years (range)	12 (0–51)	28 (17–68)	28 (13–63)
Gender, female, %	53	71	60
No identified complement mutation or autoantibody, %	53	24	30
Prior kidney transplant, %	37	41	40
LDH >ULN, %	67	59	20
Platelet count×10^9/L, mean±SD	171±83	109±32	228±78
eGFR ≥90 mL/min/1.73 m^2, %	13	0	0
Historical complications by system			
Renal, %	90	71	80
Extrarenal thrombi, %	40	6	50
Cardiovascular, %	50	94	95
Gastrointestinal, %	73	53	45
Neurologic, %	53	29	30
Pulmonary, %	50	47	40
Complications in >1 system, %	100	82	90
Complications in >2 systems, %	40	59	70
Complications in >3 systems, %	25	35	20

[a]Platelet count <150×10^9/L after ≥4 PE/PI sessions with average platelet count decrease >25% in the prior week prior to most recent TMA presentation. [b]Receiving chronic PE/PI on a stable regimen with no platelet count decrease >25% during 8-week observation period before eculizumab treatment. CKD=chronic kidney disease; LDH=lactate dehydrogenase; ULN=upper limit of normal; SD=standard deviation; eGFR=estimated glomerular filtration rate.

Methods: Medical record data were collected retrospectively for 30 aHUS patients (children, n=15; adolescents, n=4; adults, n=11) who received eculizumab treatment from 2007 to 2009 outside the setting of a controlled clinical trial. The 002 clinical trial (NCT00844844 and NCT00844845) enrolled 17 patients age ≥12 years with progressing TMA despite ≥4 plasma exchange/plasma infusion (PE/PI) sessions in the prior week. The 003 clinical trial (NCT00844428 and NCT00838513) enrolled 20 patients age ≥12 years on chronic PE/PI with no platelet count decrease >25% during an 8-week observation period prior to eculizumab. Patients/guardians provided informed consent, and the studies were approved by each center's institutional review board. Data prior to administration of the first eculizumab dose are described. All complications, not only TMA-related complications, were included in the analysis.
Results: Disease characteristics for the groups at baseline are shown in the Table. Medical records of 90% of patients in the retrospective study and 71–80% of clinical trial patients demonstrated evidence of a history of kidney disease prior to the current TMA presentation. Extrarenal, systemic organ complications were reported in the majority of patients, with up to 100% experiencing complications in >1 body system (Table). Twenty-three patients across the 3 studies (34%) experienced extrarenal thrombi, including deep vein thrombosis (n=10). In addition, cardiac morbidities occurred in 75% of patients, including cardiomyopathy (n=6), myocardial infarction (n=2), transient ischemic attacks (n=2), pulmonary embolism (n=1), and cardiac arrest (n=1). Gastrointestinal disease was seen in 40 patients (60%) and included diarrhea (n=11), vomiting (n=9), and constipation (n=8). Neurologic complications occurred in 27 patients (40%), including headache (n=8), seizures (n=4), migraine (n=3), epilepsy (n=3), and facial paralysis (n=2). Thirty-one patients (46%) had pulmonary complications, including asthma (n=5), pneumonia (n=2), and respiratory failure (n=1).
Summary / Conclusion: aHUS is a devastating and progressive disease in which TMA and the accumulation of complications from renal failure and historical treatments may be associated with sudden and potentially fatal systemic aHUS morbidities. Based on the three studies in the present analysis, extra-renal systemic organ involvement (including cardiac, gastrointestinal, neurologic, and pulmonary complications, as well as peripheral thrombi) should be expected in aHUS. Historic data from these patients confirm the need for a treatment approach outside of plasma-based therapies should systemic aHUS organ involvement occur.

B1775

VISMODEGIB – AN HEDGEHOG PATHWAY INHIBITOR INDUCES CELL DEATH IN AN ALL CELL LINE

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Background: Conserved embryonic signaling pathways such as Hedgehog (Hh), Wingless (Wnt) and Notch, critical for stem cell self-renewal and differentiation in hematopoiesis, have been implicated in the pathogenesis of several hematological malignancies. Acute lymphoblastic leukemia (ALL) is characterized by the abnormal proliferation and accumulation of immature lymphoid cells within the bone marrow and lymphoid tissues, which can develop from the aberrant activation of the Wnt/β-catenin, Notch and Hedgehog signaling pathways. On account of that, these pathways may constitute new potential candidate targets for ALL therapy.
Aims: The main goal of this study was to evaluate the therapeutic potential of the hedgehog inhibitor, vismodegib, in an ALL cell line.
Methods: To evaluate the effect of this signaling pathway inhibitor on cell viability, we use an ALL cell line, the CEM cells, submitted to different concentrations of vismodegib (GDC-0449). The IC50 (half maximal inhibitory concentration), was determined using the blue trypan assay. The cell death was assessed by optical microscopy (May-Grunwald staining) and by flow cytometry (Propidium Iodide/Annexin V staining, BAX and BCL-2 levels and mitochondrial membrane potential). We also analysed, by flow cytometry, some proteins related with cell cycle regulation, as p53 and Cyclin D1.
Results: Our results showed that, vismodegib induced a cytostatic and cytotoxic effect in CEM cells in a time- and dose-dependent manner. The half maximal inhibitory concentration (IC50) is attainted with 150 μM, after 24h of treatment. This compound induces cell death mainly by apoptosis that may be related with the observed increase in caspases levels and decrease in BAX/BCL-2 ratio and mitochondrial membrane potential. The observed antiproliferative effect may be related with the decrease in p53 and cyclin D1 levels.
Summary / Conclusion: In conclusion, our results suggest that vismodegib (GDC-0449) may be a new potential targeted therapeutic approach that could be efficient in ALL treatment.

B1776

SELECTIVE SMALL MOLECULE AXL INHIBITOR BGB324 FOR THE TREATMENT OF ACUTE MYELOID LEUKEMIA

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Background: Axl, a member of the TAM family of receptor tyrosine kinases comprising Tyro3, Axl and Mer, has been implicated in the development and metastasis of many cancers, including haematological malignancies. A high level of Axl is associated with worse progression-free and overall survival in AML patients, and represents an independent prognostic marker. Growth arrest specific gene-6 (GAS6) is the known ligand with highest affinity for Axl. AML cells induce expression and secretion of GAS6 by bone marrow-derived stromal cells (BMDSCs), and this Gas6/Axl paracrine axis promotes proliferation, survival and chemoresistance of AML cells in the bone marrow microenvironment. Axl also contributes to the pathogenesis of *FLT3*-internal tandem duplication (*FLT3*-ITD) AML, suggesting Axl as an attractive target in AML. BGB324, a potent and selective small molecule inhibitor of Axl with low nanomolar potency in biochemical assays and exceptional kinase selectivity in cellular assays (at least 50 fold difference over Tyro and Mer), will be evaluated in a phase I clinical trial in coming months.
Aims: We aimed to explore the potential of the first-in-class Axl inhibitor BGB324 for the treatment of *FLT3*-ITD (+) AML.
Methods: The Axl inbibitor BGB324 was tested in the Mv4-11 cell line either as a single agent or in combination with chemotherapeutic agents such as Cytarabine and doxorubicin in: cell proliferation assays in the absence or presence of adherent HS-5 human stroma cells; Flow cytometric analyses of cell cycle and apoptosis; *in vivo* Mv4-11 subcutaneous xenograft and bone marrow engraftment models in NOD/SCID mice. *in vivo* target validation and PD biomarker detection were performed with Western blot, immunoprecipitation, and IHC.
Results: BGB324 treatment effectively inhibited Mv4-11 AML cell growth, induced cell apoptosis, and potentiated the killing effect of chemotherapeutic agents such as Cytarabine and doxorubicin against Mv4-11 cells. BGB324 treatment caused tumor regression, and significantly prolonged animal survival.
Summary / Conclusion: These data support further clinical evaluation of BGB324 as a single agent or in combination with established treatment regimen in AML patients.

B1777

BICYCLIC DERIVATIVES OF LIDONOJIRIMYCIN AS PHARMACOLOGICAL CHAPERONES FOR NEURONOPATHIC FORMS OF GAUCHER DISEASE

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Background: Gaucher disease (GD), the most prevalent lysosomal storage disorder, is caused by mutations in the GBA1 gene encoding for lysosomal acid β-glucosidase (glucocerebrosidase, GCase). The mutant enzymes exhibit impaired cellular trafficking as a consequence of aberrant folding. Current investigational therapeutic strategies for include the development of ligands of the enzyme capable of promoting those conformational changes that are required for efficient folding and restoring trafficking. Although somewhat counterintuitive, competitiveinhibitors of this enzyme, at subinhibitory concentrations, can increase steady-state lysosomal levels of active GCase through this rescuing mechanism acting as "pharmacological chaperones". At the massive lysosomal substrate concentration, the inhibitor would be replaced from the active site of the enzyme and the metabolic activity recovered. However, most of the pharmacological chaperones under study are iminosugars that behave as broad spectrum inhibitors, inhibiting simultaneously several glucosidases, which represents a serious inconvenient for clinical applications. An additional problem is that iminosugars are not active as pharmacological chaperones for glucocerebrosidase mutations located outside the domain containing the active site and are associatedwith neurological involvement.

Aims: To develop molecules with high binding specificity towards GCase, high ratio of chaperone *versus* inhibitor activity and capable of producing an increase in the levels of GC mutants, including those with mutations located outside the catalytic domain.

Methods: Three bicyclic derivatives of L-idonojirimycin (L-idose-based sp^2-iminosugars) designed and chemically synthesized from D-glucose after *in silico* structural analysis and identification of the most favorable molecular features to interact with the glucocerebrosidase active site. Their chaperone potential was evaluated *in vitro*using stable transfectants of glucocerebrosidase mutants (N370S and L444P) in COS-7 cells and human skin fibroblasts of homozygous GD patients for these above mentioned mutations.

Results: Results showed an increase in GCase activity at various chaperone concentrations, ranging from 2-fold to 5-fold for the L444P mutant and from 2-fold to 3-fold for the N370S mutant in COS-7 cells and increases of 3-fold for the L444P homozygous GD fibroblasts.

Summary / Conclusion: The use of bicyclic L-idonojirimycin-based pharmacological chaperones could be considered as a therapeutic alternative for GD, mainly in patients with mutations located outside the GC active site and associated with neurologic involvement.

B1778

ORIDONIN UPREGULATES RETINOIC ACID RECEPTORS AND SHOWS SYNERGISTIC EFFECT WHEN USED IN COMBINATION WITH ALL-TRANS RETINOIC ACID IN T(8:21) LEUKEMIA

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Background: The t(8;21)(q22;q22) translocation, which generates AML1-ETO (AE) fusion gene, is one of the most frequent chromosomal abnormalities detected in acute myeloid leukemia (AML, 12-20%), especially in FAB subtype M2 (AML-M2, 40-50%). The resultant AE fusion protein immortalizes hematopoietic progenitors and induces a myeloproliferative disorder in mice. Additional mutations are required to cooperate with full-length AE to cause full-blown leukemia. An isoform of AE with an alternatively spliced exon (exon 9a) of ETO (AE9a) could induce leukemia in mice. Therefore, AE oncoprotein plays a critical role in leukemogenesis, and agents that target AE could be useful in treating t(8;21) leukemia. Arabinosylcytosine and anthracycline-based chemotherapy has been the principal frontline treatment for t(8;21) AML, which results in a median survival of less than 2 years and a 5-year overall survival of no more than 40%. Targeted therapies against oncoproteins crucial for leukemia pathogenesis represent a new and promising approach to cancer treatment. In our previous work, oridonin, a diterpenoid isolated from the medicinal herb *Isodon rubescens*, was identified to have a relatively selective effect against t(8;21) leukemia cells. Further studies showed that oridonin specifically bound to AE, causing caspase-3-mediated cleavage of AE to generate a truncated version that acted as a tumor suppressor. Oridonin significantly prolonged life span of mice bearing t(8;21) leukemic cells. These results demonstrate the potent antileukemia efficacies of oridonin on t(8;21) leukemia which might provide benefits for patients.

Aims: To explore whether oridonin could induce differentiation besides apoptosis and the potential synergistic effect of oridonin-based combinatorial therapies in t(8:21) leukemia.

Methods: Cell growth and proliferation assays, differentiation assay, flow cytometry, quantitative RT-PCR, luciferase reporter assay, western blotting, hematopathologic analysis, murine bone marrow transplantation by tail-vein injection and *in vivo* treatment studies were performed.

Results: In this study, we show that oridonin upregulates retinoic acid receptors α2 (RARα2) and RARb2 in t(8;21) leukemia cells. Oridonin also increases the activation of retinoic acid (RA) signaling regulated by all-trans retinoic acid (ATRA). It exerts enhanced differentiation effect when ATRA was used in combination with oridonin in both t(8;21) leukemia cell line and primary leukemic cells isolated from t(8;21) AML patients. In murine models of t(8;21) AML, combined use of oridonin and ATRA synergetic prolongs the survival of leukemic mice.

Summary / Conclusion: Oridonin upregulates retinoic acid receptors and shows synergistic effect when used in combination with all-trans retinoic acid in t(8:21) leukemia, suggesting potential benefits of oridonin/ATRA combinatorial therapy for patients with t(8:21) AML.

B1779

ROLE OF LENALIDOMIDE IN MANAGEMENT OF MYELODYSPLASTIC SYNDROMES (MDS) WITH DEL(5Q) ASSOCIATED WITH PURE RED CELL APLASIA (PRCA)

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Background: Myelodysplastic syndromes associated with PRCA is a rare condition characterized by severe anemia, transfusion dependence, reticulocytopenia, reduction of erythroid precursors and multilineage dysplasia. In PRCA erythroid precursors are nearly absent, while megakaryocytes and granulocytic precursors are usually present at normal levels. Damage to erythroid progenitors appears to be immune-mediated and in about 10% of cases acute myeloid leukemia represents the latest evolution. Conventional immunotherapy is ineffective while alemtuzumab combined with CyA seems to be a valid therapy.

Aims: Nearby 25 cases of MDS with PRCA have been described until today and 5 of them were associated with del(5)(q14q34). 2008 WHO classification defined MDS with isolated del(5q) as a syndrome characterized by bone marrow blast count < 5%, isolated del(5q) and absence of Auer rods.

Methods: Here we report 3 cases of severe transfusion-dependent macrocytic anaemia in which del(5) (q14q34) was associated with erythroblastopenia and myelodysplasia.

Results: (M/61 y.o.) with a transfusion dependence of 4 units/month, received diagnosis of PRCA and underwent 12 cycles of alemtuzumab + CyA during 3 years: transient remissions from transfusion dependence were followed by relapses; after 3 years del(5q)was evident in a bone marrow that appeared dysplastic: lenalidomide treatment was started, after few months AML merged with fatal evolution. (F/35 y.o.) received diagnosis of PRCA after 1 year treatment with steroids and transfusions (2 Units /month). She underwent three courses of CyA and alemtuzumab with short transient periods of transfusion independence: a second bone marrow investigation, performed after one year,

showed del(5q) and lenalidomide therapy was started: transfusion independence was obtained after 2 months. (M/65 y.o.) with a transfusion dependence of 4 units/month, received diagnosis of PRCA and was treated with a single course of alemtuzumab and CyA without any result, cytogenetic revision of bone marrow highlighted the presence of del(5q) and treatment with lenalidomide was started 3 months after diagnosis. No hematological improvement was observed and after 9 courses therapy was stopped. Nowadays patient is transfusion-dependent after 21 months from diagnosis.
Summary / Conclusion: Here we stress the difficulty of diagnosing PRCA within unilineage myelodysplastic syndromes and focus on the relationship among MDS with erytrhoid aplasia and del(5q) in order to speculate on the role that lenalidomide could play in such entities.

B1780
GLYCOENGINEERED ANTIBODIES MEDIATE SUPERIOR MONOCYTE/MACROPHAGE-MEDIATED PHAGOCYTIC AND CYTOTOXIC ACTIVITY COMPARED TO PARENTAL ANTIBODIES
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Background: Therapeutic monoclonal antibodies eliminate tumor cells by different mechanisms including blocking of receptor signaling pathways, induction of antibody dependent cellular cytotoxicity (ADCC), antibody dependent cellular phagocytosis (ADCP), complement dependent cytotoxicity (CDC), and induction of adaptive immunity.
Aims: In view of the important role of phagocytic cells in the mechanism of action of therapeutic antibodies, we analyzed the FcgR-dependent effector functions mediated by monocytes and macrophages upon treatment with glycoengineered (GE) and wild-type (WT) antibodies under different experimental conditions.
Methods: GA101 (obinutuzumab, a humanized type II, glycoengineered anti-CD20), GA201 (glycoengineered anti-EGFR) and GA601 (glycoengineered anti-HER2) monoclonal antibodies displaying enhanced binding affinity to human FcgRIIIa (CD16) were compared to corresponding parental antibodies bearing unmodified antibody Fc part for their capacity to induce monocyte/macrophage-mediated phagocytic and cytotoxic activity under several experimental conditions.
Results: GE and WT antibodies displayed comparable binding and induced similar monocyte/macrophage-mediated ADCC and ADCP in absence of competing endogenous IgGs. Interestingly, in their presence, i.e. in a situation that more closely mimics physiological conditions, GE antibodies displayed significantly superior binding and induced higher levels of ADCC/ADCP.
Summary / Conclusion: These data show, for the first time, that in addition to enhancing FcgRIIIa-dependent NK-cell cytotoxicity (ADCC), glycoengineering also enhances monocyte and macrophage cytotoxic and phagocytic activities through enhanced binding to FcgRIIIa under conditions that more closely resemble the physiological setting.

Cellular immunotherapy and vaccination

B1781
MONITORING OF T-LYMPHOCYTE SUBPOPULATIONS IN PERIPHERAL BLOOD OF PATIENTS WITH ACUTE LEUKEMIA (AL) DURING DONOR LYMPHOCYTE INFUSION (DLI) AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (ALLO-BMT)
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Background: Delayed Reconstitution of T-cell component of the immune system after allogeneic bone marrow transplantation is a risk factor for post-transplant relapse in AL patients. DLIs for inducing the "graft versus leukemia" effect to prevent and treat the relapse of the disease.
Aims: To investigate subpopulations of T-lymphocytes in peripheral blood derived from patients with AL after allo-BMT during DLI, followed by intravenous interleukin-2 (IL-2) infusion, as therapy of hematological relapse and mixed chimerism progression.
Methods: The study included 10 patients (9 - AML, 1 - ALL): 7 - male, 3 - female, aged 27 to 57 years (median age 34 years). The pre-transplant conditioning was performed by myeloablative regimen (BU + TF) in 6 patients and by low-intensity regimen in 4 pts: Flyud + BU + ATC - 3 patients, BU+Flyud+Cytosar+Idarubicin in 1 patient. All pts were recipients of bone marrow from HLA-matched related donor. The number of tranfused myelokaryocytes ranged from 2.5 to 5.4 x 10^8/kg (median 3.95 x 10^8/kg). DLI after allo-BMT were administered in the case of mixed chimerism progression (4 pts) and hematologic relapse (6 pts). The time between the allo-BMT and a statement of the growth of mixed chimerism / relapse ranged from 3 to 51 months (median 7.2 months). Pts with hematologic relapse were administered DLI during myelosupressive aplasia after reinduction chemotherapy. The median number of DLI procedures was 2 DLI (range from 1 to 4). The total number of transfused CD3+ cells ranged from 0.1 to 1.74 x10^8/kg (median 0.35x10^8/kg). Intravenous IL-2 (6 MUE) was administered after DLI in all pts.
The quantity composition of T-lymphocyte subpopulations in the peripheral blood was estimated using the method of immunophenotyping and flow cytometry (cytometry FACS Canto II, BD). Monoclonal antibodies to the following antigens were used: CD3 (PerCP), CD4 (PE), CD8 (FITC), CD 25 (FITC), CD 56 (FITC) (BD). Immunophenotypes of the following T-lymphocyte subpopulations: T-lymphocytes (absolute CD3+ count), T-helper cells (CD3+/CD4+); T-cytotoxic cells (CD3+/CD8+), regulatory cells (CD4+/CD25^{high}), T-NK cells (CD3+/CD56+) and NK-cells (CD3-/CD56+) were identified.Control points: statement of increase of mixed chimerism/relapse, then 1 time every 2 weeks for 10 weeks.
Results: Complete remission with full donor chimerism was achieved in 7 pts, in 3 pts the treatment was not effective. The median duration of response was 9 months (range 1 to 16 months). Acute GVHD occurred in 4 patients (I-II degree - 3 pts, III - IV degree - 1 pt).
The absolute number of T-helpers, T-cytotoxic cells, TNK-cells, NK-cells were reduced at the time of relapse (in 83%, 50%, 50%, 100 % of pts respectively), however the amount of T-regulatory cells remained normal at the same time. The number of T-cells subpopulations reduced in 2 weeks after the chemotherapy+1DLI in relapse pts. Normal values of those T-subpopulations gradually recovered after 6 weeks. Patients had received DLI for mixed chimerism progression have reduced number of CD3+ and T-helper cells (50% and 100% of pts respectively), and low levels maintained for 10 weeks after the first DLI.
Summary / Conclusion: DLI is an effective treatment of leukemia relapse after allogeneic BMT. Absolute number of T-helper cells, T-cytotoxic cells, T-NK cells and NK-cells were decreased at the time of relapse and after chemotherapy. Normal number of T-lymphocyte subpopulations recovered after 6 weeks of the first DLI if the remission was achieved. Absolute number of T-lymphocytes didn't recover in cases of DLI treatment failure.

Red blood cells and iron; physiology and disease (anemia) - Biology

B1783

ERYTHROPOIETIN LEVEL IN SICKLE CELL DISEASE PATIENTS NOT IN CRISIS

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Background: Patients with sickle cell disease (SCD) have lower than expected values of erythropoietin for the extent of their anemia. SCD may be complicated by hypoxia, renal insufficiency, and inflammation, all of which may change erythropoietin levels. We previously examined the relationship between erythropoietin level and hemoglobin and renal function as measured by glomerular filtration rate (GFR) in patients with SCD not in crisis and found that the expected correlation between erythropoietin level versus hemoglobin and renal function was not clearly maintained in SCD.
Aims: Here, we examine the relationship between erythropoietin and inflammation and hypoxia.
Methods: Charts of patients treated in the outpatient hematology clinic were reviewed. Patients who had sickle cell anemia (SCA), SC disease, or sickle-thalassemia and in whom erythropoietin had been measured on an outpatient basis while they were not in crisis and who did not receive exogenous erythropoietin were eligible for inclusion. Erythropoietin level, hemoglobin (hgb), platelet count, white blood count (WBC), mean corpuscular volume (MCV), oxygen saturation, reticulocyte count, serum creatinine, and patient characteristics including age, gender, and disease type were recorded. GFR was calculated using the CDK-EPI equation. Because direct measurements of inflammation such as erythrocyte sedimentation rate and c-reactive protein are not measured as part of standard of care, WBC and platelet count were used as proxy markers for this preliminary study.
Results: Data from a total of 54 patients was obtained, including 39 with SCA, 9 with SC disease, 5 with sickle-thalassemia, and one with S-O(Arab). Median hemoglobin was 8.3 and median erythropoietin level was 62 for all patients. Oxygen saturation ranged from 90% to 100% on room air. All patients had at least adequate iron stores. Because hemoglobin levels were higher and therefore corresponding erythropoietin levels lower in patients with SC disease, patients with SCA and sickle-thalassemia null were examined separately. Among patients with SCA and sickle-thal^0, lower hemoglobin levels (<7 versus 7+) were only marginally associated with higher erythropoietin levels (96 versus 64, P=0.07). When patients with oxygen saturation of less than 96% were compared to patients with an oxygen saturation of greater than 96%, no statistically significant difference in erythropoietin level was observed. However, patients with higher oxygen saturation had slightly higher hemoglobin levels (8.7 gm/dl versus 6.9 gm/dl, P=0.02). Smoking status (nonsmoker/former smoker versus current smoker) had no effect on erythropoietin level. Similarly, no difference in erythropoietin level was observed for patients with normal versus elevated WBC and platelet count. However, in contrast to hypoxia, high WBC was not associated with differences in hemoglobin. Interestingly, patients receiving hydroxyurea had a higher erythropoietin level, despite showing no difference in hemoglobin level, GFR, oxygen saturation, or WBC. Median MCV was higher in patients taking hydroxyurea, suggesting that compliance was acceptable.
Summary / Conclusion: Patients with sickle cell disease have low levels of erythropoietin, even when no kidney disease is evident. Hypoxia did not correlate well with erythropoietin levels. This may reflect the worsening clinical state of patients with hypoxia and sickle cell disease, but hypoxic patients did not show lower GFR than patients without hypoxia. Inflammation, as measured by the proxy measures of WBC and platelet counts, did not appear to affect erythropoietin levels. Hydroxyurea appears to preserve erythropoietin levels in patients with SCA and sickle-thal^0, but the mechanism by which it is protective is unclear.

B1784

EFFECTS OF IRON POLYMALTOSE COMPLEX, FERROUS FUMARATE AND FERROUS SULFATE THERAPIES IN ANEMIC PREGNANT RATS, THEIR FETUSES AND PLACENTAS

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Background: Iron deficiency anemia (IDA) during pregnancy is associated with adverse consequences in the mother, fetus, and placenta, such as preterm delivery and placenta insufficiency. Ferrous salts are commonly used to prevent and to treat mild to moderate IDA during pregnancy. The rapid release of iron from ferrous compounds and its uncontrolled plasma uptake can result in non-transferrin bound iron (NTBI) [1,2]. NTBI is taken up in an uncontrolled way, leading to oxidative stress [3]. Iron(III)-hydroxide polymaltose complex (IPC) is a well-tolerated, orally administered non-ionic oral Fe(III) preparation that effectively prevents and corrects IDA during pregnancy [4]. The iron from IPC is essentially taken up via active and controlled mechanism resulting in negligible levels of NTBI [1].
Aims: This study compared the effects of oral IPC, ferrous fumarate (FF) and ferrous sulfate (FS) treatments in anemic pregnant rats, their placentas and fetuses.
Methods: There were five experimental groups (n=8): untreated non-anemic control group, untreated anemic group and three anemic groups receiving 2 mg iron/kg body weight/day as IPC [Maltofer-Fol®, Vifor (International) Ltd., St. Gallen, Switzerland], FF (Anemidox Ferrum®, Merck Sharp & Dohme, Inc, Buenos Aires, Argentina), or FS (Fer-In-Sol, Mead Johnson, Buenos Aires, Argentina) from the start of pregnancy (day 0). On day 21, the animals underwent Caesarian section and were sacrificed. Pregnancy outcome was measured by number of fetuses, and by neonate and placenta weight. Maternal hepatic damage was assessed by analyzing aspartate aminotransferase (AST). Lipid peroxidation (MDA) and the ratio of reduced to oxidized glutathione (GSH:GSSG) were analyzed from homogenised fractions of the livers of mothers and fetuses and whole placentas. Interleukin 6 (IL6) and hypoxia inducible factor-1α (HIF-1α) were analyzed from homogenized fractions of the whole placentas.
Results: All therapies were comparably effective in correcting anemia. FS and FF resulted in higher lipid peroxidation in dams, fetuses and placentas vs. IPC. The FS group presented lower GSH:GSSG values in dams, fetuses and placentas than IPC and FF groups. IPC, but not FF or FS, restored normal IL6 expression levels in placentas whereas FS-treated animals presented the highest IL6 levels, suggesting a local inflammatory reaction. Anemia-induced high levels of HIF-1α were partially lowered by IPC and FF but further elevated by FS. Especially FS treatment was found to elicit hepatic damage in the dams and oxidative stress in the dams, fetuses and placenta as well as inflammation and high levels of HIF-1α in the placenta. Pregnancy outcome was worse in the FF and FS groups than in the IPC group.
Summary / Conclusion: Anemia-associated oxidative stress and inflammation, and also in part hypoxia, were resolved by IPC therapy. Treatment with ferrous salts, in particular with FS, was found to elicit maternal hepatic damage, oxidative stress in dams, fetuses and placentas, as well as high levels of IL6 and HIF-1α in the placenta.

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B1785

ROLE OF REGULATORY T CELLS IN BETA THALASSEMIA

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Background: Many immune defects in different compartments of immune response have been documented in patients with beta thalassemia. Ineffective erythopoiesis with chronic hemolysis, splenectomy, iron overload, infection with hepatitis B/C or HIV and the constant stimuli of transfusion have all been implicated in the pathogenesis of the immunologic abnormalities found in thalassemia patients
Regulatory T cells (Treg) characterized by expression of the transcription factor Foxp3 are responsible for maintaining peripheral immune tolerance and homeostasis. Data in the literature regarding the role of Tregs in thalassemia is very limited.
Aims: The aim of the study was to investigate the regulatory T cells compartment in beta thalassemia patients and to find factors which could influence their expression.
Methods: We studied 21 chronically transfused patients with beta thalassemia major (TM) (13 males and 8 females with a mean age of 48.8 years) , 9 thalassemia intermedia (TI) patients, who have never received transfusion (4 males and 5 females, with a mean age of 44.1 years) and 10 normal controls.
Iron overload was assessed with T2* of liver and heart. Peripheral blood samples were analyzed with five colour flow cytometry (CyFlow SPACE, PARTEC) using the following monoclonal antibodies. CD3- FITC, FOXP3- PE,CD127-PeCy5, CD4- PeCy7, CD25-APC. The regulatory T cells were characterized as CD3+, CD4+, CD25+ high, CD127 low/- ,FoxP3 +. Statistical analysis was performed by using the paired t-test.
Results: Twelve out of 21 TM patients and 2/9 TI patients had undergone splenectomy. Hepatitis C infection was recorded in 3 TM patients. The majority of TM patients (12/21) showed only mild to moderate hemosiderosis (7-13.9 mg/g of iron in liver T2*) , while 5 TM patients showed successful chelation therapy with < 2.9 mg/g of liver iron and only one showing increased liver hemosiderosis (>14 mg/g). There was no statistically significant difference of percentages of CD+, CD25+, Foxp3+ cells between controls (mean 0.226, SD 0.108) and TM patients (mean 0.39 ,SD 0.32) (P=0.13) neither between TM and TI patients (mean 0.2, SD 0.128) (P=0.59).

No difference of percentage of CD+, CD25+, Foxp3+ cells was found between the group of patients that had undergone splenectomy and the unsplenectomized ones. Hepatitis C infection did not seem to have significant effect on T regs percentag

Summary / Conclusion: It was previously suggested (by only one study) that increased percentage of the regulatory T cells found in TM could be explained by the chronic stimuli of transfusion but in our study we did not observe a difference of Tregs between the regularly transfused TM and the TI group that has never received transfusion. Controlled body iron stores, by eliminating the chronic inflammation background, could blunt the diversion of immunoregulatory balance. Further studies of the cytokines' pathways through which Tregs exert their effect can clarify the role of the nature of the disease (thalassemia) itself in immunomodulation

B1786

ENDOTHELIAL DYSFUNCTION IN YOUNG TRANSFUSION DEPENDENT B-THALASSEMIA PATIENTS

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Background: In patients with beta thalassemia an increased level of circulating endothelial cells reflecting endothelial damage and denudation. Repair of the denuded endothelial might be crucial for the restoration of endothelial function

Aims: The aim of this work is to study the endothelial dysfunction among patients with beta thalassemia, the ability to repair the denuded endothelium and the effect of iron load on these cells number correlating with the efficacy of iron chelation

Methods: twenty five patients aging more than 12 years with transfusion dependent β-thalassemia were recruited from the haematology/oncology clinic, Children hospital Ain Shams University and compared with 15 healthy age and sex matched controls. The quantity of circulating Endothelial microparicles (CD144+ /VE-cadherin+endothelial MPs) and Von willebrand factor Ag (vWF Ag) as markers of endothelial dysfunction, and the quantity of circulating CD34+VEGFR2+ cells were determined in relation to erythropoietin(EPO) level, echocardiographic cardiac parameters and carotid intimal thickness.

Results: The quantity of circulating CD34+VEGFR2+ cells was significantly higher among beta thalassemia patients than the controls (P=0.001) , however no significant difference existed between the patients and the control as regard quantity of Endothelial microparicles and vWF Ag level (P=0.66,P=0.33) . We found no significant correlation between Endothelial microparicles, vWF Ag level and circulating CD34+VEGFR2+ cells nor between EMPS , vWF Ag level, circulating CD34+VEGFR2+ cells and EPO level, mean ferritin in last 2 years , type of chelation, and compliance. Patients with increased left ventricular dimensions had a significantly higher circulating CD34+VEGFR2+ cells(P=0.005) and a lower vWF Ag level(P=0.02). Although 72% of the patients had increased carotid intimal thickness, yet it did not correlate with Endothelial microparicles , circulating CD34+VEGFR2+ cells quantities and vWF Ag level.

Summary / Conclusion: In this study the levels of procoagulant MPs of endothelial origins was not significantly elevated in patients with beta thalassemia, indicating probably the greater involvement of platelet or RBCs MPS in the process of endothelial damage among these patients. Patients with the greatest iron overload did not show a significant increase in the quantity of circulating CD34+VEGFR2+ cells suggesting a more significant qualitative effect of iron overload on these cells

B1787

PEROXIREDOXINS PLAY AN IMPORTANT ROLE IN THE SURVIVAL OF ERYTHROID CELLS IN BETA THALASSEMIA AND SICKLE CELL DISEASE PATIENTS.

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Background: The oxidative stress, occurs mainly by Reactive Oxygen Species (ROS) and is responsible for aggravating the symptoms of several diseases, including hemolytic anemias such as sickle cell disease (SCD) and beta thalassemia (BT). Peroxiredoxins (PRDXs), are a group of enzymes involved in the detoxification of ROS. In erythroid cells, their abundance and high reactivity can be highlighted. For example, in erythrocytes, PRDX2, is the third most abundant protein, indicating its possible role in the cell development and homeostasis. However, there are few studies connecting these proteins to hemolytic anemias, which remarkable feature is an increased production of ROS.

Aims: This study evaluated the expression and production of PRDXs in reticulocytes of SCD and BT patients compared to healthy blood donors.

Methods: To evaluate the difference in gene expression of *PRDX*s, Real Time PCR was used and Western blot analysis was used to evaluate the protein production.

Results: Our results showed that the levels of transcript and PRDX1 protein were increased in BT patients and decreased in SCD. The *PRDX2* transcript showed no differences in both diseases. However western blot analysis showed a decrease in PRDX2 protein in SCD reticulocytes, indicating a possible pos transcription regulation process for this gene in SCD. High levels of *PRDX5* transcript were found in BT patients and no difference was observed for SCD, however the protein was not found in western blot analysis. The PRDX5 protein is located at mitochondria, an organelle absent in reticulocytes and only residues of mRNA could be observed in these cells. A reduction in mRNA and protein levels for PRDX6 was observed in BT and SCD patients. Besides acting in the ROS detoxification, PRDX6 also has a phospholipase A2 activity, thus regulating the phospholipid turnover at the cell membrane. The decrease of this enzyme found in both patients could indicate that the erythroid cells membrane are not being properly renovated leading to hemolysis.

Summary / Conclusion: This is the first study correlating gene expression of peroxiredoxins in these hemolytic anemias. The results could contribute for a better understanding of the role of these proteins and for the identification of new targets that could help in the management of diseases and improve the survival of these patients.

B1788

COMPARISON OF THE PHYSICO-CHEMICAL CHARACTERISTICS OF ORIGINATOR IRON POLYMALTOSE COMPLEX AND FOUR IRON POLYMALTOSE COMPLEX SIMILARS

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Background: The originator iron(III)-hydroxide polymaltose complex (IPC_{ORIG}, Maltofer®) is a macromolecular iron-carbohydrate complex for oral treatment of iron deficiency (anemia) [ID(A)]. It has well-defined physico-chemical properties that enable a controlled release of iron within the gastrointestinal (GI) tract, resulting in good GI tolerability, low toxicity, and reliable correction of ID(A) [1]. The physico-chemical properties and pharmacological activity of iron-carbohydrate complexes, such as IPC, are highly dependent on the manufacturing process [2,3]. IPC copies, so-called IPC similars (IPCSs), have been shown to display different physico-chemical characteristics than those of IPC_{ORIG} [4], likely to affect their bioavailability, tolerability, and efficacy in correcting ID(A) [4]. Indeed, in a comparative study of IPC_{ORIG} versus an IPCS in infants with iron deficiency anemia, IPC_{ORIG} showed a favorable efficacy profile with a significantly higher increase in hemoglobin and serum ferritin levels vs. IPCS [5].

Aims: This study assessed the physico-chemical properties of IPCS preparations marketed in Israel and China, and compared them with those of IPC_{ORIG}.

Methods: The physico-chemical analyses of Ferri care ($IPCS_{FERRI}$), Iron Baby ($IPCS_{IBABY}$), Iron-3 Drops ($IPCS_{IRON3}$), and Niferex® ($IPCS_{NFRX}$) were carried out in the Quality Control and Analytical Development Laboratory of Vifor (International) Inc. The molecular weight distribution parameters were measured by gel filtration chromatography. Turbidity point and *in vitro* degradation kinetics were determined according to methods described previously [6]. The results were compared with published [6] and recently measured data of IPC_{ORIG}.

Results: IPCSs from Israel had 7.5-9 times higher weight average molecular weight (Mw) and significantly higher number average molecular weight (Mn) than those of IPC_{ORIG}. Significant differences in Mw and Mn were also found between the lots of a given product. Mw of $IPCS_{NFRX}$ was almost double and Mn significantly higher than that of IPC_{ORIG}. All IPCSs had significantly higher polydispersity (P) than that of IPC_{ORIG}. The differential rate parameters (k), determined when 10, 50, or 80% of the complex was degraded (q = 0.1/0.5/0.8), were significantly smaller for all the IPCSs vs. IPC_{ORIG}, indicating a slower degradation rate for IPCSs vs. IPC_{ORIG}, a result that was in line with the larger size of the IPCSs. $IPCS_{NFRX}$ presented with a turbidity point at pH 2.8, indicating that it might not be stable under GI conditions.

Summary / Conclusion: The analyzed IPCS preparations showed physico-chemical characteristics significantly different from those of the IPC_{ORIG}. In addition, the variable physico-chemical characteristics between different lots of a given IPCS preparation suggest that they were not produced under standardized conditions. The large size and the high stability of the analyzed IPCSs indicate that iron from these IPCs may not be utilized efficiently, which may lead to poor correction of Hb in patients with ID(A). We suggest that incorporation of isotope-labeled iron into erythrocytes and/or 3-month efficacy studies should be performed to demonstrate the efficacy of IPCS preparations.

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B1789

BCL11A POLYMORPHIC VARIATIONS INFLUENCES FETAL HEMOGLOBIN LEVELS IN PORTUGUESE SUBJECTS WITH COMMON HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN

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Background: Common forms of hereditary persistence of fetal haemoglobin (HPFH) (HbF levels 2-30%) occur in some adult individuals with normal haematological parameters. This form of HPFH typically results from polymorphic variations in the fetal globin genes (*HBG1* and *HBG2*) or along the β-globin cluster. Recent genetic association studies found other loci involved in HbF expression, including polymorphisms in *BCL11A* gene (chr. 2p) and *HBS1L-MYB* (HMIP) intergenic region (chr. 6p), in patients with β-globin disorders (sickle cell disease and β-thalassaemia) originated from different populations.
Aims: To evaluate whether genetic variability in *loci BCL11A*, *HMIP* and *HBG2* (XmnI) are involved in common forms of HPFH.
Methods: Sixty subjects of Portuguese origin, with normal haematological parameters and HbF levels ranging from 0.2% to 7.4%, aged 2-61 years, were recruited. Informed consent was provided by all the participants. HbF levels were determined by HPLC (Variant[2] Bio-Rad Laboratories, Hercules, CA, USA) and log transformed. SNPs rs11886868, rs766432, rs9399137, rs6934903 and rs7482144 were genotyped by PCR-RFLP or TaqMan assays. Statistical analysis was performed by using the PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/).
Results: Allele frequencies, Hardy-Weinberg p-values and association results between SNPs and HbF were assessed for all the polymorphisms. Linear regression used to test the association between SNPs and HbF levels showed statistical significance for *BCL11A* SNPs rs11886868 ($P=7.3x10^{-5}$) and rs766432 (P=0.002), even after age and gender adjustment ($P=2.9x10^{-5}$ and P=0.005, respectively). No significant interactions were observed between HbF levels and the *HMIP* or *HBG2* (XmnI) SNPs (P>0.05). In concordance with linear regression models, a case (HbF>2%; n=15) *vs.* control (HbF<1.7%; n=45) study, showed significant associations for *BCL11A* rs11886868 (OR =4; 95% CI, 1.6-9.6; P=0.001) and *BCL11A* rs766432 (OR=3.7; 95% CI, 1.5-8.9; P=0.002) but not for *HMIP* rs9399137 (OR=1.6; 95% CI, 0.6-4.0; P=0.318), *HMIP* rs6934903 (OR=1.8; 95% CI, 0.6-5.3; P=0.290) or *HBG2* (XmnI) rs7482144 (OR=1; 95% CI, 0.4-1.4; P=1).
Summary / Conclusion: Our results suggest that the increase of HbF levels in Portuguese individuals with common forms of HPFH is associated with *BCL11A* polymorphisms, but not with *HMIP* or *HBG2* (XmnI) *loci*.

B1790

NGAL (NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN): A NEW BIOMARKER FOR THE DIFFERENTIAL DIAGNOSIS OF ANEMIA?

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Background: NGAL (neutrophil gelatinase-associated lipocalin), a small 25-kD peptide identified as a component of neutrophil granules, inhibits bacterial growth by depleting their intracellular iron stores. NGAL is also a promising marker of kidney injury that predicts acute renal impairment. Its overexpression is associated with a poor prognosis in a variety of cancers including breast carcinomas. In addition, NGAL was shown to induce apoptosis of primary bone marrow cells including erythroid progenitor cells and inhibit erythroid cell production leading to anemia. Finally, it has been proposed as a new tool in the assessment of iron deficiency and in the management of iron therapy for hemodialysis patients.
Aims: The aim of the present study was to evaluate its utility as a biomarker for the differential diagnosis of iron deficiency anemia and chronic disease anemia, and to compare it with other conventional anemia parameters.
Methods: 35 patients with IDA (iron deficiency anemia) of whom serum hemoglobin <12 gr/dl, transferrin saturation (TSAT) <20% and serum ferritin <20ng/ml and 35 patients with chronic disease anemia (CDA) of whom serum hemoglobin >12 gr/dl, TSAT >%20 and serum ferritin >50 ng/ml were enrolled.
Results: Median serum NGAL values were 0.44 ng/ml(0.32-0.66) in IDA group and 2.16 ng/ml (1.39-3.22) in CDA group and a statistically significant difference was observed between two groups in respect to NGAL (P<0.001). NGAL showed a significant positive correlation with hematocrit, MCV, MCH, MCHC, serum iron, TSAT and ferritin (P<0.001) and a significant negative correlation with transferrin and total iron binding capacity (P<0.001). A 'cut-off' value of NGAL was determined as 1.02ng/ml (97.1% sensitivity, 83.9 % specifity) by ROC analysis (NCCS 2007, PASS 2005 ve GESS 2006).
Summary / Conclusion: Various biochemical parameters are being used for the diagnosis of iron deficiency anemia. However, there might be some difficulties in the assessment of these conventional parameters. For example, ferritin behaves as an acute phase reactant which limits its diagnostic accuracy greatly. The serum ferritin level is frequently increased independent of iron status by factors such as acute/chronic inflammation, infection, malignancy, liver disease and alcohol use. Serum iron levels also decrease with infection, inflammation, and malignancy and increase with liver disease.Transferrin saturation is a calculated parameter, and therefore reflects confounding effects on individual components. Therefore, besides the current conventional parameters that we use in routine practice to diagnose IDA, there is still need for more sensitive and powerful parameters. Our results show that NGAL is a useful parameter that can be confidentially used in the diagnosis of IDA and NGAL cut-off value of 1.02ng/ml predicts iron deficiency anemia.

B1791

HEMOGLOBIN BART'S LEVELS IN CORD BLOOD OF BAHRAINI NEONATES AND ITS CORRELATIONS WITH GENOTYPES OF ALPHA THALASSEMIA

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Background: Alpha-thalassemia (α-thal) is common in the Arabian Peninsula. The majority of previous studies have used electrophoretic methods and demonstrated correlations between Hb Bart's levels in neonates and deletional α-thalassemia genotypes. However, there is a notable lack of information related to (a) the quantitation of neonatal Hb Bart's levels with more sensitive techniques such as HPLC and (b) correlations of Hb Bart's levels with the non-deletional genotypes prevalent in this region. This has practical significance because many countries in this region have embarked upon neonatal screening programmes.
Aims: This prospective study was done with the specific objectives of determining (1) the extent to which neonatal Hb Barts levels correlate with α-thalassemia genotypes (2) the feasibility of establishing a cut-off value of Hb Barts that would be useful for diagnosis and (3) alterations in the erythrocyte indices in relation to α-thalassemia genotypes.
Methods: We screened umbilical cord blood samples of 435 Bahraini neonates with the aim of selecting cases with normal and elevated Hb Barts for molecular genotyping. Hb fractions were quantified by HPLC (Primus CLC 385). Hb Bart's levels showed a tri-modal distribution curve with a major peak occurring between 0.2% and 1.9% followed by a smaller peak between 2% and 9% and a minor one comprising cases with values greater than 9%. Representative samples from these three regions were selected for genotyping. Therefore, 78 of the screened cases were tested by an established polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) strategy to identify the four common alpha-thalassemia abnormalities prevalent in this region: (1) $-\alpha^{3.7}$ (2) $-\alpha^{4.2}$ (3) $\alpha^{Hph}\alpha$ and (4) $\alpha^{T\text{-}Saudi}\alpha$. Erythrocyte indices were estimated by an automated cell counter. This report describes the observations in these genotyped cases.
Results: Hb Bart's levels ranged from 0.2-18.2%. The distribution of various genotypes were as follows: αα/αα (normal, 29 cases); $-\alpha^{3.7}/\alpha\alpha$ (10); $-\alpha^{4.2}/\alpha\alpha$ (5); $\alpha^{Hph}\alpha/\alpha\alpha$ (1); $\alpha^{TS}\alpha/\alpha\alpha$ (2); $-\alpha^{3.7}/-\alpha^{3.7}$ (16); $-\alpha^{4.2}/-\alpha^{4.2}$ (2); $\alpha^{Hph}\alpha/\alpha^{Hph}\alpha$ (2); $-\alpha^{3.7}/-\alpha^{4.2}$ (1); $-\alpha^{3.7}/\alpha^{Hph}\alpha$ (2); $-\alpha^{3.7}/\alpha^{TS}$ (4); $\alpha^{TS}\alpha/\alpha^{TS}\alpha$ (4). These cases were classified into four genotypic groups based on the number and type of defects as follows: (I) normal genotype (αα/αα), 29 cases (II) 1-gene defect (-α/αα or αTα/αα), 18 cases (III) 2-gene defects excluding cases with the $\alpha^{T\text{-}Saudi}$ mutation (TS-), 23 cases and (IV) 2-gene defects with homozygous or double-heterozygous $\alpha^{T\text{-}Saudi}\alpha$ (TS+); 8 cases. Sickle cell trait was an associated feature in 13 cases (group II, 1; group III, 8; group IV, 4). Hb Bart's clearly discriminated between cases with 2-gene abnormalities and the normal group (Mann-Whitney test, P<0.001). Although there was a minor overlap of Hb Bart's values between the normal group and the group with 1-gene defects the difference between the mean values was also highly significant (P<0.001). A cut-off value of Hb Bart's 1.6% showed 83% sensitivity, 100% specificity, 100% positive-predictive value and 78.9% negative-predictive value in the diagnosis of α-thalassemia. Markedly elevated Hb Barts levels clearly separated the most severely affected group with 2-gene defects (TS+) from the other α-thalassemia genotype-groups. There was no difference between cases with and without sickle cell trait within the same genotypic group. Among red cell indices, MCH was the best discriminator in differentiating α-thalassemia from the normal group with 72.9% sensitivity and 96.7% specificity.
Summary / Conclusion: Quantitation of Hb Bart's in cord blood by HPLC has the ability to identify α-thalassemia in neonates with high discriminatory power provided a valid threshold value is established by prior molecular studies. Genotype-phenotype correlations are highly significant and Hb Bart's levels can accurately identify the most severely affected cases belonging to the group with homozygous or double-heterozygous $\alpha^{T\text{-}Saudi}$ mutation. This observation has added significance in view of the fact that this mutation is commonly associated with HbH disease in this geographic region.

B1792

THE HIGH FREQUENCY OF HB GROENE HART IN SPAIN: FIRST CASE OF ASSOCIATION OF THIS VARIANT WITH HB J-PARIS-I

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Background: Thalassemias are the most frequent monogenic disorder around the world. α-Thassemias should to a deficiency of synthesis in the α globin chain of the hemoglobin (Hb), andcharacterized by a hypochromic microcytic anemia. Around 5-10% of α-thassemias are due to punctual mutations. When they affect to posttranslational processing, originate hyper unstables Hbs, which cannot be detected by most electrophoretic or chromatographic methods.
Hb Groene Hart, also called Hb Bernalda, is a variant hyper unstable which precipitates in an early stage of erythroid precursors and is characterized by CCT>TCT mutation at codon 119 of the α1 gene, resulting in the change of proline (Pro) by serine (Ser) at position H2 of the peptide chain. This variant was described for the first time in 2 patients from Morrocco. These patients showed a phenotype of mild α-thalassemia minor with microcytosis and hypochromia.
Aims: In this abstract we show the study of 21 cases belonging to fifteen families affected by Hb Groene Hart, one of them associated with Hb J-Paris-I.
Methods: 21 patients from 15 unrelated families (6 of them from North African) were included in this study because they showed microcytosis without iron deficiency. Hematological data were obtained on a hematologic counter. Quantification of A2 and F hemoglobin were conducted by ion exchange HPLC. Hemoglobins were studied by capillary electrophoresis and ion exchange HPLC and the globin chains by reversed-phase HPLC. Most frequent mutations in α globin genes were ruled out by *a-globin StripAssay*. Molecular characterization was performed by specific sequencing.

Fam	Age	Sex	Sub	Hb	MCV	MCH	HbA_2	Hb F	Ret.	Genotype
A	55	M	I1	16	82,3	27,6	2,2	2	0,8	$\alpha\alpha^{Gr}/\alpha^{J\text{-}Pa}\alpha$
	30	M	II1	16,1	80,1	26,4	3,1	0	1,1	$\alpha\alpha^{Gr}/\alpha\alpha$
	26	F	II2	12,9	77,6	26,7	3,5	0	0,8	$\alpha\alpha^{Gr}/\alpha\alpha$
B	35	F	I1	11,8	77,3	26,3	2,5	1,4	---	$\alpha\alpha^{Gr}/\alpha\alpha$
C		M	I1	13,7	77,9	26	2,9	0,5	3,1	$\alpha\alpha^{Gr}/\alpha\alpha$
D	28	F	I1	13,1	78,5	26	2,9	0	0,19	$\alpha\alpha^{Gr}/\alpha\alpha$
	2	M	II1	8	70,2	23,9	3,3	0,4	1,9	$\alpha\alpha^{Gr}/\alpha\alpha$
E*			I1	13,9	77	24,5	3,4	0,3	1,6	$\alpha\alpha^{Gr}/\alpha\alpha$
F		F	I1	12,9	82,4	24,5	2,9	0,6	2,2	$\alpha\alpha^{Gr}/\alpha\alpha$
G*	37	M	I1	13,9	79,5	26	3,2	0,4	1,75	$\alpha\alpha^{Gr}/\alpha\alpha$
H*			I1	14,2	71,3	23	2,2	0,2	1,85	$\alpha\alpha^{Gr}/\alpha\alpha$
I		M	I1	14,4	81,5	26,4	3,1	0,2	0,27	$\alpha\alpha^{Gr}/\alpha\alpha$
J		M	I1	15,4	80,6	26,3	2,6	0,3	0.58	$\alpha\alpha^{Gr}/\alpha\alpha$
K	16	F	I1	13	79,9	26,9	3	0,4	0,77	$\alpha\alpha^{Gr}/\alpha\alpha$
	11	F	I2	12,9	71,9	24	3	0,4	1,14	$\alpha\alpha^{Gr}/\alpha\alpha$
L	26	M	I1	14	84,4	27,5	2,7	0,2	0,99	$\alpha\alpha^{Gr}/\alpha\alpha$
M*		M	I1	12,9	80,8	27,4	3	0,2	0,67	$\alpha\alpha^{Gr}/\alpha\alpha$
N*	7		I1	12,3	76,4	25,4	3	0,3	0,32	$\alpha\alpha^{Gr}/\alpha\alpha$
	5		I2	12,3	76,7	25,1	3	0,4	0,41	$\alpha\alpha^{Gr}/\alpha\alpha$
Ñ*	43	F	I1	13,7	85,6	26,4	2,8	0,3	0,37	$\alpha\alpha^{Gr}/\alpha\alpha$
	9	M	II1	13,7	81,1	26,3	3,4	0,4	0,31	$\alpha\alpha^{Gr}/\alpha\alpha$

Results: α_1 gene selective sequencing showed CCT>TCT mutation (Pro>Ser) at codon 119 (Hb Groene Hart). Moreover, we also observed a patient with GAC>GCC mutation (Ala>Asp) at codon 12 of the α_2 gene (Hb J-Paris-I). All mutations were heterozygous. Hematimetric parameters, Hb A2, Hb F and alpha genotype are listed in Table 1.
Summary / Conclusion: Hyper unstables Hbs carriers present a thalassemic syndrome which is due to anomalous chains posttranslational precipitation. This type of Hb variants accounts for 9.5% of structural hemoglobinopathies and it is very difficult to distinguish by electrophoretic techniques and ion exchange HPLC. In the Hb Groene Hart [CD119 (H2) Pro>Ser α_1], the residue 119 of α globin chain is affected, which has a key role to preserve the stability of α globin chain when it interacts with α hemoglobin–stabilizing protein (AHSP). AHSP is a chaperone which binds alpha globin chains to protect their precipitation. In the case of Hb Groene Hart, hyper unstability is due to its decrease in affinity for AHSP. Many others Hbs in which is affected residues in G or H helix H show a similar unstability. Finally, it is remarkable the presence of this variant in both immigrant and native population. Native population is a carrier of the mutation because during seven centuries of the Middle Ages the Iberian Peninsula was occupied by Muslims. Both contributions (immigrant and native population) maintain a great frequency of Hb Groene Hart in our area, still relatively high compared with other less common variants and therefore the molecular identification of Hb Groene Hart mutation carriers should be considered in the study of α-thalassemia cases in the context of microcytosis screening programs, as is done in Northern Africa.

B1793

ASSOCIATION IN THE SAME GENE OF TWO MUTATIONS INTERFERING WITH BETA GLOBIN RNA SPLICING IN A BETA MAJOR THALASSEMIC PATIENT.

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Background: Beta thalassemia is characterised by the decrease or absence of beta chain production. Mutations interfering with either the donor or the receiving splicing site of both IVS of beta globin gene have been described. The IVS-1-nt5(G>C) leads to β^+ thalassemia while mutation of the IVS-1-nt130(G>C) leads to β^0 thalassemia. Both mutations have never been described in the same chromosome, while their association (double heterozygous state), is the cause of beta major transfusion depended thalassemia. These mutations are frequent in the Indian subcontinent region.
Aims: We investigated the molecular pathology of a severe beta thalassemia major found in a patient of Bangladesh origin living in Fribourg Switzerland.
Methods: This 39 years old male patient is regularly transfused for beta thalassemia major since his very young age. Furthermore he is receiving chelation therapy by Deferasirox. DNA was extracted from his peripheral blood leucocytes just before the next programmed transfusion. Amplification of the whole beta globin gene and alpha2 and alpha1 globin genes followed by bi-directional sequencing was done.
Results: The following mutations were found in the two beta globin genes: 1. IVS-1-nt5(G>C) in homozygous state and 2. IVS-1-nt130(G>C) in heterozygous state. Sequencing of both alpha2 and alpha1 genes showed no mutations. The molecular analysis of the beta globin gene was confirmed twice.
Summary / Conclusion: The above mentioned mutations at IVS-1 of beta globin gene have been described by Kazazian et al, EMBO J. 1984 Mar;3(3):593-6 and Yamamoto et al, Hemoglobin 1992;16(4):295-302. However both mutations were described in a beta globin gene not presenting any other nucleotide substitution or deletion. The association of both mutations in the same beta globin gene to our knowledge has never been described before. Because both of them are relatively frequent in that region of the globe, we can admit an abnormal crossing over of two chromosomes carrying the two mutations, as the genesis mechanism of the new beta thalassemic mutation. As the patient is adopted by a Swiss family, studies of his parents' beta globin genes at a molecular level are not possible to realize. It will be interesting to investigate the result of the above mentioned mutation at the mRNA level.

B1794

ACERULOPLASMINEMIA IN A TURKISH ADOLESCENT WITH A NOVEL MUTATION OF CP GENE: THE FIRST DIAGNOSED CASE FROM TURKEY

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Background:
Ceruloplasmine (CP) is required for proper iron transport in the plasma**.** CP gene mutations cause the lack of *in vivo* ferroxidase activity of which enables theoxidation of ferrous iron (Fe^{+2}) into ferric iron (Fe^{+3}). Hence, iron in the plasma can not bind to the transferrin in aceruloplasminemia (aCp). Elevated free iron leads to the generation of free radicals causing oxidative stress. This process ultimately results pathological iron overload, especially in basal ganglia, pancreas, liver and retina. The most early symptoms of disease is hypocromic anemia and retinal degenerations.
Aims: Here, we discuss a case of aCP in a teenage with a novel mutation prior to the onset of neurologic symptoms
Methods: A 16 year old girl was admitted to our clinic with refractory anemia. Her medical history revealed that she had complains of pallor and fatigue and received intermittent oral iron supplementation within the last four years. Her full blood count and peripheral blood smear showed hypochromic microcytic anemia with a low serum iron level (14 µg/dl; 25-156µg/dl) and normal serum iron binding capacity (305 µg/dl; 100-370µg/dl) and transferrin level (263mg/dl; 200-360mg/dl). However, repeated measurements of serum ferritin levels were found high, ranging between 326 and 433 ng/dl in the absence of inflammatory state.
Results: Clinical and laboratory signs related to lead accumulation (1 µg/dl; 0-20 µg/dl) and zinc (12.8 µmol/L; 10.7-19.5 µmol/L) deficiency were not found. She had no clinical signs of copper deficiency, but, her serum copper and ceruloplasmin levels were extremely low (serum copper:<25µg/dl (80-155 µg/dl), ceruloplasmin:<2 mg/dl (20-60mg/dl) whereas 24 hours urinary copper excretion was normal (4.2 µg/d; 3-35µg/d). Kayser-Fleischer ring in the cornea was not observed. The diagnosis of aCP was suspected on the basis of the biochemical data. Ceruloplasmin gene was amplified in Tokyo University, Japan. Direct sequence analysis was performed by using the ABI 3700 instrument. A homozygous nonsense mutation at exon 7 of the CP gene was determined at 1306th base of CP mRNA. This mutation produces an early stop codon (X: TGA) at 436th amino acid which is originally arginine (R:CGA) (Figure I). We could not find a similar mutation in the aCP database**,** hence accepted as a nov-

el mutation. The fasting blood glucose level was determined within normal limits (84mg/dl; 70-126 mg/dl). Magnetic resonance imaging (MRI) of both liver and heart was performed for detecting iron overload. Mild iron overload in liver was determined and quantified as 3.8ms (Normal:>6.3ms). No iron accumulation was observed in the heart (28.2ms; Normal:>20ms). Cerebral MRI findings and examination of the eye including retina and fundus were found normal. Family history revealed that it was a fifth degree consanguineous marriage.
Summary / Conclusion: the diagnosis of aCp in early stages before the onset of typical symptoms is quite difficult. Although it is a rare disease, one should keep in mind the differential diagnosis of aCp with persistent hypochromic microcytic anemia with high serum ferritin level.

B1795
THE EFFECT OF IRON OVERLOAD ON THE RAT BONE MARROW ERYTHROBLAST DIFFERENTIATION AND THE EXPRESSION OF IRON REGULATORY GENES
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Background: Erythroblastic differentiation is closely related with the iron metabolism both in bone marrow and liver. Investigation of the cellular transport and storage of iron were showed the importance of signaling pathways which are sensing iron availability and regulating the iron metabolism by the needs of erythropoiesis. It has also been found that the effect of iron overload on bone marrow and liver tissues has had a therapeutic importance on iron overload diseases.
Aims: The effect of iron overload on distribution of erythroblasts in bone marrow was assayed and the expression of iron regulatory genes were investigated at the bone marrow and liver tissues of iron overloaded rats.
Methods: Rats were treated with 100 mg/kg iron dextran for 2 weeks and than bone marrow and liver samples were collected. Bone marrow cells were analyzed with flow cytometry for the CD71 transferrin receptor expression. The mRNA expression levels of iron regulatory genes were measured by Q-PCR method in bone marrow and liver tissues. Plasma levels of hepcidin and bone morphogenic protein 6 (BMP6) were measured with ELISA method.
Results: Distribution of CD71 (+) erythroblasts among total bone marrow cells were not changed but the size distribution of CD71 (+) erythroblasts has showed a tendency towards to higher FSC levels with iron overload. mRNA expression levels of CD71 in bone marrow cells were decreased by iron overload but erythropoietin receptor (EpoR) and type II transferrin receptor (TfRII) expressions were significantly increased. Contrary to these results, expressions of CD71, TfRII and HFE genes were decreased in liver tissue. Hepcidin and hepcidin-regulatory gene BMP6 were remained unchanged between control and iron overloaded rat livers. Additionally, plasma levels of hepcidin were slightly decreased although BMP6 levels were not changed.
Summary / Conclusion: Flow cytometry analysis of bone marrow cells has implied that late erythroblast differentiation was blocked with iron overload. Decreased expression of CD71 was considered as a cellular response of bone marrow cells to iron excess and as a cellular effort to limit the intracellular levels of iron. Liver cell response to iron overload was indicated that both iron uptake and the signaling receptor components of transferrin were down-regulated. Its possible to conclude that this down regulation of iron uptake related genes may responsible for the suppression of hepcidin expression.

B1796
HB CIBELES [ALPHA2 CD25(B6)(GLY>ASP)]: A NOVEL ALFA CHAIN VARIANT CAUSING ALPHA- THALASSEMIA
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Background: Thalassemias are the most common monogenic disorders worldwide, representing a serious health problem in areas where the incidence is higher. The α-thalassemias are caused by a deficiency or absence of hemoglobin (Hb) α-chain synthesis, and are characterized by a hypochromic microcytic anemia. Its main molecular mechanisms are large deletions. However, around 5-10% of the α-thalassemias are caused by point mutations (non deletional α-thalassemia) caused by a defect in the transcription, translation or post-translational processing. This latter mechanism originates Hb hyper unstables, which cannot be detected by most electrophoretic or chromatographic methods.
Aims: In this study we show a case with a new alpha chain structural variant in heterozygous state.
Methods: A four years old patient from Ethiopia was studied by presenting microcytosis and hypochromia with Hb A_2 and Hb F normal and without iron deficiency. Hematological data were obtained on a hematologic counter. Quantification of A_2 and F hemoglobin were performed by ion exchange HPLC. Hemoglobins were studied by capillary electrophoresis and ion exchange HPLC and the globin chains by reversed-phase HPLC. The most common forms of α thalassemia deletion and no deletion were ruled out by a-globin StripAssay and molecular characterization was performed by specific sequencing.
Results: α2 gene specific sequencing showed the mutation GGT>GAT (Gly>Asp) at codon 25 (B6). This finding leads to a new structural hyper unstable hemoglobinopathy named Hb Cibeles. Mutation was found in heterozygous state. Hematimetric parameters: Hb 12.6 g / dL, MCV 70.1 fL, MCH 22.6 pg, RDW 14.4%, 2.6% Hb A_2 and 0.5% Hb F. The abnormal Hb was not detected by capillary electrophoresis or by ion exchange HPLC. No abnormal α chains are separated by reverse phase HPLC.
Summary / Conclusion: Hyper unstables Hbs carriers present a thalassemic syndrome due to anomalous chains precipitation postraducional. This kind of alterations represent almost 10% of the structural hemoglobinopathies. They are very difficult to distinguish by electrophoretic techniques and ion exchange HPLC. This is the first description of a structural change that affects the residue 25 of the alpha globin chain, however others mutations were described in adjacent residues. Some of them, like Hb Luxembourg [alpha 24 (B5) Tyr>His] or Hb Shenyang [alpha 26 (B7) Ala>Glu], are also unstable because they affect to inner residues changing their charge and therefore their conformation. In the Hb Cibeles a larger, polar and negative aminoacid (Asp) is placed instead of Gly (smaller and apolar), turning the molecule hyper unstable.

B1797
HAPLOTYPE ANALYSIS OF COMMON HFE MUTATIONS IN THE PORTUGUESE POPULATION AND ASSOCIATION WITH IRON OVERLOAD
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Background: Hereditary Haemochromatosis (MIM:235200), an autosomal recessive disorder caused by increased iron absorption, is one of the most common genetic diseases among individuals of European origin. In Portuguese population, the haemochromatosis HFE gene mutations C282Y and H63D were found at frequencies (average) of 3.3% and 17%, respectively, however associated haplotypes using intragenic polymorphisms remains to be established.
Aims: To investigate i) the HFE intragenic haplotype background associated with C282Y, H63D and S65C mutations in the Portuguese population; ii) weather particular SNPs or haplotypes are associated with iron overload.
Methods: Three internal HFE SNPs IVS2(+4)T/C, IVS4(-44)T/C and IVS5(-47)G/A were analysed in a total of 150 subjects after informed consent was obtained: homozygous C282Y (n=12), H63D (n=19); heterozygous C282Y (n=18), H63D (n=34), S65C (n=6); compound heterozygous C282Y/H63D (n=17), S65C/H63D (n=3); subjects without HFE mutations (n=41). SNPs were genotyped by PCR-RFLP using RsaI, HaeIII and NlaIV, respectively. Sixty four adult subjects with high ferritin levels (>300 ng/mL) (excluding carriers for the C282Y mutation), with no other identified causes of iron overload, were compared with a control group of 20 adult subjects with normal/low ferritin levels (<100 ng/mL) (seven heterozygous H63D). Statistical analyses were performed with PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/).
Results: Mutations C282Y (n=59), H63D (n=92) and S65C (n=9) were associated exclusively with haplotypes TTG, CTA and CCA, respectively. In non-mutant chromosomes five of the eight possible haplotypes were found: TTG (53.3%), TTA (23.5%), CTA (9.8%), CCA (7.1%) and CTG (6.3%). In the case/control study, mutation H63D (OR=3.4; 95% CI, 1.4-8.3; P=0.0045), and alleles IVS2(+4)C (OR=3.64; 95% CI, 1.69-7.81; P=0.00061) and IVS5(-47)A (OR=2.89; 95% CI, 1.39-6.00; P=0.0035) were found positively associated with iron overload. Moreover, the TTG haplotype in non-mutated chromosomes (0.55 in controls vs. 0.25 in iron overload subjects) suggested to have a protective effect (P=0.00039).
Summary / Conclusion: The mutation associated haplotypes C282Y:TTG, H63D:CTA and S65C:CCA, are the same that have been reported in other European populations, suggesting a single origin for each HFE mutation. Regarding normal chromosomes the most common haplotypes reported for other European populations were found. The case/control study suggests that alleles IVS2(+4)C and IVS5(-47)A could be associated with risk of iron overload.

B1798
INVESTIGATION OF HEMORHEOLOGICAL PARAMETERS AT THE DIAGNOSIS AND FOLLOW UP OF CHILDREN WITH IRON DEFICIENCY ANEMIA AND MIX ANEMIA
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Background: Iron deficiency anemia (IDA) is characterized with decreased mean corpuscular volume (MCV), and in the peripheral blood smear microcytosis and hypochromia in the eryhrocytes, while in mix anemia (IDA+vitamin B12 deficiency anemia) MCV could be normal or decreased, and in the peripheral blood smear erythroctes could be morphologically normal. In the literature it has

been reported that, especially in hematological disorders which affect the morphology of the erytrocytes, hemorheological parameters are altered variously. The effects of IDA and vitamin B12 deficiency on hemorheological parameters have been investigated previously in different studies. These conditions are known to cause alterations in hemorheological parameters, but there is no data about how the hemorheological parameters are affected, when these two conditions coexist.
Aims: The aim of this study was to investigate the effects of iron deficiency anemia (IDA) and vitamin B12 deficiency anemia coexisting with IDA which is called as mix anemia (MA) in our study, on hemorheological parameters, to compare them with each other and healhty controls, and to assess the changes in hemorheological parameters after adequate treatment of these anemias.
Methods: The study enrolled 32 IDA patients (16 female, 16 male) aged 8 months-15.6 years (mean age: 6.3±5.3 years), 30 MA patients (15 female, 15 male) aged 7 months-16 years (mean age: 7.2±5.4 years), and 31 age and sex matched healthy controls (16 female, 15 male, mean age:7.1±5.2 years). Informed consent form was obtained from the parents. Elongation index (EI) which is the indicator of erythrocyte deformability was measured at 9 different shear stresses between 0.3 and 30 Pa by an ectacytometer. Erythrocyte aggregation amplitude (AMP) was also determined by an ectacytometer. Plasma and whole blood viscosities were determined by a cone-plate rotational viscometer. The differences between IDA and MA, and healthy controls were compared. Hemorheological parameters were repeated in both of the patient groups after treatment and compared with the initial results.
Results: In both of the patient groups (IDA and MA), erythrocyte deformability was found to be significantly decreased compared with the controls, and after adequate treatment it increased significantly. There was no statistically significant difference between the EI's of IDA and MA groups before treatment. No statistically significant alterations was found in erythrocyte aggregation measurements of both patient groups. Whole blood viscosities measured at either autologous or standardized hematocrit (40 %), and plasma viscosities were found to be significantly decreased in both of the patient groups before treatment compared with the controls, and after treatment these parameters showed a significant increase. There were no significant differences in these parameters between the IDA and MA group before treatment.
Summary / Conclusion: In conclusion, the results of this study indicate for the first time that hemorheological parameters except erythrocyte aggregation, are affected similarly in children with IDA and MA. The shortened lifespan of erythrocytes in these anemia types might be related with these hemorheological alterations. When vitamin B12 deficiency anemia coexists with IDA, no further hemorheological alteration occurs. The adequate treatment of these anemias helps to normalize the hemorheological parameters. We think that, further investigations are needed to identify the mechanisms of the alterations in hemorheological parameters, especially in children with mix anemia.

B1799
FLOW CYTOMETRIC TEST USING EOSINE-5 MALEIMIDE AS A FIRST-LINE SCREENING TEST FOR DIAGNOSTICS OF HEREDITARY SPHEROCYTOSIS.
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Background: Eosine 5-maleimide (EMA) test based on measuring the fluorescence intensity of red blood cells as EMA predominantly binding to band-3 and RH-proteins. In this study we evaluated the use of EMA for a rapid screening test of patients with Hereditary Spherocytosis (HS). The mean fluorescence intensity (MFI) for EMA-labelled RBCs from three groups of patients with HS were compared with 2 groups of random blood donors as a normal control group.
Aims: to find an optimal conditions for using EMA-test in diagnostic laboratories.
Methods: Flow cytometry to analyze EMA-labelled RBCs. Clinical features evaluating to identify RBCs membrane disorders. SDS-polyacrylamide gel electrophoresis and an Osmotic fragility test were used to verify diagnose of HS. ROCs analysis was done using MedCalc pro v.11.6.0.0. for Windows. In this study were included 5 groups of patients with informed consent:
- the adult control group consisted of random individuals without haematological pathology in routine blood test - 270 individuals (ages ranging 18-72 years);
- the children's control group – 18 randomly selected normal blood samples from routine donations (ages ranging 1 month – 15 years);
-children with verified HS diagnose (age ranging 1 month-18 years) -35 individuals;
-children with verified HS diagnose treated with blood transfusion one week prior to their RBCs analyses – 3 individual;
-adult splenectomised HS patients – 8 individuals (age ranging 20 - 48 years).
Results: Fresh RBCs were stained with EMA and analyzed for mean channel fluorescence (MCF) using one-colour flow cytometry. As EMA is non-sustainable dye and the results of different diagnostic laboratories are often non-comparable we offered to use index S to unify the records of EMA-tests: S=EMA MCF of examed blood sample/(total of EMA MCF for 6 normal control samples/6). RBCs from non-splenectomised patients with HS expressed a greater degree of reduction in MCF compared to those from the normal control group and splenectomised adult patient's group. Children with verified HS EMA MCF=21,2±2 units; S=0,7±0,1. Adult splenectomised patients RBCs EMA MCF was 25 u.; S=0,8±0,1.EMA MCF of adult individuals without haematological pathology and healthy children had no significant difference, EMA MCF=31±4 u., (P=0,45). We found significant difference in EMA MCF of children with HS and control groups (P<0,05). Evaluation of MCF for RBCs of patients treated with a blood transfusion showed few fluorescence peaks in the analysis and wider ranges of MCF. EMA method`s sensitivity =97 % and specificity=100%.
Summary / Conclusion: Comparing MCF of RBCs from adult and children control groups we found out that MCF is a non-age-connected characteristic and blood samples of normal individuals can be used as a normal control in this test with no connection with the age of individuals. We used the unifying index S to compare results of EMA-test. The method of EMA binding screening is speedy and a reliable diagnostic test and could be used as a first line screening test for diagnosis of hereditary RBCs membrane disorders.

B1800
A NEW FRAMESHIFT MUTATION IN THE B-GLOBIN GENE CAUSING B0-THALASSEMIA: CD 47 (-G)
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Background: β-thalassemia is one of most common autosomal recessive disorders worldwide and one of the public health problems more important in some regions of the world. It has been estimated that about 1.5% of the global population (80 to 90 million people) are carriers of β-thalassemia, with about 60000 symptomatic individuals born annually. The total annual incidence of symptomatic individuals is estimated at 1 in 100000 throughout the world and 1 in 10000 people in the European Union.The beta globin gene mutations cause a reduced or absent production of β globin chains, the large majority is in functionally important regions of the β globin gene. More than 200 mutations have been so far reported. Rarely deletions are responsible of the β-thalassemia and the common of mutations is single nucleotide substitutions or deletions or insertions of oligonucleotides leading to frameshift. A complete updated list of β-thalassemia mutations is available though the Globin Gene Server Web Site.
Aims: In this report, we described a new β-globin gene frameshift mutation
Methods: β-Thalassemia minor was diagnosed using hemoglobin high performance liquid chromatography (HPLC) analysis (VARIANT II, Bio-Rad Laboratories, Hercules, CA, USA).Genomic DNA was extracted from peripheral blood leukocytes using an automated method [Bio-Robot® EZ1 (Quiagen GmbH, Hilden, Germany)]. The β-globin gene was amplified using these primers: β1D: 5'-CCT AAG CCA GTG CCA GAA G-3' (from nt –160 to –142) and CD2: 5'-GAC CTC CCA CAT TCC CTT TT-3' (from nt +1659 to +1643) (all nt positions are provided relative to the Cap site = nt1 from NCBI GenBank®). Polymerase chain reaction (PCR) products were treated with the ABI PRISM™ BigDye® Terminator V1.1 Cycle Sequencing Ready Reaction Kit (PE Applied BioSystems, Foster City, CA, USA) with b1D and CD2 primers for sequencing, following the manufacturer's instructions, and the sequence was analyzed on an ABI PRISM™ 310 Genetic Analyzer (PE Applied BioSystems) demonstrated that the patient was heterozygous for a deletion of one nucleotide (-G) at amino acid codon 47 in exon 2 [HBB:c.142delG]. α-Thalassemia was ruled out using α-globin StripAssay.
Results: The propositus was an 82-year-old Spanish man who was referred because he showed mild microcytosis and hypocromia [hemoglobin (Hb) 10.8 g/dL; MCV 67.1 fL; MCH 21 pg]. He was an increased Hb A_2 (4.9%) and normal Hb F (1.1%) and normal Hb pattern.
Summary / Conclusion: The codon 47 (-G) mutation introduces a premature termination codon TGA at position 60 instead of codon 147 in exon3, affecting the beta globin expression resulting in abnormal mRNA translation. Phenotypically is a β^0-thalassemia, characterized by the complete absence of beta chain production. This mutation has not been reported for the β-globin gene. This kind of mutation is called frameshift, there are currently over 100 described.

B1801
A PRELIMINARY STUDY ON RISK OF A COUPLE HAVING A CHILD WITH SEVERE THALASSEMIA SYNDROME, PREVALENCE IN KHON KAEN PROVINCE, THAILAND
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Background: Thalassemia is the leading genetic disease in Thai population, and certainly it was ranked in Thai national policy for prevention and control. Prevalence of couple at risk for having a child born with thalassemia major and incidence of thalassemia disease child differ from different region.
Aims: To known of the thalassemia epidemiologic data for help supporting the thalassemic prevention and control plan in each definite area.
Methods: The study was conducted on pregnant women and spouses who

attended the antenatal clinic at 10 community hospital and Khon kaen hospital in Khon kaen province form March 2011 – May 2011. All subjects had blood taken for diagnosis of thalassemia trait or diseases, based on quantitative electrophoresis, and PCR (polymerase chain reaction) technique. After identification of couples at risk for having a child born with thalassemia major, prenatal diagnosis (amniocentesis) was proceeded in those risked couples.
Results: 410 couples were recruited. Prevalence of heterozygous α thalassemia 1 was 2.2%, heterozygous β thalassemia 4.8%, heterozygous Hb E 28.3%, homozygous Hb E 10.8% and other abnormal Hb 0.08%. There were 14 risked couples (2.9%), 10 risked for compound heterozygous β thalassemia/ Hb E and 4 risked for Hb Bart's hydrops fetalis. Amniocentesis was performed in all couples and found thalassemia major child were 5 cases, 3 were compound heterozygous β thalassemia/ Hb E and 2 were Hb Bart's hydrops fetalis.
Summary / Conclusion: Prevalence of couples at risk in Khon kaen province in preliminary study was 2.9%. And the prevalence of thalassemia carriers among pregnant women were high, just about three months of the screening thalassemia program in this center was successful for practical to prevention and control of this disease.

B1802
GENETIC OF GLOBIN DISORDERS IN RUSSIA
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Background: Hemoglobinopathies are heterogeneous group of diseases caused by qualitative (abnormal Hb) or quantitative (thalassemia) failure in hemoglobin synthesis. They are the most common monogenic disorder in the world, creating major public health problem in many countries. However in Russia they are relatively rare.
Aims: Analysis of the spectrum of mutations in the α-globin locus and β-globin gene in children from Russian Federation with suspected hemoglobinopathies.
Methods: The presence of 7 most frequent deletions (α3.7, α4.2, α20.5, MED, FIL, SEA, THAI) and 3 control sequences of the α-globin locus were tested by conventional PCR followed by electrophoresis in 1% agarose gel. Direct sequencing of β-globin gene was performed in order to determine mutations. From May 2009 to January 2013 334 patients were screened for deletions in the α-globin locus and 383 patients were examined for mutations in β-globin gene. In most cases both studies have been carried out. Family members of some patients were examined as well. Totally 537 samples have been analysed.
Results: Three known α-globin locus deletions have been found in 33 families (9.8 % of investigated), namely α3.7 (17 cases, 52%), α4.2 (4 cases, 12%) and α20.5 (6 cases, 18%). Other 4 deletions have not been detected. Compound heterozygotes for α3.7 and α20.5 were found in 6 families (18%). In 5 families the deletions in α-globin locus were combined with mutations of β-globin gene. Thirty four various β-globin gene mutations have been found in 166 families (43.3 % of investigated). Mutation CD8 –AA was detected on 69 chromosomes (33% of all mutated chromoseomes, 11 homozygotes), IVS2-1 G®A on 24 chromosomes (12%, 6 homozygotes). Frequencies of prevailing mutations were: IVS1-110 G→A 6%, CD36/37 –T 5%, CD8/9 +G 5%. Totally 34 mutations were identified on 206 mutated chromosomes. In 14 families two different mutations of β-globin gene were present simultaneously.
Summary / Conclusion: Low level of mutations found in α-globin locus indicates that further study of the region is necessary. Implementation of methods that permits more dense analysis of the locus is required. Our data revealed an even distribution of mutations in β-globin gene. Therefore direct sequencing of the whole gene is the most logic diagnostic approach.

Red blood cells and iron; physiology and disease (anemia) - Clinical

B1803
SAFETY AND TOLERABILITY OF AUTOMATED RED CELL EXCHANGE TRANSFUSIONS IN PATIENTS WITH SICKLE CELL DISEASE
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Background: Red cell exchange transfusion is an effective therapy in sickle cell disease (SCD) in the setting of both acute complications such as acute ischaemic stroke and acute chest syndrome, and chronic complications such as leg ulcers and recurrent vasoocclusive crises. Automated red cell exchange transfusion (ARCET) can achieve very low HbS levels rapidly and efficiently with minimal haemodynamic imbalance.
Aims: To assess the tolerability and complication rate of ARCET in patients with SCD.
Methods: This is a retrospective analysis of events occurring from May 2011 to December 2012 involving patients with SCD receiving ARCET at Homerton University Hospital.
Results: 40 patients have been transfused since May 2011. 26 of these patients are still on the programme. Three patients only had transfusion prior to elective surgery and one post surgery. Three patients discontinued due to ineffectiveness, 2 due to poor compliance, and 5 due to tolerability and complications that will be discussed later. Notes for 8 of the patients were unavailable so symptomatic history could not be discerned in these patients. The total number of transfusions in the remaining patients was 203. Of these, the documentation for 37 (18%) could not be found. Nine patients (28%) had no symptoms. Out of 166 transfusions with documented history, there were 10 episodes of paraesthesiae due to citrate toxicity (6%). 3 patients have each had 2 episodes of paraesthesiae. Only 4 were related to documented hypocalcaemia. 14 transfusions were associated with vasovagal symptoms (8%), but only 3 of these had a documented blood pressure drop. One patient had 2 episodes of vasovagal symptoms, one had 3. There have been only 2 episodes of rash requiring chlorphenamine (1%). One patient has had 6 symptomatic transfusions, accounting for 3 episodes of paraesthesiae, 2 of vasovagal symptoms and one with rash. However, he still continues the programme. Only one patient has their transfusion through peripheral access. All of the other patients have femoral lines on the day unit apart from 2 patients requiring a femoral line in theatre due to sedation purposes. This patient on another occasion also developed E-coli sepsis. One line tip result for another patient was positive for mixed skin flora and another for coagulase negative staphylococcus. All of the rest have been negative. There have been 6 episodes of mild bleeding (4%) from the femoral line site, of which 2 have been in one patient. Four patients have had major bleeding leading to a Hb drop requiring transfusion or readmission (2%). Only one patient (2.5%) has developed alloantibodies (Anti KpA). Out of the 5 patients that discontinued the programme due to complications, one developed hyperhaemolysis, one developed a line-related thrombosis and was also later found to have low protein C activity, one didn't like having a femoral line inserted and peripheral access was difficult, one discontinued due to repeated vasovagal episodes and one simply didn't like the procedure.
Summary / Conclusion: In general, ARCETs are well tolerated; approximately one quarter (26%) of procedures were associated with complications but the majority were mild. Only 5 (6%) patients have discontinued the programme due to problems tolerating the procedure or other complications, and more than a quarter of patients have had no symptoms.

B1804
LIVER DYSFUNCTION IN AN ADULT SICKLE COHORT
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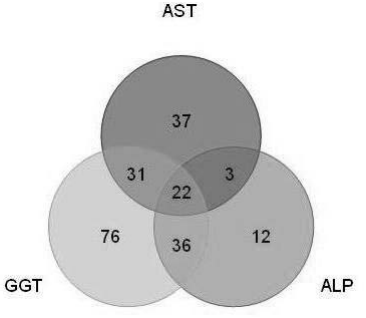
Background: In developed countries, sickle cell disease (SCD) has evolved into a debilitating chronic disorder with significant morbidity due to end-organ damage. The liver is one of the organs affected, resulting in "sickle hepatopathy". This study focuses on liver dysfunction in SCD patients at King's College Hospital ("King's").
Aims: To examine the incidence and spectrum of liver dysfunction in an adult sickle cohort.
Methods: The King's sickle database (comprising demographic, genotypic, clinical and laboratory data) was analysed. The case notes of patients with significant liver dysfunction were subjected to in-depth analysis.
Results: The sickle database comprised 581 adults regularly attending clinic with genotypes: 61% HbSS, 33% HbSC, 5% HbSβ$^+$ thalassaemia, <1% HbSβ° thalassaemia and <1% HbS HPFH. A retrospective analysis of the liver enzymes in these patients over the previous three years was conducted to find

predisposing factors to liver impairment. Mean *steady-state* values of all three liver enzymes were calculated: aspartate transaminase (AST), gamma glutamyl transpeptidase (GGT), and alkaline phosphatise (ALP). 63% of patients had no abnormal liver enzymes; 21% had one abnormal liver enzyme; 12% had two abnormal liver enzymes; and 4% (22 patients) had all three liver enzymes at elevated levels (see *Figure 1: number and distribution of SCD patients with each abnormal liver enzyme(s)*). Our focus was these 22 individuals with abnormal liver function to better understand the spectrum of liver disease in SCD. 21 of these 22 patients had genotype HbSS, and one patient, HbSC. The mean age was 39 years, range 22-78 years. Six of these patients have died: two with no other identifiable cause, hence of "sickle hepatopathy" alone, one of sickle hepatopathy and iron overload; of the remaining three, two had pulmonary hypertension and one had Hepatitis C. In the remaining 16 patients, possible causes of liver dysfunction (some with multiple aetiologies) included:evidence of *cleared* Hepatitis B in three patients; significant iron overload in three (with liver iron concentration over 7mg/g dW); right heart failure in one; intra-abdominal lymphoma in one; end-stage renal failure in two; possible hepato-toxic anti-tuberculosis medication in one; chronic cholecystitis in one. Eight patients had only SCD directly as the possible cause of hepatic dysfunction, one of whom developed end-stage liver failure, and was liver transplanted. None had significant issues with alcohol misuse. None of the patients were obese.



Summary / Conclusion: Our population reflected a modern, urban cohort in a well-resourced setting with notably lower rates of both viral hepatitis and alcoholic hepatitis than previously reported. Iron overload appears to be a major contributor to liver dysfunction, perhaps reflecting the increased use of blood transfusion, and likely to escalate with the increasing lifespan in SCD. Notably, the King's dataset reflects this: older age correlates with more liver enzyme abnormalities for both HbSS and HbSC.
Further work is needed to characterise the natural history of "sickle hepatopathy" to enable us to identify patients at risk and the contributory factors, so that monitoring and intervention can be directed appropriately, including red cell exchange transfusion and liver transplantation.

B1806

MYOCARDIAL AND HEPATIC IRON OVERLOAD IN SICKLE/THALASSEMIA PATIENTS OF ITALIAN ORIGIN

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Background: Sickle-thalassemia is an inherited hemoglobin disorder resulting from the combined heterozygosity for sickle-cell and β-thalassemia genes. Myocardial and hepatic iron overload in patients with sickle-thalassemia has been poorly studied. A small study including 10 multitransfused Arab patients has shown no evidence of cardiac iron and an higher prevalence of hepatic iron overload (6/10).
Aims: The current study aims to further evaluate cardiac iron overload assessed by a multislice approach and hepatic iron overload in a larger group of Italian patients and explore their correlation with transfusions, age and sex.
Methods: Fifty-nine sickle-thalassemia patients (29 males, mean age 35.6±14.1 years), enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) network underwent MRI (1.5T GE Signa/Excite HD, Milwaukee, WI, USA). For the measurement of iron overload, fast-gradient-echo multiecho T2* sequences were used. The left ventricle was segmented into a 16-segment standardized model and the T2* value on each segment was calculated as well as the global value. In the liver, the T2* value was assessed in a single region of interest (ROI) defined in a homogeneous area of the parenchyma. Liver iron concentration (LIC) was derived from T2* values using the formula described by Wood et al.
Results: We found 55 (93%) patients with all 16 segmental T2* values normal (>20 ms). Of the 4 patients with abnormal segmental T2* values, all showed an heterogeneous MIO (some segments with T2* values >20 ms and other segments with T2* values <20 ms) and none showed an homogeneous MIO (all segment with T2* values <20 ms). Out of the 4 patients with heterogeneous MIO, only one had a global T2* global <20 ms.
The mean global heart T2* value was 34.4±6.2 ms.
The mean MRI LIC value was 5.9±6.5 mg/g/dw and 30 patients (50.8%) had a pathological value (≥ 3 mg/g dw). Table 1 shows the comparison of iron levels among different transfusional regimens. There was not a significantly difference in terms of cardiac iron overload while patients regularly transfused had significantly higher hepatic iron than sporadically transfused patient (P=0.0001). On linear regression analysis, there was a statistically significant positive correlation between global heart T2* and age but with poor linearity (R=0.368; P=0.004) and there was not a significant correlation between age and MRI LIC (R=-0.170; P=0.197). Males and females had comparable global heart T2* values (35.6±4.9 ms vs 35.2±7.2 ms; P=0.118) as well MRI LIC values (5.1±4.6 mg/g/dw *vs* 6.7±7.9 mg/d/dw; P=0.762).

	Transfusions			P
	No (N=7)	Sporadic (N=32)	Regular (N=20)	
Global heart T2* (ms)	33.4 ± 7.3	32.4 ± 6.3	35.5 ± 5.4	0.425
Pts with global heart T2*<20 ms, N (%)	0 (0)	0 (0)	1 (5.0)	0.458
MRI LIC (mg/g dw)	3.6 ± 3.3	3.6 ± 2.9	10.5 ± 8.9	0.001
Pts with MRI LIC ≥ 3 mg/g/dw , N (%)	3 (42.9)	11 (34.4)	16 (80.0)	0.004

Summary / Conclusion: In respect of myocardial iron deposition, the sickle/thalassemia patients are similar to patients with homozygous SCD for which iron overloading is relatively rare. Hepatic iron overload may develop also in no regularly-transfused patients, maybe due to increased absorption of iron from the digestive tract, characteristic of both SCD and thalassemia intermedia patients. This finding underline the importance to monitor by MRI also no regularly transfused sickle/thalassemia patients.

B1807

UTILITY OF THE MULTIVARIATE APPROACH IN PREDICTING THE BETA-THALASSEMIA INTERMEDIA OR MAJOR TYPES IN IRANIAN PATIENTS

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Background: Recently, 5 genetic modifiers (β-globin mutations, co-inheritance of α-thalassemia, *XmnI* polymorphism, and single nucleotide polymorphisms (SNP) in the *BCL11A* and *HBS1L-MYB* loci) were used to predict the β-thalassemia (β-thal) major (β-TM) or intermedia (β-TI) types in 106 French patients. The dichotomous grouping was based on administration of 8 transfusions before (β-TM) or after (β-TI) the age of 4 years. Subsequently, multivariate regression analyses and a simple scoring system were used to predict the β-TM/β-TI with 83.2% accuracy.
Aims: Here, a similar study was conducted in a cohort of 306 Iranian β-thal patients having distinct β-globin mutations and minor allele frequencies (MAF) of key SNPs in these loci. The aim was to test the utility of this approach in predicting the β-thal types in Iranian patients.
Methods: The α1, α2, and β-globin mutations were determined by DNA sequencing. Deletions in the α- and β-globin genes were diagnosed by multiplex ligation-dependent probe amplification (MLPA) (MRC Holland, Amsterdam, The Netherlands). Genotyping of the *XmnI* polymorphism was carried out by using the PCR-restriction fragment length polymorphism (RFLP) method. Genotyping of the *BCL11A* and *HBS1L-MYB* SNPs was carried out by using the amplification refractory mutation system (ARMS)-PCR (rs4671393 and rs9399137) or tetra-ARMS-PCR (rs766432).
Results: Multivariate regression analyses and a simple scoring system were used to predict the β-TM/β-TI types in Iranian patients under three scenarios: 1) when considering only the severe β-TM and the mild β-TI cases, 2) using clinical parameters for β-thal typing, and 3) using age at first transfusion as the basis for classification. Under these scenarios, the β-thal types could be predicted in 77.6%, 75.5%, and 68.0% of the cases, respectively. Correct prediction, however, was especially difficult in the β-TI cases. When using clinical parameters for the classification, for example, 92.5% of the β-TM cases and only 45.2% of the β-TI cases could be predicted correctly.
Summary / Conclusion: Our results suggest that the multivariate approach is useful in predicting the β-thal types in Iranian patients. Furthermore, limitations in prediction are only partially due to the imprecise definition of β-TM and β-TI. However, correct prediction of β-TI remains to be difficult.

B1808

BONE MARROW IRON ACCUMULATION IN SICKLE CELL DISEASE, PAROXYSMAL NOCTURNAL HEMOGLOBINURIA AND THALASSEMIA PATIENTS ASSESSED BY MRI

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Background: Bone marrow iron accumulation, and its relationship to other organs, has not been extensively studied in diseases associated with iron imbalance owing to the difficulties in accessing this organ for quantitative measurements. Sickle cell disease (SCD), paroxysmal nocturnal hemoglobinuria (PNH) and β−thalassemia are all associated with iron overload, secondary to blood transfusion and/or disrupted iron regulation. Spin density projection assisted R2−MRI (FerriScan®) [St Pierre, T.G., *et al*., Blood, 2005. 105(2): p. 855−61] has been validated as a non-invasive tool for assessing iron load, with the proton transverse relaxation rate (R2) correlating with tissue iron concentration.
Aims: In this study we have compared bone marrow R2 data from patients with sickle cell disease, paroxysmal nocturnal hemoglobinuria, β−thalassemia, and a healthy control group.

Methods: Analysis of magnetic resonance images was retrospectively performed in patients that had already had an assessment of hepatic iron loading as part of their clinical care programme. There were 40 SCD patients (25 females, 15 males, 26.9±8.8 years), 15 PNH patients (7 females, 8 males, 45.5±15.7 years), 13 thalassemia patients (7 females, 6 males, 38.3±14.8 years), and 17 healthy control participants (4 females, 13 males, 37±7.7 years). PNH and thalassemia patients were receiving iron chelation; SCD patients were all chelation naïve. Axial images of the abdomen covering part of the thoracic and lumbar vertebrae were obtained from clinical MRI scanners operating at 1.5 T. Spin density projection assisted R2−MRI (FerriScan®) [St Pierre, T.G., *et al*., Blood, 2005. 105(2): p. 855−61] were used to assess liver iron concentration (LIC) in the participants. R2 values for the bone marrow were derived from pixel−wise mono−exponential fits to image data acquired with a single spin−echo sequence (FerriScan®). Group means were compared using Student's t test, with P=0.05 as the threshold for significance.
Results: Mean bone marrow R2 values for SCD (43.2±21.1 s−1, p <0.0001), PNH (36.1±19.2 s−1, P=0.0059) and Thalassemia patients (44.3±20.0 s−1, P<0.0001) were significantly higher than control mean bone marrow R2 values (21.8±4.9 s−1). Median LIC and ranges for these groups were: controls = 1.1 mg/g, range 0.4 – 1.8 mg/g; SCD = 4.7 mg/g, range 0.6 – > 43 mg/g; PNH = 7.6 mg/g, range 1.8 – > 43 mg/g and thalassemia = 10.8 mg/g, range 0.6 – 43 mg/g. LIC showed a significant positive correlation with serum ferritin (P<0.01)
Summary / Conclusion: These results indicate that we may be able to utilise R2 measurements of bone marrow to quantitatively assess bone marrow iron deposition in diseases such as SCD, PNH and Thalassaemia. This technique opens up the opportunities to assess the effect of chelators on bone marrow iron overload.

B1809

LONG-TERM EFFICACY OF DEFERASIROX FOR CARDIAC SIDEROSIS IN THALASSEMIA MAJOR

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Background: Iron accumulation is a consequence of long-term transfusion. The majority of transfusion-dependant TM patients will succumb to cardiac-related mortality, resulting from iron loading in the heart. Lifelong iron chelation therapy is therefore necessary to reduce iron accumulation; however, compliance issues associated with parenteral treatments remain a challenge. Deferasirox provides an attractive alternative due to its longer half-life, which permits once daily oral dosing regimens. With the development of advanced myocardial imaging modalities, there is the opportunity for early intervention to delay and indeed reverse iron loading in these patients, with the hope of preventing adverse cardiac outcomes.
Aims: The aim of this study was to examine the long-term efficacy of deferasirox in improving myocardial T2* in both adult and pediatric TM patients.
Methods: This was a multicentre retrospective cohort study of TM patients over 2 years of age receiving iron chelation treatment with once daily oral deferasirox monotherapy. Patients underwent MRI scans at baseline, with at least 1 further scan during follow-up, as determined by standard care and clinical need. This allowed quantification of myocardial and hepatic iron burden, through myocardial T2* and estimated liver iron concentrations (LIC), respectively. Data pertaining relevant clinical and routine laboratory parameters were collected at baseline and follow-up. Data are presented as mean with standard deviation unless otherwise stated. Statistical comparisons were made using paired t-tests.
Results: Data from 3 participating centers describes 52 patients with TM, with a mean age of 23±11 years. Overall, 75% of patients (n=39) were adults (age ≥16 years) and 69% (n=36) female. Mean duration of follow-up was 3.3±0.9 years. Dosing regimens were titrated according to standard practice. There was significant increase in mean myocardial T2* from baseline to latest follow-up, with an increase from 29.9±9.6 ms to 35.2±10.3 ms (mean increase: 5.3 ms, P=0.004). This remained significant following stratification by baseline myocardial T2* values <20 ms (n=7, mean increase 13.8 ms, P=0.04) and ≥20 ms (n=45, mean increase 3.9 ms, P=0.03). The improvement observed in myocardial T2* was also significant in both patients with LIC ≥7 mg Fe/g dw (n=18, mean increase 6.8 ms, P=0.04) and LIC <7 mg Fe/g dw (n=34, mean increase 4.5 ms, P=0.04). Of those with T2* <20 ms at baseline (n=7), 85.7% achieved values ≥20 ms at last follow up. Secondary analysis confirmed that the observed improvement in T2* was most pronounced in adults, males, and those achieving final deferasirox doses ≥30 mg/kg/day. There was a trend towards improved left ventricular ejection fraction in the cohort as a whole, but this did not reach statistical significance (65.3% to 67.6%, P=0.08).
Summary / Conclusion: Our findings suggest that in patients with TM, deferasirox shows long-term efficacy for preventing as well as managing cardiac siderosis. The benefits of deferasirox are not restricted to patients with low hepatic iron burdens but are also noted in patients with considerable iron overload.

B1810

AUTOIMMUNE HAEMOLYTIC ANAEMIA IN MALIGNANT DISEASES

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Background: Autoimmune haemolytic anaemias (AIHA) are diseases characterized by increased degradation of erythrocytes due to the presence of plasma proteins (globulin) that have character of antibodies' reacting with antigens of erythrocyte membranes of patients. According to the temperature at which the auto-antibodies bind to antigens in erythrocytes, AIHA are divided into: 1) AIHA caused by warm antibodies. 2) AIHA caused by cold antibodies. 3) Mixed (mixing) type. Depending on the presence or absence of the underlying disease, AIHA are divided into primary and secondary.
Aims: The aim of this paper was monitoring the appearance of secondary AIHA in malignant diseases.
Methods: During the three-year period, 154 patients of both sexes were observed, aged between 22 and 84 years with a diagnosis of a malignant disease. About 30% were patients with lymphoproliferative disorders, 9% of patients with multiple myeloma and 61% of patients with other malignancies (breast carcinoma, lung carcinoma, gastrointestinal malignant tumours, prostate cancer and carcinoma of genitourinary tract).
Results: During the monitoring, 18% of patients were reported with autoimmune haemolytic anaemia occurrence. Anaemia is characterized by reticulocytosis, increased levels of lactate dehydrogenase, indirect hyperbilirubinemia, reduced levels of haptoglobin and positive direct Coombs antiglobulin test (DAT). In 80% of patients AIHA was caused by warm antibodies and in 20% of patients with cold antibodies. We have found that AIHA may occur before, along with the appearance of carcinoma, during or after completion of chemotherapy, or as the first sign of recurrence of the disease. Most frequently AIHA was ascertained in patients with lymphoproliferative disorders (about 60%). In patients with solid tumours AIHA was slightly more common in renal carcinoma and gastrointestinal tract. Secondary AIHA is well known paraneoplastic phenomenon in lymphoproliferative disorders, but this form of anaemia may also occur in solid tumours.
Summary / Conclusion: AIHA may precede the occurrence of malignant disease, which initially has a great diagnostic significance. AIHA also may be the first, early signs of recurrence of the disease. Promptly determination of the cause of AIHA is of great importance for the diagnosis, monitoring and therapy of primary malignant disease.

B1811

COMBINATION OF DEFERASIROX AND DEFEROXAMINE IN CLINICAL PRACTICE: ALTERNATIVE SCHEMES OF CHELATION IN THALASSEMIA MAJOR (TM) PATIENTS

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Background: The availability of three iron chelators improved the scenario of chelation therapy, allowing to tailor the drugs according to the goals expected for each patient. New drug combination (Deferasirox/Deferoxamine-DFX/DFO- or DFX/Deferiprone-DFX/DFP) have been reported as applicable in clinical

practice.
Aims: To evaluate the efficacy, tolerability and safety of DFX/DFO in TM patients.
Methods: Combinated DFO/DFX. Nine patients (pts) affected by TM have started DFO/DFX for different reasons: **1)**lack of efficacy in removing liver/cardiac iron with monotherapy; **2)**restriction to use standard combination therapy (DFO/DFP) for agranulocitosis on DFP; **3)**adverse events in DFX or DFO. The study design included: at baseline, cardiac and hepatic T2*MRI, transient elastography evaluation (Fibroscan), biochemical evaluation (liver and renal function, ferritin, NTBI, creatinine, creatinine clearance and proteinuria), audiometric and ocular examination. Biochemical tests were repeated at1,3, 6 and 12 months from baseline; Fibroscan, T2*MRI, audiometric and ocular examinations at 6 and 12 months. The starting doses were: DFO 30 mg/kg/day for 3-4 days a week and DFX 20 mg/kg/day. Based on ferritin level and patient tolerability, doses were gradually increased. *Alternated DFO/DFX***.** Three pts started in 2011 alternative use of DFO or DFX for personal reasons or slight elevation of creatinine during DFX. They used DFO at the mean dose of 40 mg/kg/day for 5 days/week for 139±28 days/year and DFX at the mean dose of 22±2,9 mg/kg/day for 226±28 days/year. They repeated T2*MRI every year and biochemical evaluation monthly.
Results: *Combined DFO/DFX.* All pts (3 males, 6 women, mean age of 33±6 years) started combination therapy in 2012. The actual mean treatment period is 8±3 months. At baseline the mean pre-trasfusional Hb levels was 9.4±0.4 g/dl, the mean iron intake 0.40±0.10 mg/kg/die, the median ferritin 5258 ng/ml (range 644-17681 ng/ml), the median NTBI1,82 mM (range 0,38-3,57 mM). The median Fibroscan value was 6.2 kPa (range4,4-75). The cardiac T2* values were 19,73±10,61 ms (<10 ms in 2 pts, between 10 and 20 ms in 2 pts). The mean LIC was 15,05±12,97 mg/g dw, abnormal in 8 pts. Data available at six months showed no alteration of renal/hepatic function and no adverse events. A marked reduction in LIC level was achieved in 5 pts during a 6-months-period (4,94±3,28 mg/g dw vs 9,30±4,46 at baseline), with a concomitant reduction of NTBI (2,14±1,25 vs1,33±0,65 mM) and median ferritin (1624 vs 875 ng/ml). No changes in cardiac T2* values were detected. No patient has been submitted at 12 months controls. *Alternated DFO/DFX.* All pts (3 males, mean age of 42±11 years) showed at baseline a mean pre-trasfusional Hb levels of 9.6±1 g/dl, a mean iron intake of 0.32±0.05 mg/kg/die, a median ferritin of 876 ng/ml (range 837-1160 ng/ml). Cardiac T2* value at baseline was 39.96±7.81 ms and mean LIC value was4,41±0,5 mg/g dw, showing absence of cardiac iron overload and slight liver iron load. After 2 years, no significant changes in cardiac T2* values were detected (38.33±7.07 ms) but a normal LIC was achieved (2.90±0.54 mg/gdw) with stable median ferritin (876 vs 989 ng/ml).
Summary / Conclusion: Combined or alternated DFO/DFX can be considered when monotherapy is not able to remove the iron overload or in presence of adverse events. Cardiac changes seem to need prolonged time versus a more rapid reduction in LIC. In our experience, the use of these schemes of chelation seems to be efficacious and safe, but a prolonged observation is mandatory.

B1812
HEPATIC IRON CONCENTRATION QUANTIFICATION BY MRI: DIAGNOSIS AND MANAGEMENT IN HEREDITARY HEMOCHROMATOSIS´S PATIENTS
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Background: The magnetic resonance imaging (MRI) is the most sensitive and specific imaging modality in the iron overload diagnosis and management of hereditary hemochromatosis (HH). The effect caused by the accumulation of iron leads to signal loss in the affected tissues, particularly with T2* weighted sequences, which makes the diagnosis of iron overload, as well as determining severity and monitoring therapy in a noninvasive procedure.
Aims: 1. To evaluate the utility of hepatic MRI for the iron overload diagnosis and follow-up in HH patients. 2. To determine correlation between serum ferritin levels (SF) and transferrin saturation index (TSI) with the hepatic iron concentration (HIC) estimated by MRI.
Methods: Descriptive and retrospective study, realized in an overall period of 5 years (2008-2013). Hundred three patients, with SF >=300 µg/L and/or TSI >= 45%, and hepatic MRI study in axial planes with sequences promoted in T1, DP, T2, T2* y T2**, and calculate of the HIC according to protocol of Rennes University (N.V: 36±30 µmolFe/g). The patients were divided into 2 groups: Group A (n=20): C282Y homozygotes and compound heterozygotes C282Y/H63D or C282Y/S65C; Group B (n=34): other homozygotes (H63D, S65C), simple heterozygotes (C282Y, H63D, S65C). Twenty six patients were excluded because of no mutations in HFE gene. The levels of iron concentration were classified while HIC estimated by MRI as follows: Group I: <20 µmolFe/g no iron overload and/or 20-39 µmolFe/g light iron overload, Group II: 40-79 µmolFe/g moderate iron overload and Group III: >79 µmolFe/g high iron overload. The data were then compared with SF and TSI levels for each group. Statistical analysis with SPSS 18.0 version.
Results: Seventy seven patients met the inclusion criteria. Mean age was 53 years (range, 27 to 75). Male 84.4% (n=65) and female 15.6% (n=12). The SF levels mean were 553.96 (38.60-1781) and the TSI mean were 41.83 (16.6-85.64). Group A: 36.4% (n=28); homozygotes C282Y (n=12), compound heterozygotes C282Y/H63D (n=15) and heterozygotes C282Y/S65C (n=1). Group B: 63.6% (n=49); homozygotes H63D (n=11) and S65C (n=1), heterozygotes C282Y (n=12), H63D (n=24) and S65C (n=1). Group I: 10.4% (n=8) all of as with HIC < 20 µmolFe/g, Group II: Group III: 40.3% (n=31), Group IV: 49.4 (n=38). The most frequent genotype in the group III (high iron overload) were C282Y homozygotes (n=12) and compound heterozygotes C282Y/H63D (n=10). The HIC in group A (high genetic risk) were 137.22±85.9 and in group B (low genetic risk) were 75.3±51.4. The relation between SF levels and TSI in each iron overload group is shown in table 1.

Descriptive study by risk group				
		Group A (n=28)	Group B (n=49)	
Age [a]		50 (27-66)	55 (29-75)	
Male [b]		66,7%(n=18)	94% (n=47)	
Female [b]		33,3% (n=9)	6% (n=3)	
SF [c]		468.37 (38.6-1185.8)	602.87 (137.8-1781)	
TSI [c]		52.03 (27.85-85.64)	36.00 (16.6-67.3)	
HIC [c]		137.22 +/- 85.9	75.3 +/- 51.4	
Correlation SF – TSI vs. HIC				
HIC µmolFe/g		I (<36)	II (37-79)	III (>79)
		Mean +/- ST	Mean +/- ST	Mean +/- ST
A	TSI	NV	42.13 +/- 17.81	54,21 +/- 17.71
	SF		445.23 +/- 135.10	533.54 +/- 228.20
B	TSI	34.10 +/- 11.12	34,63 +/- 9,4	38,76 +/- 13,14
	SF	370.7 +/- 155.25	590.78 +/- 226.23	690.36 +/- 338.19

Summary / Conclusion: Elevated values of TSI were more observed in high genetic risk group (A) than in low genetic risk group (B), according to awaited. In contrast, the elevated values of SF showed no correlation with genetic risk. Similar results were obtained in HIC correlation. The transferrin saturation index was a stronger parameter related with hepatic iron concentration than serum ferritin levels. This differences, probably in relation with others inflammatory disorders that influenced in SF levels; and no translated in hepatic iron overload, estimated by MRI. The assessment of HIC estimated by MRI with the Rennes university protocol has been a useful tool to follow up patients with hereditary hemochromatosis and with iron overload.

B1813
TRANSIENT ELASTOGRAPHY TO ASSESS LIVER FIBROSIS IN PATIENTS WITH SICKLE CELL DISEASE
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Background: Liver failure is a rare but potentially very severe complication of Sickle Cell Disease (SCD) in adult life.Liver involvementin SCD is multifactorial, often asymptomatic for long time but eventually can lead to liver failure and death. Assessment of liver fibrosis by liver biopsy (LB) is not a common practice in such patients being an invasive procedure and non-invasive tests such as Transient Elastography (TE) have not yet been validated in these conditions.
Aims: To early identify patients with SCD who could be at risk of hepatic fibrosis and cirrhosis development by using TE.
Methods: Seventy-eight patients affected by SCD, including Sickle Cell Anaemia (SCA), HbS/β-thalassaemia, (T-SCD) and HbS/HbC disease, regularly followed at the Haereditary Anaemia Center in Milan underwent concomitant blood tests and TE, a non-invasive test for liver stiffness measurement (LSM). According to those validated in the setting of chronic viral hepatitis, (Fraquelli *et al*, Gut 2007**)** the following cut-off were used: as follows: < 5.0 KPa no fibrosis (F0), 5.1-7.9 KPa mild fibrosis (F1), >7.9 kPa moderate fibrosis (F2), >10.3 advanced fibrosis (F3), >11.9 kPa cirrhosis (F4).
Results: Seventy-eight patients were evaluated; TE was not available in 19 (24%) and failed in 4 (5%); and therefore we analyzed data from 55 patients (20% HbS/HbC, 22% SCA, 58% T-SCD). Patients were: females (71%), Italians (60%), with a median age of 37 (19-57) years. Sixteen (29%) patients had a BMI >25. Median Hb values were 9.8 (7.2-13.3) g/dL and ferritin median was

411 (31-7238) ng/ml. Alanine amino-transferases (ALT) were increased in 8 patients (14.5%) and gamma-glutamil transferases (gGT) were increased in 16 (29.1%) respectively. Overall median TE value was 5.6 (2.8-21.3) kPa, with 4 (7%) patients showing a TE >11.9 kPa (F4); T-SCD patients had the highest LSM values (HbS/HbC vs SCA vs T-SCD, *P=0.03*). LSM was elevated in 8 of the 47 (17%) patients with normal ALT (4 F2, 1 F3, 3 F4) and in 2 out of 8 (25%) patients with elevated ALT (1 F2 and 1 F4) (*P=0.6*). Moreover, TE values were increased (2 F2, 3 F4) in 42% of the patients with increased gGT values and in 10% (3 F2, 1 F4) of those with normal gGT values (*P=0.018*). Diagnosis of cirrhosis was confirmed by liver histology in all the patients with TE >11.9KPa; HCV RNA was positive in 2 out of 4 cirrhotic patients. Overall regression analysis showed a statistically significant correlation between TE values and AST levels (*P<0,001*), whereas only in T-SCD patients a correlation between TE values and LDH was observed (*P=0,003*). In all patients who underwent TE, Liver Iron Concentration (LIC) derived by T2* MRI was measured and no correlation was observed with LSM.

Summary / Conclusion: Patients with SCD could benefit from regular non invasive assessment of liver fibrosis, independently of ALT and gGt values; increased GGT combined to TE, may identify patients who can be at risk of developing fibrosis and eventually cirrhosis. Further study to validate TE in SCD are worrented.

B1814

QUALITY OF LIFE IN THALASSEMIA PATIENTS: ALMADINAH EXPERIENCE

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Background: Thalassemia major is a genetic disorder affecting beta globin chain synthesis with various clinical manifestation, requiring lifelong regular blood transfusion. common complications related to high iron load are heart failure, liver fibrosis, diabetes mellitus, growth retardation and delayed puberty. Quality of life (QOL) is consider as an important health outcome and index of effective treatment. Few studies conducted on QOL worldwide on thalassemia. To the best of our knowledge this had not been studied in Saudi Arabia or Middle East .

Aims: The present study aimed to find out levels of QOL among thalassemia patients in Saudi Arabia at hereditary blood disorders center in AlMadinah

Methods: A cross-sectional study was performed on 43 transfusion-dependent thalassemia patients compared with 43 normal subjects, as a control, using World Health Organization Quality Of Life (WHOQOL)-Bref questionnaire; Arabic version. Data were analyzed by Microsoft Excel 2010 and SPSS version 19.0. Chi^2 test was used for qualitative variables and t-test was applied for quantitative variables. Binary Conditional Logistic regression was used to determine the differences between cases and controls and QOL domains. P<0.05 was considered significant.

Results: 43 thalassemia patients has been examined, 23 males and 20 females age 15-31 years. Compared to 43 peered control group 24 males and 19 females. The level of education was high in 8 patients, secondary school in 14 and 21 had basic education. 26 had serum ferretin < 2500 and 17 were > 2500. 28 were using desferroxamine as iron chelator. 13 were employees, 17 were students. Parent level of education were 23 basic education, 9 secondary and 11 had higher education. There was no statistical difference between patients and control group at Psychological domain (53.4 vs 56.9, P=0.059) and Environmental domain (56.6 vs 57.0, P=0.884). Control group had better Physical QOL than patients (55.4 vs 61.9, P=0.047) while patients had better social QOL (39.3 VS 31.7, P=0.003). Further analysis has shown, basically educated control group had better Physical QOL (P=0.01) while basically educated patients had better social QOL(P=0.00), There was no statistical difference between patients and control group at other domains or higher level of education. Control males had better QOL at Physical (P=0.03) and Psychological domain(P=0.03) with no statistical difference in other domains. Female patients had better QOL at social domains(P=0.04) with no statistical difference in other domains. There was no statistical difference between Saudi patients and Saudi control group. Non-Saudi controls had better QOL at Physical domain(P=0.02) and patients had better QOL at social domains(P=0.00). Older patients had better QOL at social domains(P=0.00), with no statistical difference between patients and control group in other domains or younger age.
Among patients group, There was no statistical difference in QOL domains at variables of sex, nationality, level of education, use of Desferroxamine, health status, family history, socioeconomic, Parents level of education and employment. There was a statistical correlation between school, employment and physical health with a better QOL (P=0.01,0.01,0.01 respectively)

Summary / Conclusion: QOL in thalassemia patients is similar to control group particularly social life, though physical health is less. Improvement of patients care from all aspects will improve their QOL. More studies in this field is needed with a bigger sample size.

B1815

CORTISOL RESPONSE TO LOW DOSE VERSUS STANDARD DOSE (BACK-TO-BACK) ADRENOCORTICOTROPHIC STIMULATION TESTS IN CHILDREN AND YOUNG ADULTS WITH THALASSEMIA MAJOR.

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Background: Patients with thalassemia major with repeated blood transfusion have high prevalence of endocrinopathies due to iron overload. Both adrenal and pituitary iron overload can affect cortisol secretion and risk patient life during stress conditions

Aims: To study cortisol response to low dose and standard dose ACTH test in thalassemic children and adolescents.

Methods: We examined the adrenocortical function in 23 thalassemic patients (10 children and 13 young adults) aged 8 to 26 years. Serum cortisol and dehydroepiandrosterone sulfate (DHEA-S) concentrations were determined in each subject before blood transfusion both in basal condition and after low dose (LD) (1 microgram) followed by standard dose (SD) (250 micrograms) respectively) with synthetic corticotrophin beta 1-24 ACTH (Synacthen Ciba). Normal controls were a group of 13 age and sex matched normal subjects.

Results: Using a peak total cortisol cutoff level of 550 nmol/L and increments of 200 ug above basal cortisol, adrenal insufficiency was demonstrated in 8 patients (34.7%) after the LD ACTH and in 2 patients (8.7%) after SD ACTH test and in none of the controls. Using a peak total cortisol cutoff level of 420 nmol/L and increments of 200 ug above basal cortisol, adrenal insufficiency was demonstrated in 5 patients (21.7%) after the LD ACTH and in 2 patients after SD ACTH test (8.7%) but none of controls. All patients with biochemical adrenal insufficiency were asymptomatic with normal serum sodium and potassium concentrations and no history suggestive of adrenal pathology. The peak cortisol concentrations in thalassemic patients with impaired adrenal function both after 1ug and 250 ug cosyntropin, (294±51 nmol/L and 307±58.6) were significantly lower than those with patients with normal (454±79.7 nmol/L and 546.1±92.2 nmol/L respectively) and controls (460.2±133.4 nmol/L and 554.3±165.8 nmol/L respectively). Adolescents and young adults but not children with thalassaemia had significantly lower peak cortisol concentration after SD cosyntropin test versus controls. Peak cortisol response to LD cosyntropin test was correlated significantly with peak cortisol response to SD in all patients (r = 0.83, P<0.0001). In adolescents and young adults with thalassemia DHEA-S levels before and after LD ACTH stimulation were significantly lower and the cortisol/DHEA-S ratios significantly higher than controls

Summary / Conclusion: The use of LD ACTH test diagnoses more adrenal abnormalities versus SD ACTH in thalassemic patients. The relatively high prevalence of AI in thalassemic adolescents and young adults necessitates that these patients have to be investigated for AI before major surgery and those with impaired cortisol secretion should receive stress doses of corticosteroids during the stressful event

B1816

EVALUATION OF THE EFFECTIVENESS OF HEMOGLOBIN MEASURED BY A NON-INVASIVE SPECTROPHOTOMETRIC PORTABLE DEVICE AS A SCREENING METHOD FOR ANEMIA DURING PREOPERATIVE ASSESSMENT

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Background: To date, there are no data available concerning non-invasive measurement of spectrophotometricHb in the preoperative setting. Standard laboratory testing may take considerable time to identify patients with preoperative anemia and hence may delay the appropriate management and accordingly the operative procedure.

Aims: To evaluate Hb as measured by portable spectrophotometric device compared to standard laboratory as a screening tool for the preoperative population.

Methods: This is a parallel prospective assessment of instant Hb versus standard Hb which was conducted between March 2012 and January 2013 at the Launceston General Hospital, Launceston, Tasmania.
We screened 322 patients in the pre-operative clinicwith spectrophotometricHb (Pronto-7, version 2.1.9, Masimo Corporation, Irvine, USA) in correlation to the Hb as measured on the same day using Cysmex XE5000 automated analyzer ((Sysmex Corporation, Kobe, Hyogo, Japan). A control group of 87 patients with a known low Hb (≤ 100 g/L) was also assessed simultaneously by both methods in order to assess the effect of low Hb on the spectrophotometric Hb (SpHb) reading. The study was approved by the Tasmanian Human Research Ethics Committee and registered in the Australian New Zealand Clinical Trials Registry (http://www.ANZCTR.org.au/ACTRN12611001256965.

Results: Median age of recruited patients was 66 years (range, 26-92) with a male to female ratio of 155:143, while the median age of the control group was 57 years (range, 21-89) with a male to female ratio of 29:26.
The median and mean Hb as measured by SpHbwas 132 and 131 g/L versus 140 and 138 g/L respectively by the standard laboratory. In the control group, the median and mean Hb as measured by SpHbwas 102 g/L versus 96.5 and 98.5 g/L respectively by the standard laboratory. Our data showed that the correlation between spectrophotometricHb and standard Hb measurement was highest among males compared to females (P=0.001).
Summary / Conclusion: Our data suggest that SpHb correlates well with the standard Hb particularly in males and especially in detecting true low Hb values. Therefore, instant Hb measurement can be integrated into the routine preoperative work-up for screening patients with low Hb and hence offers a prompt management for patients with anemia. Addressing adequately the important issue with preoperative anemia will result in a major health saving and improvement of outcome of surgery. Further studies to confirm these findings are warranted.

B1817

EVALUATION OF QUALITY OF LIFE AND ANALYSIS OF EFFICACY AND SAFETY OF TWO IRON CHELATORS IN PATIENTS WITH IRON OVERLOAD

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Background: Iron excess in blood and tissues causes irreversible tissue damage.It has been demonstrated that removal of excess iron has a positive influence in the response to treatment and survival in patients with overload.
Aims: To evaluate the efficacy and the safety of two iron chelators (intensity and time to response), presence and frequencies of adverse events and quality of life (QOL).Quantify biomarkers of macrophage activation: Chitotriosidase (CT) and CCL18/PARC and to evaluate if they can be used as markers of response of chelation therapy
Methods: A comparative, randomized, open, non-inferiorityexperimental study into two arms (EUDRACT: 2009-017799-26). A total of27 patients with maintained iron overload (ferritin>600 mg/L), MDS/AML (13) iron overload after transfusion, alo-BMT(8) and type 1 Gaucher Disease (6), were compared with a healthy control group of 27 subjects with normal serum iron profile stratified by age and sex to analyze comparatively the biomarkers. The study was approval by the Ethical Commitee of Aragon and supported by a grant (TRA-158). All participants signed informed consent. The analysis included physical exam, blood counts, iron profile, protein profile, natriuretic peptide (proBNP) concentration, CT activity and CCL18/PARC, study of *HFE* genes, quantitation of iron liver deposits by MRI, calculating Liver Iron Concentration (LIC) by the ratio of strength signal between liver parenchyma *vs* the paravertebral muscles, and QOL using SF36 questionnaire.Treatment period: 4 months. Patients were weekly monitorizared in the first 4 weeks and every month after. Two randomized groups: A: Deferasirox 20 mg/Kg/day p.o. and B: Desferoxamine: 30 mg/Kg/day sc for 8 hours, three times a week.Statistical analysis: Descriptive statistics and frequency distribution. Comparison of means in independent groups using t-Student considering significance level <0.05
Results: Mean age: 56.9 y (29-77), 52% female. From 26 patients analyzed, 31% were heterozygous for *H63D,* 4% were homozygous,11% were double heterozygous *C282Y/7H63D* and 54% were no mutation. Mean at baseline; Hb: 12.4 g/dL (7.2-15.2), Hematocrit: 36.3% (20-45.2), Ferritin: 1042.8 ng/mL (635-1461), ProBNP: 381.1 pg/mL (18.8-2804), LIC: 83.2 mmol/g (0-240), Chitotriosidase: 65.2 nmol/mL/h (0.81-186) and CCL18/PARC: 150.3 ng (56-393). Fifteen patients were included in A group and twelve in B.Nine patients required transfusion of packed red cells during the study period (mean 14 units).After 4 months on therapy, 80% of patients showed a significant reduction in ferritin levels in both groups A: 490.7 ng/mL (155-1090) (P<0.001), B: 662.7 ng/mL (312-1395) (P<0.05), the differences in reduction grade were similar in the two series. proBNP 368 pg/mL (30-2705), LIC: 50.6 mmol/g (0-190). According the diagnosis, the patients with GD have a significant baseline ferritin leves lower than the other groups (P<0.04) but the grade of reduction after therapy was similar. Related to QOL, A patients group showed significant better scores in SF (P=0.04), RE (P=0.05), MH (P=0.025) MCS (P=0,031) than B group.Adverse events: In A group three patients experimented a reversible mild increase in creatinine level, one of them was grade 3 and mild digestive intolerance in other 3 cases
Summary / Conclusion: There were no differences in the severity iron overload regarding to the *HFE*genotype.Efficacy: Significant decrease of ferritin and iron liver deposits after four months on chelation therapy but more significant in arm A.No relationship were observed between ferritin decrease and plasma biomarkers of activated macrophage: CT and CCL18/PARC.Respect to LIC, were observed decrease of iron deposits, but non statistical differences in reduction of iron between both types of chelator. We have not observed differences in ProBNP concentration.Consequently, and according to the results of this study, ferritin concentration remains the best indicator, in clinical practice, of iron overload Quality of life: Patients under oral therapy, arm A, best scores that arm B, and statistical differences in: SF, RE, MH and MCS_SP.Safety: 11 patients had, at least, one adverse effect, patients in oral therapy had gastrointestinal disturbances and slight and reversible increase of creatinine levels (<2 mg/dL) in the first weeks.

B1818

PYRUVATE KINASE DEFICIENCY: CLINICAL AND MOLECULAR CHARACTERIZATION OF 11 PEDIATRIC CASES FROM ARGENTINA

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Background: Pyruvate kinase (PK) deficiency is the most common enzyme defect of erythrocyte glycolytic pathway causing hereditary nonspherocytic chronic haemolytic anaemia. The severity of thedisorder is highly variable, ranging from mild to severe anaemia, which can be life-threatening and requiring continuous transfusion therapy. PK deficiency is transmitted as an autosomal recessive trait and more than 200 mutations associated with the disorder have been so far reported in the gene encoding the red cell pyruvate kinase (*PK-LR*). Pyruvate kinase deficiency has a worldwide distribution with the higher prevalence of 1 in 20,000 in the Caucasian population. No reports were so far available on PK deficiency in Argentina.
Aims: We report the clinical, haematological and molecular characteristics of 11 PK deficient Argentinean children.

Table 1.

Pts	Ethnic Group	Age at admission (years)	Splenect. age (years)	Hb g/dL	Retics %	Hb g/dL	Retics %	SF ng/ml	Mutation	Effect
				Pre splenectomy		Post splenectomy				
TM-S	Gypsy	0,2	1,0	5,5	n.a	7,9	75	548,17	Gypsy/Gypsy	del ex11/ del ex11
TM-Y	Gypsy	0,1	0,8	7,1	n.a	7,8	87	392,68	Gypsy/Gypsy	del ex11/ del ex11
CR	Gypsy	0,1	3,9	6,6	24,6	8,5	94	2347,71	Gypsy/Gypsy	del ex11/ del ex11
J-L	Gypsy	0,2	2,2	6,7	8,9	8,1	98,8	1561,39	Gypsy/Gypsy	del ex11/ del ex11
J-D	Gypsy	18 days	0,7	5,5	n.a	7,2	102	1445,2	Gypsy/Gypsy	del ex11/ del ex11
GMH	IT- ES	24 hours	2,0	9,8	5	8,5	60	102,69	**347A / 1232T**	Arg166Gln/ Gly411Val
RJ	IT-IT	0,6	3,5	7,6	17,8	7,8	63	1012,67	1483A / 1518A	Ala 495Thr/ Val506Ile
VJA	ES-ES	0,4	3,8	3,4	20	7,6	22,2	2915,99	1594 T/**364-369 GGCTCC dupl.**	Arg532Trp/ Gly122-Ser123 dupl
SAM	Gypsy	2,4	2,9	7,9	23,2	11,5	80	785,08	Gypsy/Gypsy	del ex11/ del ex11
CK	Gypsy	0,3	No	10,7	3,2			1800	Gypsy/Gypsy	del ex11/ del ex11
CB	ES-ES	0,4	No	6,9	14			475,66	**1021A /** 1456T	Gly341Ser/ Arg486Trp

IT : Italian, ES: Spanish, SF : Serum Ferritin, n.a : not available; new mutations are reported in bold.

Methods: Eleven patients (4 males, 7 females) from 9 families, with a median age of admission 0.2 yrs (range 0-2.4yrs) were studied. Seven patients were of Gypsy ethnic group and consanguinity of parents was declared. The remaining 4 patients had Italian or Spanish origin. Haematological parameters and red cell enzyme activity were determined according to standard methods. The entire coding region and promoter of the *PK-LR* gene were amplified by PCR and automatically sequenced.
Results: The most important clinical and haematologic parameters and the results of the molecular characterization are reported in Table 1. All the patients displayed a severe clinical picture at birth with hyperbilirubinemia and need of phototherapy, and 7 required exchange transfusion. Because of the high transfusion requirement (median 15/year, range 6-29) , 9 patients were splenectomised (median age at splenectomy 2.2, range 8 months- 4 yrs). Splenectomy did not lead to normalization of anaemia, but resulted in stabilisation of the haemoglobin to slightly higher levels in all patients but one (median Hb increase 1.8 g/dL, range 0.2-4.2) with a drastic reduction of transfusion requirement (median 2/year, range 1-4). All patients but one had increased serum ferritin levels (median SF 1013 ng/ml), and 6 underwent chelation therapy. As typically reported in PK deficiency, a conspicuous rise of reticulocytes after splenectomy was observed. Nine different mutations were found in *PK-LR* gene, four of them never described before: three missense mutations (Ala495Thr, Val506Ile Gly341Ser) and a 6 nt duplication (c.364_369 ins GGCTCC), resulting in the in-frame duplication of amino acids Gly122-Ser123. In all the patients of Gipsy origin we confirmed the presence at homozygous level of mutation c.1437-518_1618+440 del 1149 bp (already reported as "Gypsy mutation").
Summary / Conclusion: This is the first comprehensive report of molecular characterization in PK deficiency from Argentina. Four novel mutations were identified in the patients of Italian and Spanish origin. For the first time the clinical and haematological parameters of a large group of Gypsy subjects were described.

B1819

HB RIAD/LAUSANNE, A NEW B-VARIANT RESULTING FROM A DOUBLE MUTATION TGC >TCT, (ALA>LEU), AT CODON 71. IDENTIFICATION BY CLONING AND SEQUENCING

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Background: Four different mutations have been previously described at the amino acid position 71 of human beta-globin gene (initiation codon included): Hb Abington (c.211G>C, p.Ala71Pro), Hb Seattle (c.212C>A, p.Ala71Asp), Hb Hershey (c.212C>G, p.Ala71Gly) and Hb Marineo (c.212C>T, pAla71Val).
Aims: The aim of this study was to identify a new variant resulting in an abnormal HPLC pattern, never described up to date and with a normal isoelectric focusing (IEF) of the hemolysate. Preliminary molecular analysis localised this variant to codon 71. This abnormal Hb was found in a Saudi Arabian patient from Riad
Methods: A 26 year old woman was investigated because of slight anemia: Hb 118 g/L; MCV 96.7 g/l; MCHC 311 g/L. WBC and platelets count was normal. An hemoglobinopathy was investigated by IEF and HPLC of the hemolysate as well as by gel agar electrophoresis at acidic pH. An α-globin variant was looked by sequencing both α2 and α1-globin genes. A β-globin variant was looked by sequencing of β-globin. Sequencing was performed by classical capillary Sanger sequencing.
Results: No abnormal Hb was found in IEF. In HPLC we found 43% of HbA and 43% of an abnormal Hb migrating adjacent to HbA. This fraction has never been described before and was not separated by pH acidic electrophoresis. Sequencing of α-globin genes was normal. Sequencing of β-globin made in evidence two mutations at positions c.211G>C and c.212C>T in an heterozygous state. As blood from the parents was not available and in order to rule out that each mutation is located on different chromosome, we performed cloning of the first PCR product using pCR™4-TOPO TA vectors (Invitrogen). 8 colonies were analysed by sequencing of the insert. We found that 5 were positive for both mutations while 3 were wild type for the β-globin exon 2 region.
Summary / Conclusion: Our molecular studies made in evidence a new β-globin variant resulting from a double mutation at codon 71 leading to a substitution of Alanine to Leucine in a heterozygous state. Taking into consideration the already described variants at codon 71 (see background) our patient combines in the same codon the two already described mutations c.211G>C and c.212C>T. This raises the question concerning the genesis of this new hemoglobine: Is this new variant the result of a crossing over of the previous Hb Abington and Hb Marineo between nucleotides 211 and 212? Studies of the parents and analysis of further β-globin genes of the Riad region are needed to check this hypothesis or to see if this new Hb is endemic. Because the percentage of this variant equals the percentage of HbA in HPLC analysis and because the Hb value is in the lower limit of the normal for a young lady, we postulate that this variant is asymptomatic. This new variant is called Riad/Lausanne from the origin of the patient and from the location of the identification.

B1820

EFFECT OF RHUEPO THERAPY ON LONG-TERM SURVIVAL OF ANEMIC CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA, UNDERGOING CHEMOTHERAPY

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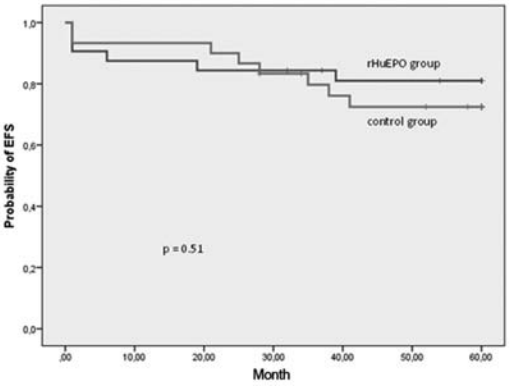
Background: Anemia is one of the most frequent problems during treatment of children with acute lymphoblastic leukemia (ALL). Up to now recommendations for use of erythropoiesis stimulating agents (ESA) in children with the malignant disease (MD) are absent. There are only several publications, which have reported about safety and efficiency of the option in correction of anemia in ALL children, undergoing chemotherapy. However serious contradictions in a question of impact of ESA treatment on survival of adult patients with MD are available. We analyzed long-term survival of ALL children with chemotherapy-induced anemia, who recieved ESA treatment during phase of intensive chemotherapy.
Aims: To estimate 5-year event-free survival (EFS), relapse-free survival (RFS) and overall survival (OS) in chemotherapy-induced anemia children with ALL, treated with rHuEPO.
Methods: Sixty two patients undergoing chemotherapy according to the program ALL BFM-90m were enrolled in the clinical trial. Thirty two children received rHuEPO (epoetin-alfa) throughout an intensive phase of chemotherapy (mean duration was7,6±0,17 month) in dosage of 200 IU/kg subcutaneously 3 times a week or 600 IU/kg intravenously weekly. Thirty children comparable on age and clinical manifestations were controls (without rHuEPO). Kaplan-Meier analyses were used to compare the EFS, RFS and OS.
Results: At the minimum term of supervision more than 5 years 27 (84,4%) patients in rHuEPO treated group and 22 (73,3%) patients in control group were in complete remission (P=0,347).The 5-year EFS for patients treated with rHuEPO was 81±4% compared to 73,3%±3% in control group (P=0,513). Increasing RFS in rHuEPO treated group in comparison with control was noted (93±3% and 79±4% respectively), however difference wasn`t significant (P=0,130). Probability of 5-year EFS of children with ALL in rHuEPO-group and control group shown on the figure.
Summary / Conclusion: Long-term survival analysis didn't reveal negative effect of rHuEPO treatment on survival of ALL children with chemotherapy-induced anemia, undergoing chemotherapy.



B1821

EPIDEMIOLOGICAL SURVEY OF ALPHA-THALASSAEMIA SYNDROMES IN NORTHERN GREECE

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Background: Greece is a Mediterranean country with a high frequency of thalassaemia and haemoglobinopathies. The α-Thalassaemia syndromes are due to mutations of the linked pair of α globin genes on chromosome 16 and result in imbalance in the production of α- and β- globins. Clinically they range from a lethal condition (hydrops fetalis) to a silent carrier state of no hematologic significance. Hb H disease is the cinical significant form of α-thalassaemia syndromes with a phenotypic variation due to the different molecular basis.
Aims: The aim of the study was to investigate the incidence of α-Thalassaemia syndromes in Northern Greece
Methods: The carrier identification is carried out by a standard scheme which includs CBC and red cell indices, a Cation Exchange HPLC variant system to determine HbA, HbA2 and HbF levels. Hb H identification is made by electrophoretic techniques. Hb H inclusion bodies after incubation and serum ferritin levels are also investigated. The molecular basis of Hb H disease was investigated in some cases and in heterozygous carriers in cases of genetic counseling.
Results: A total of 88003 subjects were screened for heamoglobinopathy in our unit from 1986 to 2011 at the regions of Central and Western Macedonia in Northern Greece with a population of around 2.5 million. We found 1390 heterozygous carriers of α-thalassaemia, (1,5% of the population screened). From 1986 to 1999 hemoglobin biosynthesis was performed in cases of genetic counseling. Since 2000 molecular tests are mostly used for the identification of α mutations in order to consult couples at risk. 62 heterozygous carriers of α- thalassaemia had had molecular identification due to genetic counseling. 65% (40) carried the –α3,7/ αα, 12,9%(8) carried the mutation –med/αα, and 9,7%(6) had the mutation α2pPoly A. We found 9 heterozygous carries of both α and β thalassaemia, 2 heteroygous carriers of Sickle cell and α- thalassaemia, 1 heterozygote for both α and δ thalassaemia, 1 heterozygote for both α and E thalassaemia, all DNA tested. Moreover we found 28 patients with HbH disease (14 males and 14 females). The most prevalent genotype was the -α 3.7/—med, (5 cases), 2 cases of –α3,7/-α5,2, 1 case of –med/α Poly A, 1 case of –α3,7/Hb Adana. 10 patients (36%) were under the age of 14. 7 patients (25%) were transfused occasionally (3 due to pregnancy, 2 due to infection, 1 due to surgery and 1 due to severe haemolytic crisis at the age of 10). 17 patients (61%) presented splenomegaly and only one was splenectomized. Hepatomegaly was present in 5 (18%). 4 patients had chololithiasis and 2 of them underwent cholocystectomy. Half of the patients have an annual follow up, 25% a rare follow up, 18% have no follow up at all and 7% come to our unit 3 times a year. The phenotype suggests that HbH disease in Greece has in general a moderate presentation.
Summary / Conclusion: The molecular characterization of the patients with HbH disease is useful for the prediction of the clinical outcome and the genetic counselling of the couples at risk.

B1822

SCREENING PARADIGM FOR B-THALASSEMIA CARRIERS; FROM CLINICAL TO MOLECULAR

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Background: β thalassemia (β-thal) represents a major health problem worldwide and particularly in Egypt. Its prevention, when compared to treatment, is found to be cost-effective, possible and practical. This work aimed to evaluate the effectiveness of diagnostic techniques in detection of the β-thalassemia carrier state.
Aims: This work aims to evaluate the effectiveness of automated HPLC followed by PCR in screening for β-thal carriers before nationally adopting a premarital screening strategy for β-thal carriers.
Methods: The present work included 1627 child and adolescent of both sexes presenting as outpatients to clinics of Ain-Shams University Hospitals during period from 1/11/2009 to 30/6/2010. In the first phase microcytic hypochromic for age CBCs were selected. In the second phase, Iron profile and HPLC testing were performed. Molecular characterization by PCR reverse hybridization for detection of 22 common β-globin gene mutations was done as a final step.
Results: Clinical characteristics of the studied population The population selected for having microcytosis & hypochrom ia was 280/1627 cases (17.2%). It comprised representative groups from infancy to young adults (30.7% infant, 21,8% young child, 21.8% older child, 9.6% adolescent and 16.1% young adult) including both males (64.3%) & females 35.7%). First & second phases of screening for β-TT; Phenotypic diagnoses There were 14 possible causes or phenotypes for the microcytic hypochromic indices based on clinical and hematological parameters including CBC, iron profile and HPLC. 44.6% (125 cases) were suspected to be βTT by CBC, 35.4% (99 cases) were suspected to be βTT after iron profile & HPLC. Iron deficiency anemia was the commonest cause of microcytosis and hypochromia (38.6%) followed by βTT (35.4%) (21.8% had HbA_2 >4% & were considered classic β-TT and the rest were suspected β-TT but required confirmation by genotyping). Final Phase; Genotypic confirmation of the thalassemia carrier state - In our study, 74% (37/50) of the genotyped cases exhibited presence of β-thalassemia mutations. The commonest four mutations were; IVS 1.6 (27%, 10/37 cases) followed by IVS 1.110 mutation and IVS 1.1 mutation (21.6%, 8/37 cases for each) followed by IVS 2.745 mutation (13.5%, 5/37 cases). Four cases were homozygous for IVS 1.6 mutation, one case was homozygous for IVS 2.745 mutation and one case was compound heterozygous for IVS 1.110 mutation and IVS 1.6 mutation. The commonest four genotypes constituted about (83.8%, 31/37 cases), followed by other less frequent mutations as IVS 2.848 and codon 5 (5.4%, 2/37 cases for each) then IVS 2.1 mutation, -87 mutation and codon 39 mutation (2.7%, 1/37 case for each). Diagnostic performance of hematologic parameters in screening for βTT. By utilizing the Receiver Operating Characterestic (ROC) curve HbA >3.5% provided 100% sensitivity, 70% specificity, 75% PPV, 100% NPV and accuracy of 70% to identify βTT and at a cut off 4%: it provided 97.4% sensitivity, 72.7% specificity, 92.6% PPV, 88.8% NPV and a diagnostic accuracy of 92%. As regards MCV, a cut off 61fl revealed 72% sensitivity, 20% specificity, 25% PPV, 71% NPV and accuracy of 40%.
Summary / Conclusion: In conclusion, HPLC proved to be a reliable screening tool for βTT to be followed by molecular characterization for selected cases in which HbA_2 lied in the interval from 3.6% to 4%**.** It is our belief that such a strategy would capture most of the βTT cases with minimum costs making it applicable for a national βTT screening programs.

B1823

EFFICACY OF RITUXIMAB IN AUTOIMMUNE HEMOLYTIC ANEMIA

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Background: Autoimmune hemolytic anemia (AIHA) affects males and females of all age groups, but more frequent in young adult females. Most patients respond initially to corticosteroids and intravenous immunoglobulin (IV IgG) as first line therapy. Splenectomy is recommended for those who do not respond well to first line treatment. For patients who do not respond to Splenectomy, several single immunosuppressant or immune modulator agents are tried. These include danazol, mycophenolate, colchicine, dapson, azathioprine, vincristine, cyclophosphamide, and Thrombopoitin receptor agonists such as Romiplostim and Eltrombopag.
Aims: The aim this non-randomized, prospective study is to explore the safety and efficacy of Rituximab as a new modality treatment for refractory AIHA.
Methods: Treatment with rituximab was explained to patients and an informed consent was obtained from a series of 15 patients (7 males and 8 females, aged 33 +I- 15 years), who agreed to take rituximab as an off label treatment. Their Hb level was 73 +I- 14 g/l (range 35-95). Each patient was given rituximab 375 mg/m2 intravenously weekly for four weeks, then monthly for four months, then maintenance every three months for two to three years.
Results: All patients showed a rapid and sustained response with significant rise in Hb level to 128±10.8 g/l within one to four months. Rituximab was tolerated very well without any major adverse effect during the follow up period, which was 27.6±21.8 months
Summary / Conclusion: Rituximab is probably highly effective in the treatment of patients with refractory AIHA with sustainable response. It could be considered for refractory patients prior to Splenectomy. However, larger studies are needed to verify the optimal utilization of rituximab in AIHA cases.

B1824

THE EFFECT ON WEIGHT, BODY MASS INDEX AND SUBCUTANEOUS ADIPOSE TISSUE FAT OF DIFFERENT CHELATING AGENT: A SURVEY FROM MIOT DATABASE

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Background: Several studies showed that the use of Deferiprone (DFP) alone and in combination with desferioxamine (DFO) was associated with both an increase in food intake and body weight. However, very few data are available on the long term effect of different chelation therapies on some nutritional parameters.
Aims: This study aimed to evaluate the effect of DFP versus DFO and deferasirox (DFX) in monotherapy on Body Mass Index (BMI) and body composition assessed by magnetic resonance imaging (MRI) .
Methods: Among the first 1506 thalassemia major (TM) patients enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) project, 792 performed a MRI follow up study at 18±3 months, according to the protocol. Patients under 18 years were excluded. We evaluated prospectively the 159 adult TM who had been received each monotherapy between the two MRI scans and three groups of patients were identified: 66 treated with DFO, 37 treated with DFP and 57 treated with DFX. BMI and Body Surface Area (BSA) were calculated from anthropometric parameters available according to the protocol. Subcutaneous adipose tissue (SAT) measurement was obtained by MRI (HIPPO FAT*)performing a retrospective analysis of selected SPGR imagesof homologous axial abdominal slices at baseline and follow up and evaluation of the SAT/Body size ratio.
Results: Basal weight and BMI in the tree groups are indicated in Table 1. At baseline 53/57 DFP patients were taking the considered therapy from > 6 months. The percentage of patients overweight or obese (BMI ≥ 25 Kg/m2) was significantly higher in the DFP (40.5%) group than in both DFO (18.2%) and DFX (16.1%) groups (P=0.013 and P=0.028, respectively). Due to technical reasons the analysis of the ratio SAT/body size was carried out on 64 (40%) patients (29 patients in DFO, 14 in DFP and 20 in DFX) and showed that at baseline patients under DFP treatment had the highest ratio SAT/body size (20.1±8.1) with respect to that observed in DFO (19.1±6.9) and in DFX (19.1±6.7) groups but the difference among three groups was not statistically significant. Table 1 shows also weight and BMI in the tree groups at follow up examination. Apart from a significant increase in weight (P=0.011) in DFO users, not other statistically significant differences at intra-treatment and inter-treatment prospective comparisons were observed. Similarly, at follow up, the changes in the ratio SAT/body were not of relevance among the three groups of patients.
Summary / Conclusion: Although the differences in the previous chelation treatments limit the applicability of our conclusions, the data at baseline suggest that in clinical practice, DFP users had highest weight, BMI and ratio SAT/body probably as a result of a steady and stable effect of the drug on nutritional parameters. Differently from what observed in DFO users, the prolonged (18±3 months) treatment with DFP seemed not to be associated to a further progressive increase in body weight, BMI and ratio SAT/body. Further longitudinal studies are needed to better clarify these effects of DFP treatment on naïve patients from the start of the therapy.

	DFO (N=66)	DFP (N=37)	DFX (N=56)	P
Basal MRI				
Weight (kg)	59.67 ± 10.45	63.02 ± 9.77	58.73 ± 12.89	0.182
BMI (Kg/m²)	22.53 ± 2.61	24.14 ± 2.88	22.78 ± 3.55	0.031
Follow up MRI				
Weight (Kg)	61.23 ± 11.30	62.57 ± 10.07	58.68 ± 13.05	0.256
BMI (kg/m²)	22.91 ± 3.00	23.78 ± 2.96	22.47 ± 3.64	0.163

B1825

LONGITUDINAL MRI AND SERUM FERRITIN MONITORING OF IRON OVERLOAD IN CHRONICALLY TRANSFUSED CHILDREN WITH HEMOGLOBINOPATHIES

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Background: Iron overload is an ineluctable complication in chronically transfused children with hemoglobinopathies. Accurate assessment of iron overload is therefore crucial to avoid iron related morbidity and mortality and to monitor chelation therapy. Pediatric studies are scarse and have provided contradictory results regarding correlation between serum ferritin levels and MRI imaging.
Aims: We investigated longitudinally the relationships between ferritin levels and hepatic and cardiac T2*MRI imaging in a cohort of chronically transfused children receiving chelation therapy.
Methods: Data regarding chronically transfused children, 30 affected with sickle cell anaemia (SCA) and 7 with thalassaemia major (TM), were collected between June 2005 and April 2010. Data included in particular annual numbers and cumulated volumes of transfusion and blood withdrawal, calculated iron intakes before first and before each annual MRI measurement, types and dosages of chelation therapy, and mean ferritin levels in the semester preceding MRI imaging. The same chelation protocol was applied in SCA and TM patients and used deferasirox.
Results: Sex ratio, age, and median duration of transfusion programs (5 years [2-14]) were comparable in both TM and SCA children. Median iron load received by each child was 0.54 mg/kg/day [0.27 -0.74]. It was not significantly different in SCA and TM patients (P=0.26). Median ferritin level in the whole population was 1550 mg/L [184-6204] and differed significantly between SCA (1917 mg/L [184-6204]) and TM patients (842 mg/L [266-5332]), P=0.002. A correlation (rho=0.27, P<0.001) was found between iron load and ferritin level.
Results of 73 hepatic T2*MRI were analysed. Severe hepatic iron overload was found in 38.3% cases (7 TM, 21 SCA) and was more frequent in TM patients when compared to SCA patients (P=0.037). A positive correlation was found between serum ferritin level and hepatic iron overload (rho = 0.57, P<0.001) in the whole population and in both SCA and TM groups. 55 cardiac T2* MRI measurements were analysed. No cardiac overload was found in the majority of cases. Iron overload was found moderate in 4 cases (5.4%) and severe in 1 case (1.8%), regarding exclusively TM patients. There was no correlation between serum ferritin levels and cardiac iron overload. Comparison of trends of ferritin levels and hepatic MRI results were analysed in 37 cases. In 19 cases trends were similar: the direction of changes of both ferritin and hepatic overload was congruent. Among them, 7 showed both improvement in ferritin levels and hepatic iron overload (3 TM, 4 SCA), 8 demonstrated aggravated results (3 TM, 5 SCA) and 4 showed (4 SCA) stable trends for both parameters. Conversely, opposite trends were observed in 3 cases, interestingly only in SCA patients: ferritin levels increased whilst hepatic overload decreased in one case, and the contrary in 2 cases. In all the other cases, analysis of trends did not allow conclusion. Comparison of trends of ferritin levels and cardiac MRI results were analysed in 19 cases. Similar trends were found in 5 (26%) cases: in 2 cases of TM patients both trends decreased, in 3 cases trends were stable (2 SCA, 1 TM). Opposite trends (concerning exclusively SCA patients) were observed in 5 cases (26%): In3, ferritin levels increased whilst MRI measurement improved; in 2 cases ferritin level decreased whilst MRI cardiac overload increased. In all the other cases, analysis of trends did not allow conclusion.
Summary / Conclusion: This study shows that iron overload is not controlled in all regularly transfused and chelated children with hemoglobinopathies. These findings emphasize the importance of MRI imaging for iron load assessment as ferritin trends may fault in both over or underestimating tissular iron load. Our results indicate in particular that data drawn from TM cohorts may not be extrapolated to SCA patients. This study argues for tailoring chelation therapy according to MRI results and not ferritin results.

B1826

RELATIONSHIP BETWEEN QT DISPERSION, PRO-BNP, T-WAVE ALTERNANS, HEART RATE VARIABILITY AND BODY IRON BURDEN DETECTED BY THE FERRITIN, HEART AND LIVER MRI IN ASYMPTOMATIC PATIENTS WITH THALASSEMIA MAJOR

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Background: The most important cause of mortality in patients is iron accumulation. Cardiac complications due to iron overload is the major cause of death in patients with thalassemia major. Increased QT dispersion and reduced heart rate variability has been reported to be associated with ventricular arrhythmias and sudden cardiac death.
Aims: Aim of the study is to evaulate the relationship between the non-invasive methods (heart rate variability, QT dispersion, T-wave alternansproBNP) that can be used to determine cardiac damage at early stagesand ferritin, heart and liver iron overload in patients with thalassemia major.
Methods: Thirty-one patients with thalassemia major, fifteen boys and sixteen girls (12-37 years old) were included in the study. By calculating the difference between the largest and the smallest corrected QT (QTc) on standard 12-lead ECG, QTc dispersion (QTcd) was found respectively. Limit value for QTcd was set as 50 ms. Automatically measured time-based heart rate variability parameters from 24-hour Holter recordings and serum ferritin values of the pateints were recorded. Heart and liver MRI was performed by 1.5 Tesla Philips device considered as T2 *in the Department of Radiology, Istanbul Faculty of Medicine. The iron overload indicators, ferritin levels, heart and liver MRI results, were compared with age, sex, heart rate variables (SDNN 24, SDANN1, SDNN1, RMSDD, pNN50) QTc dispersion (QTcd) and pro-BNP values. Statistical analysis was performed with SPSS program, Pearson's correlation test.
Results: The mean ferritin level of the patients was 2033±1970 ng / dL. There was not a correlation between cardiac and liver MRI. There was a significant relation between ferritin levels and liver iron overload. Ferritin was not related with cardiac iron detected by MRI with Pearson's correlation test. Cardiac iron overload was associated with age. In 3 patients with severe iron overload in the heart (cardiac T2 * values <10 ms), we found that the heart rate variables were associated with age, gender, proBNP and ferritin. Corrected QT dispersion was associated with age and ferritin (p <0.01). In 5 patients with moderate iron overload in the heart (cardiac T2 * values 10-20 ms), heart rate variables associated with age; corrected QT dispersion associated with ProBNP, and Liver MRI associated with ferritin and Aver HR (p <0.01). In 23 patients without iron overload in the heart (MRI T2 * values> 20 ms), Liver MRI was associated with ferritin. In 9 patients with hepatic iron < 5 mg, ferritin was associated with QTc, proBNP, TWA. Cardiac MRI was also associated with pNN50 and RMSDD (P<0.01). In 16 patients with liver MRI 5-20 mg; Cardiac MRI associated with age, LIC associated with ferritin (p <0.01). In 6 patients with severe liver iron overload (LIC> 20 mg), ProBNP was associated with Aver HR (p <0.05). LIC was assoociated with age. Cardiac iron overload was associated with sex (p <0.05).
Summary / Conclusion: Although ferritin shows the liver iron load, it is not enough to assess cardiac state. Cardiac iron overload may seen in patients with low ferritin levels. In patients without any cardiac symptoms, to predict the risks of lethal arrhythmias, sudden cardiac death and heart failure due to iron toxicity, should be performed liver MRI, pro-BNP, QTc dispersion and HRV at regular intervals without considering ferritin levels.

B1827

STUDY OF THE ALTERATIONS OF PLATELET FUNCTIONS IN CHILDREN AND ADOLESCENTS WITH IRON DEFICIENCY ANEMIA AND RESPONSE TO THERAPY

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Background: Iron deficiency anemia (IDA) is the commonest type of malnutrition. Several changes in platelets in IDA have been reported, so, a relationship between iron metabolism and thrombopoiesis should be considered**.**
Aims: to study the alterations of platelet functions in patients with IDA by assessment of platelet aggregation with collagen, ADP and ristocetin and by measuring platelet function analyzer (PFA-100) closure time together with the effect of iron therapy on the same tests.
Methods: A case control study was conducted in Ain Shams University Children's hospital in the period from June 2011 to June 2012 including 20 patients with confirmed IDAand 20 healthy age and sex matched control. Their ages ranges between 2-18 years.Patientswith coexisting chronic disease,decompensated severe IDA, thrombocytopenia and inherited platelet function defect were excluded. All patients and control were subjected to history takingwith emphasis on socioeconomic standard according to Park classification, Dietetic history of iron intake with Food frequency questionnaire and Diet Analysis Program1995*;* Symptoms suggestive of anemia, history of blood transfusion, history of bleeding tendency and drug history.Laboratory analysis included complete blood count to assess the erythrocyte indices(Hb, MCV, MCH, MCHC, RDW) and platelet parameters including(count ,size anddistribution width), assessment of iron status by measuring serum iron, total iron binding capacity and ferritin, assessment of platelet functions by PFA-100 closure time and platelet aggregation with collagen, ADP and ristocetin. Patients with IDA were treated by oral iron therapy 6mg/kg/day of ferrous sulphate for 12 weeks. Post therapeutic assessment of patients clinical improvement including bleeding, and laboratory iron status,erythrocyte indices , platelet functions after 48 hours of discontinuation of oral iron therapy.
Results: Mean age of IDA patients was 5.7±4.2 years compared 7.6±4years in control group (P>0.05). Bleeding manifestations were more common in patients group. One patient was diagnosed as Glanzmannthrombasthenia and excluded from statistical analysis. Mean PFA-100 closure times (with epinephrine) were significantly longer in patients(179.1±86.4sec)compared to control group(115±28.5sec)(P <0.05). Platelet aggregation by ADP(38.1±22.2%), epinephrine(19.7±14.2%) and ristocetin(58.8±21.4%)were significantly reduced in patients compared to control (62.7±6.2, 63.3±6.9, 73.8±8.3 respectively)

(P<0.001). After treatment all hemoglobin parameters and iron profiles were improved, platelet aggregation tests induced by ADP(64.78±18.25%), and epinephrine(55.47±24%) were significantly increased in patients with IDAcompared to before treatment(39.44±21.85%, 20.33±14.58%)(P<0.001).PFA 100closure time as well showed significant decreased after treatment(118.4±27.242) compared to before treatment(186.2±90.35) (P<0.05). A negative correlation between platelet aggregation induced by ADP and mean values of serum ferritin before treatment (r=0.042, P<0.05) and)was detected.
Summary / Conclusion: A mutual effect is considered between iron deficiency and platelet functions. Sublte bleeding manifestations can occur in patients with IDA with delay in platelet aggregation and prolongation in PFA-100 closure timeswhich can be reversed by iron therapy.Iron containing enzymes of the platelets including cycloxygenase and lipoxygenase might play a role.

B1828
SUCCESSFUL CHELATION THERAPY WITH THE COMBINATION OF DEFERASIROX AND DEFERIPRONE (3 PATIENTS) AND DEFEROXAMINE AND DEFERASIROX (2 PATIENTS).
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Background: Iron overload occurs commonly in patients with congenital anemias mainly as a result of the frequent blood transfusions. Without adequate iron chelation therapy, almost all patients will accumulate iron levels that are toxic to the heart, liver and endocrine glands.
Deferoxamine (DFO) has been the standard of care for transfusional iron overload for more than 40 years, although subcutaneous infusion negatively affects patient compliance. The oral iron chelators, deferiprone (DFP) and deferasirox (DFX) are effective in reducing iron burden, while at the same time they improve compliance and patients´quality of life.
Extensive long-term experience has shown that combined chelation with DFP and DFO improves cardiac function and reduces cardiac mortality. However, there are very limited data in the literature for effective combinations of two oral chelators. Two chelators can be used either sequentially (on different days) or in combination (both given on the same day).
Aims: To evaluate combined therapy with more than one chelator in 5 patients with congenital anemias transfusion-dependent and refractory severe iron overload.
Methods: Demographic data, diagnosis, prior chelation therapy, combined chelation regimens and results are listed in Table I.
Refractory iron overload was defined if ferritin levels>2000 ng/mL and/or Liver Iron Concentration (LIC) > 10 mg/g and/or cardiac T2*<20 mseg.
Results: Efficacy: After a media time of 11 month treatment of combined therapy (4-16m), ferritine level had decreased in 4 patients, from a media value of 6196 ng/mL (2600-11948) to 2808 ng/mL (1872-5360) (see Table 1).
Security: All combination regimens were very well tolerated and no kidney function alterations, arthralgias, skin reactions, neutropenias, gastrointestinal adverse effects or other safety problems were documented in none of the 5 patients. The adverse effects disappearance is probably related to the lower necessary dose of each chelator drug when prescribing it combined with other chelator drug. Patients compliance was excellent.

	DIAGNOSE	PRIOR THERAPY	PRIOR ADVERSE EFFECTS	COMBINED CHELATION	MONTHS OF THERAPY	FERRITINE ng/ml	
						BASELINE	Feb 2013
Patient 1 female/51y	hb SS Refractory iron overload LIC 19mg/g	DFO DFP	audiologic, ophthalmologic	DFP 75mg/k/d + DFX 10mg/k/(2d/w)	16	11948	5360
Patient 2 female/23y	Pyruvate Kinase deficiency Refractory iron overload LIC18mg/g	DFO DFP	gastrointestinal	DFP 75mg/k/j + DFX 10-20mg/k/d	6	2600	1872
Patient 3 male/32y	Thalassemia major Myocardial siderosis T2 *16ms	DFO + DFP DFX	gastrointestinal	DFP 75mg/k (4d/w) + DFX 30mg/k (3d/w) alternating days	4	452	
Patient 4 female/36y	Thalassemia major Refractory iron overload LIC 18mg/g	DFO DFP		DFO 30mg/k (3d/w) + DFX 30mg/k/d	18	7450	2016
Patient 5 female/35y	Thalassemia major Refractory iron overload LIC 10mg/g	DFO DFP	gastrointestinal	DFO 40mg/k (4d/w) + DFX20mg/k (3d/w) alternating days	12	2786	1964

Summary / Conclusion: The use of combination therapies for overcoming toxicity and improving efficacy over the use of single agents has been estab lished in several patiens. The combination regimen was very well tolerated and no adverse events were documented. Our patients were treated with two drugs simultaneously or in a daily alternating regimen and the combination was always very efficacious and very safe for all the patients. Larger studies are needed to know if dual-chela tor therapy at different schedules, may improve compliance and enhance iron removal if an additive or synergistic effect occurs.

B1829
PREGNANCY AND SICKLE CELL DISEASE: A SINGLE GREEK CENTER EXPERIENCE
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Background: Pregnancy in women with sickle cell disease is considered to be a challenging condition. Pain crises become more frequent during pregnancy, particularly in the third trimester. A pregnant woman with sickle cell disease is more likely to have a miscarriage, preterm labor or a low-birth-weight baby.
Aims: In the present retrospective study we report on the pregnancy course and outcome in women with sickle cell disease being treated in our hospital.
Methods: A retrospective study of the medical records from 74 sickle cell women older than 18 years (57 Hb S-Beta and 17 Hb SS) revealed 25 pregnancies characterized by 12 Hb S-Beta and 3 Hb SS haemoglobinopathy. The course and outcome of pregnancy including pain episodes and transfusions, the types of labor and delivery as well as the clinical characteristics of the neonates were evaluated.
Results: All the women were in a month scheduled come to our unit for exams during pregnancy. None of them received hydroxyurea for six months before pregnancy. Overall, in twelve Hb S-Beta haemoglobinopathy women with a maternal age between 24-36 years old, we recorded sixteen successful pregnancies and five spontaneous abortions. Conception was spontaneous in all pregnancies. All but one woman (91%) suffered from more than one pain episodes during pregnancy, were hospitalized, received analgesics therapy and were transfused when their haemoglobin level dropped to 7-8 gr/dl. Five women (41,6%) gave birth by Caesarean section. The weight of the neonates was between 2600-3200 gr. Only one neonate was pre-term due to premature rupture of membranes. Among three Hb SS haemoglobinopathy women with a maternal age between 32-36 years old, we recorded four successful pregnancies. Only one woman experienced pain episodes, hospitalized and received transfusion during pregnancy. Conception was also spontaneous. The average weight of the neonates was normal.
Summary / Conclusion: Our study suggests that pregnancy is safe and usually has a favorable outcome under close monitoring. The interesting in our study is that the women with HbS-Beta haemoglobinopathy present more complications during pregnancy than the Hb SS women despite the fact that Hb SS type is the most severe disease. The small sample size in the current study precludes any definitive conclusions.

B1830
FACTORS AFFECTING ANEMIA IN NORTHEAST THAI VEGANS
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Background: Practicing vegan lifestyle for a long time may increase the risk of anemia due to micronutrient deficiencies, especially vitamin B12 deficiency and iron deficiency (ID).
Aims: To investigate factors attributed to anemia among northeast-Thai vegans.
Methods: A cross-sectional study was conducted at a vegan community in northeast Thailand. A total of 260 vegans (121 males and 139 females within the age range from 5 to 85 years) participated. The duration of practicing a vegan lifestyle varied from 3 months for young children to more than 30 years for the elderly. All participants were healthy based on their physical appearance and self-report. Informed consent was obtained from all participants including the guardians of all children and adolescents aged below 18 years. Hemoglobin (Hb) concentration and red blood cell (RBC) parameters were measured using an automated blood cell counter. Serum ferritin (SF) and vitamin B12 levels were determined by chemiluminescent immunoassay. Thalassemia and hemoglobinopathies were identified using standard methods including DNA analysis for a-thalassemia.
Results: Based on the WHO criteria adjusted for age and sex, 140 out of 260 (53.9%) vegans were anemic, 62 (23.9%) had ID (SF < 15 ng/ml), and 117 (45%) had vitamin B12deficiency (B12 < 160 pg/ml). Thalassemia and hemoglobinopathies were detected in 51.2 % of participants. Calculating the proportions by age revealed that 36.7% of adolescents (age 13-19 years) and 36.2% of reproductive age participants (age 20-45 years) had ID. The proportions of vitamin B12 deficiency of 54.4% and 50% in adolescents (age 13-19 years) and reproductive age (age 20-45 years) were significantly higher than in the other age groups. The highest proportion of anemia of 73.7% was found in the elderly (age > 60 years), and 35.7% of them had anemia of unknown causes. Based on a multiple logistic regression analysis, factors affecting anemia among vegans included age, sex, ID and thalassemia. No significant association was obtained for vitamin B12 status and the duration of practicing a vegan lifestyle.
Summary / Conclusion: Adolescents and reproductive age vegans are most

vulnerable not only ID but also vitamin B12 deficiency. Though participants were apparently healthy at the time of investigation, iron and vitamin B12 supplementation as well as modification of food consumption patterns might be necessary to prevent the adverse clinical outcomes due to a long term negative balance of iron and vitamin B12 status.

B1831
RENAL DYSFUNCTION MONITORING IN ADULT BETA-THALASSEMIC PATIENTS
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Background: Iron deposition and organ failure are the most important cause of mortality and morbidity in patients with beta-thalassemia. While cardiovascular and liver damage have been frequently described, little is known about renal involvement in patients with beta-thalassemia. The main underlying cause of renal damage are chronic anemia, iron overload and chelating therapies.
Aims: The aim of this study is to evaluate renal dysfunction in adult beta-thalassemic patients with different chelating treatment.
Methods: We described the glomerular and tubular function of 64 adult beta-thalassemia (βT) patients (13 βT major, βT-M, and 51 βT intermediate, βT-I) in comparison with age matched healthy control group (25). For each patients and healthy control urinary samples were collected and analyzed (three times monthly) for protein, urine osmolality, urobilinogen, hemoglobin and NAG activity, whilst blood samples were analyzed for complete blood count, blood urea nitrogen, serum creatinine, electrolytes uric acid, albumin, total cholesterol, calcium/phosphate ratio (C/P), beta2-microglobulin, and ferritin. Patients were divided into 5 groups depending on the chelating therapy they received: group I with deferoxamine (DFO) and deferiprone (P) chelation (n=7, 2 βT-M and 5 βT-I), group II with deferasirox (E) chelation (n=20, 8 βT-M and 12 βT-I), group III with DFO chelation (n=11, 1 βT-M and 10 βT-I), group IV with P chelation (n=5, 2 βT-M and 3 βT-I) and group V without any chelation therapy. The patients included in the study had either tubular dysfunction or glomerular dysfunction.
Results: Data demonstrated that glomerular renal dysfunction is a common complication (52%) of βT-M and βT-I, independently from the chelation therapy. Moreover, renal tubular dysfunction represents the majority of renal damage and NAG activity evaluation allows to detect early renal tubular damage since the alteration of its activity precedes other markers of renal damage. Noteworthy, tubular injure depends on chelating therapy: patients receiving DFO and deferiprone (43%) or deferoxamine alone (36%) are less damaged than patients receiving deferasirox (75%) or deferiprone (80%) alone.
Summary / Conclusion: Renal tubular dysfunction represents the majority of renal damage in thalassemic patients and the monitoring of this renal dysfunction my be made by NAG activity evaluation since the alteration of NAG activity precedes other markers of renal damage.

B1832
MEDICAL RADIATION EXPOSURE IN SICKLE CELL DISEASE PATIENTS WITH FREQUENT HOSPITAL ATTENDANCE FOR PAINFUL CRISES
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Background: Patients with Sickle cell disease (SCD) experience frequent episodes of painful vaso-occlusive crises, often requiring emergency department (ED) attendance, involving radiographic imaging such as chest x-rays (CXR), skeletal x-rays and CT scans. Many SCD patients frequently attending our ED are young adults. Due to the increased risk of malignancy from medical radiation exposure, we analysed the numbers of radiographic studies performed and the resulting radiation exposure in this group. To our knowledge, this is the first such study in adults with SCD.
Aims: We evaluated the number of ED attendances and radiographic studies performed for young adult SCD patients, assessing radiation exposure and investigating strategies to minimise this.
Methods: We reviewed the electronic patient records for 11 adult SCD patients who frequently attend ED for painful crises. We determined the number of hospital admissions and number and type of radiographic studies performed per patient from 2007 to January 2013. We analysed the maximum numbers of studies performed in 1 or 12 months per patient and calculated the average rate of CXR exposure to allow comparison despite different follow-up durations. We also calculated the total radiation dose received.
Results: 11 patients were evaluated, aged 19-37 years (mean 25), with 840-2112 days follow-up (mean 1703 days). 62.5% patients were female and 37.5% male, 73% patients had HbSS SCD, 18% HbSC and 9% HbS/beta-thalasseamia. Hospital admissions during the study period ranged from 11-42 (mean 22), with a maximum of 18 in any 12 month period. A total of 377 radiographic studies were performed in the study period (range 13-64 per patient); most commonly CXRs, totalling 304 (mean 27.6 per patient, range of 8-58). Other studies included AXRs (6) and skeletal films (65), including spinal, hip, shoulder and extremity. The highest numbers of CXRs for one patient in a 1 and 12 month period were 6 (mean 3.1) and 31 (mean 11.5) respectively. This corresponded to an average CXR rate of 6.2 per year (range 1.6-13.1) and 0.5 per month (range 0.13-1.1), giving an exposure rate of 0.124 mSv per year (range 0.032 - 0.262). The maximum radiation dose received by one patient from plain x-ray studies was 0.62mSv in a year (0.12 mSv in a month). The average total exposure over the study period was 0.55mSv (range 0.16-1.16). We extrapolated the expected CXR and radiation exposure over 10 years as 62 CXRs and 6.2mSv per patient.
Summary / Conclusion: Patients with SCD who experience frequent painful crises are exposed to increased medical radiation , particularly from CXRs. Many of these patients are young adults and females, and iatrogenic risk of malignancy is increased in this population therefore it's essential to limit this exposure. If imaging continues at the current average rate, patients will be exposed to >6mSv in 10 years from CXRs alone (approximately 1 CT scan). Currently, there are no set criteria for appropriate imaging requesting. Principles such as reviewing recent imaging, only requesting CXRs in the presence of chest signs, symptoms or fever and carefully considering the indication for skeletal imaging (rarely of diagnostic benefit in early sickle bone complications) could help reduce radiation exposure but can be easily forgotten in a busy ED.

B1833
CHALLENGES AND IMPACT OF INTRODUCING AN INTEGRATED CLINICAL PATHWAY AT THALASSEMIA CENTRE – DUBAI
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Background: Dubai thalassemia centre was established in 1995 to meet the increasing needs of patients with β-Thalassemia major in UAE. In May 2010 Thalassemia centre has developed the first integrated clinical pathway (ICP) to standardize care and to facilitate the use of guidelines. The authors believe that this is the first report of such an occurrence.
Aims: To describe the challenges and the impact of introducing an ICP to guide the care of the chronically transfused β-Thalassemia major patients.
Methods: We began in January 2010 with a team being created to investigate and adapt a methodology that meets the specific needs of the centre. Series of educational and orientation sessions involving nurses and physicians were held. Chronic blood transfusion program was selected being high volume, high risk and high cost process. The chosen process of care was reviewed against the guidelines. The various aspects in the process that were challenging and needed improvement or monitoring were included. Patients' education and medication compliance monitoring were thoroughly stressed throughout the pathway. Prior to pathway implementation, staff roles were clearly defined and both physicians and nurses took part in the process. The pathway was finally implemented in May 2010 and was evaluated through variance analysis, patients' satisfaction, patients' awareness and staff's satisfaction. Variance analysis was the tool used to monitor variation in chronic care management. The data from 2010 through 2012 was analyzed.
Results: The ICP introduction was met with physician resistance. The format had to be acceptable to physicians and nurses and good enough to be used as permanent medical record. Another major challenge was developing a variance reporting tool that identifies appropriate variances and that is easy to understand and complete by the staff. Upon implementation, we were challenged by the huge number of variances being reported without knowing what level of variance is meaningful to our practice. We couldn't find a leading practice to help defining thresholds or ranges so we tweaked these ranges as appropriate to support proper analysis and decision-making. A variance of 3% or less occurrence was considered minor, while an occurrence of 15% or more was considered significant. Monthly analysis of variances was time consuming and not useful, so we decided to perform annual analysis. Patients' visits guided by the ICP went from 3171 in 2010 to 5639 in 2012. Significant variances with occurrence rate of 15% or more declined from 76.9% in 2010 to 16.8% in 2012. Reduction in significant variances occurrence was considered as an indicator of a more uniform care provision. Patients' satisfaction data in 2012 showed significant improvement in patients' satisfaction with counseling sessions and with the time spent with health care provider when compared to 2010; from 68% to 92% and from 87 % to 98% respectively. Patients' awareness of the disease and its complications has improved from 85% in 2010 to 95 % 2012. Staffs' satisfaction survey conducted at the end of 2010 revealed 98% satisfaction with the ICP.
Summary / Conclusion: This report has provided evidence that introducing an ICP to guide the care of β-Thalassemia major patients undergoing chronic blood transfusion may improve the quality of care and patient's satisfaction. Analyzing the impact of the ICP on patients' clinical outcomes is yet to be studies as well as discovering the root causes for the repeatable elements that lead to positive variances.

B1834

PARTNER TESTING FOR PREGNANT WOMEN AT RISK AND PRE-NATAL DIAGNOSIS OF SICKLE CELL DISEASE. A SINGLE CENTRE 5 YEAR EXPERIENCE.

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Background: Pregnant women suffering of sickle cell disease (SCD) or are carriers of the HbS gene or other haemoglobin variants that combined with HbS can give rise to the sickle cell phenotype, are at risk of having a child affected by SCD if their partner is also affected in a similar way. It is therefore essential for partners of such women to be tested. Despite this, the number of partners testd remains unsatisfactory. Pre-natal diagnosis (PND) can help couples decide the future of the pregnancy but the decision to proceed with it depends on the ethical, cultural and social background of the family.
Aims: To assess the level of paternal testing in pregnancies where the mother is at risk of having a child with SCD and proportion of "at risk" pregnancies proceeding to PND in a London area with high prevalence of SCD..
Methods: Retrospective analysis of maternity data at Homerton University Hospital over a five year period (2007-2012).
Results: There was a mean of 5536 bookings / year (range 5036 – 5909) (table). The mean number of women at risk every year was 445 (8%). The number of partners tested improved over the years but remains less than 50%: Only 49 partners of the 400 women-carriers (12%) were tested in 2007-2008 but this figure picked in 2010-2011 at 229 partners of 492 women-carriers (46.5%) and the latest from 2011-2012 was 186 partners of 417 women-carriers (44.6%). The pregnancies "at risk" identified after partner testing ranged from 16% to 24.3%. No prenatal diagnosis (PND) was attempted in 2007-2008. Subsequently more "at risk" pregnancies as identified after partner testing underwent PND with a peak of 36% in 2008-2009. The number of terminations of pregnancy (TOP) after PND ranged from 0 to 25%. The number of affected children as identified by PND ranged from 17% to 50%.
Summary / Conclusion: We find a relatively stable number of bookings / year in our institution with a stable number of women who are at risk of having an offspring affected by SCD (7.1 – 9.1%, mean 8%). Even though the number of partners tested in recent years has increased, it remains below the UK average as less than half of partners are tested. Several social, cultural or religious factors may hinder the process but a more rigorous approach in identifying partners and pursuing their timely testing is essential. An increasing number of couples at risk seek PND.

B1835

STUDY OF THE RENAL FUNCTIONS IN BETA (B)-THALASSEMIA (B-THALASSEMIA MAJOR, B-THALASSEMIA INTERMEDIA AND B–THALASSEMIA MINOR) AND COMPARISON WITH THE TOTAL ANTIOXIDANT CAPACITY

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Background: Beta-Thalassemia is a chronic disorder, characterized with defective hemoglobin synthesis and ineffective erythropoiesis, causing microcytic anemia. It is divided into three groups; thalassemia major (TM), thalassemia intermedia (TI) and thalassemia minor. The iron that accumulates in beta-thalassemia as a result of the increased iron absorption in the intestines, hemolysis and erythrocyte transfusions causes functional impairment in the liver, pancreas, and heart, as well as in the kidneys, particularly in the proximal tubules, and results in alterations in the concentrations of some substances in the urine and blood. Although the pathological effects on the organs including heart, endocrine glands and liver have been focused on till now, there are a limited number of studies concerning the kidneys, which are important organs of the body.
Aims: The aim of this study was to measure the renal functions and the antioxidant system established in the body against the oxidant substances in the β-thalassemia group and the healthy control group, and to compare the similarities and differences of the groups.
Methods: The study enrolled total 49 transfusion-dependent TM patients (23 boys, 26 girls), 18 TI patients (11 boys, 7 girls), and 51 thalassemia minor patients (28 boys, 23 girls), aged between 4-17 years. The control group consisted of age and sex matched 51 healthy individuals (29 boys, 22 girls). Informed consent form was obtained from the parents. Blood and urine samples were collected and hemogram parameters were studied, sodium, potassium, magnesium, phosphorus, uric acid, protein and creatinine were measured in serum and centrifuged urine samples; in addition, total antioxidant capacity (TAOC), cystatin-C were studied in serum samples, and retinol binding protein (RBP), α-1 microglobulin (α-1MG) and β-2 microglobulin (β-2MG) were studied in urine samples. The tubular reabsorption and excretion was calculated from the results obtained, and the glomerular filtration rate (GFR) was calculated using the Shwartz formula.
Results: FENa values in TM and TI groups were found higher compared with the thalassemia minor and the control groups. No differences were found between the groups in the urinary potassium excretion (FEK), urinary magnesium excretion (FEMg) and the tubular phosphorus re-absorption (TPR). The uric acid-GFR values were significantly higher in the TM group, compared to the thalassemia minor and control groups. RBP was significantly higher in the TM group compared to the control group, while the urinary β-2MG was significantly higher in the TM group compared to the thalassemia minor and the control groups. No differences was found between the groups in GFR, while the mean values of cystatin-C were similar in the thalassemia minor and control groups and in TM and TI groups respectively, and the values of TM and TI were higher compared to the other two groups. The urinary protein/creatinine ratio was found higher in the TI and TM groups. Additionally, a negative correlation between TAOC and FENa, and a positive correlation between ferritin and FENa was found. There was a negative correlation between α-1MG and TAOC, while there was a positive correlation between α-1MG and ferritin. Negative correlation was found between cystatin-C ,ferritin and TAOC.
Summary / Conclusion: In conclusion, both tubular and glomerular functional renal impairments can be found in varying levels in all three β-thalassemia groups. FENa can be considered as a marker of the tubular function in all the β-thalassemia types, uric acid-GFR value as an indicator of early tubulopathy in TM and TI, α-1MG and β-2MG as parameters strongly related with the oxidative damage with ferritin in TM and TI; and RBP can be considered as a parameter indicating the relation of the tubular functions with iron accumulation. However, TPR and GFR may not be good markers. Cystatin-C can be used as a perfect marker of the glomerular functions in all the β-thalassemia types. Urinary protein/creatinine ratio can be used to show the relation between the glomerular function and the iron accumulation in the TM and TI groups. Additionally, we have the opinion that studies in molecular levels could provide important data.

B1836

EVALUATION OF IMMUNE STATUS IN IRON DEFICIENCY ANEMIA

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Background: Zinc and iron deficiency significantly disturbs the structural and functional state of erythrocytes as well as of immunocompetent cells. In the recent years, a special significance is attached to the role of microelements status as a regulator of immunologic homeostasis. Development of iron deficiency aggravated with zinc insufficiency (critical level in serum equal to 12mmol/l) brings to deep disorders of immune system, cellular and humoral links of immunity with development of secondary immunodeficiency. It is well known that the iron and zinc disbalance regulates the activity of T and B-lymphocytes. In the process of proliferation and differentiation of lymphocytes the zinc containing enzyme ecto-5'-nucleotidase (e5N, CD73) takes part.
Aims: The aim of the research was to study the changes of immunoregulatory protective system in iron deficiency anemia aggravated by zinc insufficiency.
Methods: The research group consisted of female patients aged from 20 to 55 years. The average age of the patients was 37, 0 ±1, 65 years. The analysis was conducted between the control and two groups of patients with iron deficiency anemia with various degrees of zinc insufficiency (in group 1 the content of zinc in blood serum was <12mmol/l, in group 2 - >12mmol/l).
Results: We have found out a significant decrease in the e5N activity in membrane lymphocytes in group 2 patients (Zn<12mmol/l). It should be also noted, that acute decrease of immune index CD4/CD8 is typical for these patients.
The obtained results demonstrate presence of a positive correlation between T-cellular immunosuppression and activity of e5N (CD73) in case of development of iron deficiency anemia and zinc insufficiency.
Summary / Conclusion: New possible ways of regulation of ineffective erythropoiesis in iron deficiency anemia are discussed.

B1837

HIDDEN VITAMIN B12 DEFICIENCY

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Background: Mild to moderate vitamin B12 (Cobalamin) deficiency is common due to inadequate intake of animal products, gastro-intestinal disorders (including inefficient digestion, parietal cell disorders), systemic comorbidities such as autoimmune diseases, and medications including proton-pump inhibitors and metformin. In most cases, hematological values are not affected due to the wide range of normal values and to the presence of other hematological disorders such as thalassemia trait and co-existence of iron deficiency which prevent increase in MCV values beyond normal range. In most cases, patients suffer from vague, non-specific neurological and/or psychiatric symptoms such as fatigue and mode disturbance. For all of these reasons, vitamin B12 deficiency is commonly overlooked and the diagnosis is missed or delayed
Aims: The aim of this retrospective study was to find the prevalence of hidden

Vitamin B12 deficiency among patients with normal hematological finding
Methods: We reviewed Vitamin B12 results that were tested from 1st of January to the 30th of December 2012. The total number cases was 2698 patient
Results: we found 3 cases with severe B12 deficiency and megaloblastic anemia (B12 < 22 pmol/l), 380 patients (14%) with moderate B12 deficiency (B12 =45-140 pmol /l), and 2315 with normal or elevated B12 (145-1400 pmol/l). We compared B12 level, Hb and MCV between the 380 patients with Vitamin B12 deficiency (B12 45-140 pmol/l) 124 males and 256 females, aged 36.01±18.1 years and 418 patients with normal Vitamin B12 level (200-400 pmol/l),157 males and 261 females, aged 39.7 ±22 years. There was no difference in Hb or MCV between the two groups. However, there was a significant difference in Vitamin B12 values, 111.32±19.72 pmol/l versus 273.39±54.05 pmol/l. Despite normal Hb and MCV values in both groups, the difference in B12 level was significant, indicating that vitamin B12 deficiency may exist despite normal hematological parameters.
Summary / Conclusion: Moderate B12 deficiency is common without hematological; manifestations. Diagnosis is commonly missed or delayed. Therefore due to lack of awareness and clear symptoms and signs., physician needs to be aware of hidden B12 deficiency

B1838
PREVALENCE OF 25-HYDROXYVITAMIN D DEFICIENCY IN KOREAN PATIENTS WITH ANEMIA
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Background: Vitamin D deficiency is a very common health problem in Korea. Vitamin D is suggested to play an important role in non-skeletal functions, including cellular proliferation and differentiation, muscle function, immunity, and erythropoiesis. Recent studies have reported that vitamin D deficiency is associated with anemias of iron deficiency (IDA), chronic disease (ACD), and inflammation.
Aims: This study investigated the prevalence of vitamin D deficiency in Korean anemia patients and analyzed the association between vitamin D status and specific anemia subtypes.
Methods: We included 200 anemic patients (median 66 years, range 19~91 years) and 300 nonanemic controls (median 65 years, range 23~91 years). Anemia was defined according to WHO criteria. Serum 25-hydroxyvitamin D [25(OH)D] was measured using electrochemiluminescence immunoassay. Deficiency of 25(OH)D was defined as <20 ng/mL and severe deficiency was defined as <10 ng/mL. We compared serum 25(OH)D levels based on the presence and subtypes of anemia.
Results: There was 90% (179/200) and 87% (262/300) 25(OH)D deficiency in the anemic (median Hb, 9.7 g/dL) and nonanemic groups (median Hb 13.8, g/dL), respectively. Severe 25(OH)D deficiency was significantly higher in the anemic group than in the nonanemic group [43% (85/200) vs. 13.7% (41/300), *P*<0.0001]. The odds ratio for severe 25(OH)D deficiency in anemic patients was 4.67 (95% confidence interval, CI 3.03–7.20, *P*<0.0001). The 25(OH)D deficiency prevalence was not different between IDA and ACD groups. However, severe 25(OH)D deficiency was significantly higher in the ACD group than in the IDA group [50.8% (61/120) vs. 34.3% (24/70), *P*=0.03].
Summary / Conclusion: This study demonstrated that severe vitamin D deficiency is associated with anemia in Korea. Although vitamin D deficiency is also very common in nonanemic Koreans, anemia is related to a poor vitamin D-deficient status. Multiple factors, including poor nutritional status and the potential roles of vitamin D in inflammation and erythropoiesis may be considered.

B1839
ORAL IRON CHELATION ENHANCES PYRIDOXINE EFFECT IN ONE PATIENT WITH INHERITED SIDEROBLASTIC ANEMIA
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Background: Inherited Sideroblastic anaemia is a rare disease, characterized by decreased haem synthesis and mitochondrial iron overload, which are diagnosed by the presence of ringed sideroblasts in the bone marrow aspirate.
Aims: In our study we aim to evaluate the effect and safety of oral iron chelation in lowering the ferritin level and improving the response to pyridoxine treatment in a case of inherited sideroblastic anemia
Methods: Serum ferritin , Hb ,LFT,creatinine & urine protein were done as baseline and every month after initiation of oral iron chelation with deferasirox along with pyridoxine treatment for one year..The starting deferasirox dose was 10 or 20 mg/kg per day depending on baseline iron burden. Dose adjustments were permitted based on serum ferritin trends (rises of >=1000 µg/l on 2 visits or >2500 µg/l without decreasing trend) and reduced for elevated levels of creatinine, urinary protein and transaminases, ,in response to adverse events and drop of Hb level.
Results: An improvement to pyridoxine treatment noted and elevation of Hb level to 113g/L and drop of S ferritin to 1076ug/L compared to the baseline of Hb level of 80g/L and S ferritin level of 4000ug/L. In general, adverse events were mild and consistent with that documented throughout the registration studies of deferasirox. We noticed that the maximum enhancement of pyridoxine effect, in the form of higher Hb concentration ,seen with the lower dose of deferasirox 10mg/kg.
Summary / Conclusion: Inherited sideroblastic anaemia usually have hypochromic and microcytic red cells, reflecting a reduction of haem synthesis in the erythroid precursors. In those not requiring blood transfusions, transfusion-independent iron loading may occur, usually in adulthood, as in thalassemia intermedia because of ineffective erythropoiesis and of increased intestinal iron absorption. Iron depletion may be an effective treatment in inherited siderblastic anemia and can be undertaken after pyridoxine supplementation, when the haematological response is stable. Iron chelation with deferasirox proved to be a safe and effective means of substantially lowering ferritin levels in case of transfusion independent inherited sideroblastic anemia.

B1840
ROLE OF IRON DEFICIENCY IN CHILDREN WITH THROMBOTIC EVENTS.
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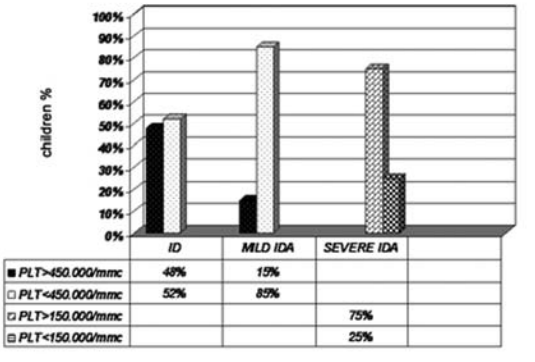
Background: Iron deficiency (ID) and iron deficiency anaemia (IDA)have been associated with increased risk of developing thrombotic events in both children and adults.
In children with ID reactive and often severe thrombocytosis was frequently observed even if the mechanisms causing thrombocytosis are not completely understood.
Aims: The aim of this study is to evaluate the effects of thrombocytosis due to ID or IDA on development of venous thromboembolism (VTE) in children and possible interaction with some genetic risk factors .
Methods: In the last 7 years we have studied 125 children, of both sexes aged between 5 and 15 years, affected by ID or IDA.
Diagnosis of ID and IDA was performed on the basis of WHO criteria for Hb, MCV, serum ferritin and transferrin saturation values. Secondary thrombocytosis was diagnosed at platelet counts ≥ 450.000/mmc, without infectious diseases, while thrombocytopenia at platelet counts ≤ 150.000/mmc.
Polymorphisms in MTHFR C-677-T, PT G20210A and G1691A FV Leiden have been also assayed.
Informed consent was obtained from parents. Principles outlined in the Declaration of Helsinki were followed. Procedures followed were in accordance with the ethical standards.
Results: In our cohort of patients 70 children had ID (Hb11.5±0.5g/dl), 35 mild IDA(Hb 9,5±0,2 g/dl) and 20 severe IDA(Hb 6.5±0.4g/dl). Furthermore our data showed that 48% of patients with ID had secondary thrombocytosis (PLT 650.000±110.000 /mmc) as well as 15% of children with mild IDA (PLT 520.000±55.000/mmc) while 25% of patients with severe IDA showed mild thrombocytopenia (PLT160.000±20.000/mmc) (Graph 1). Two female patients, aged 12 and 15 years respectively, with ID and thrombocytosis (PLT 620.000 and 580.000 /mmc), werereferred to us because they had developed VTE characterized by deep venous thrombosis (DVT) particularly saphaenous vein thrombosis, confirmed by Doppler ultrasonografy and magnetic resonance.
In both casesthe G1691A polymorphism in the Factor V Leiden gene (FV) in heterozygous form was detected.

graph 1 : Platelet count in children whit ID, mild IDA, severe IDA



Summary / Conclusion: Our data show that thrombocytosis is frequently associated with ID or mild IDA while severe IDA is associated with thrombocytopenia.. So normal iron levels are required to prevent thrombocytosis by inhibiting thrombopoiesis but a minimum amount of iron is required to maintain platelet production. Furthermore ID could cause increased oxidant stress with tendency to platelet aggregation. We observed 2 cases of VTE in adolescent females with secondary thrombocytosis associated with G1691A polymorphism of FV

Leiden in heterozygous form. Some genetic factors at homozygous and/or compound heterozygous state have been frequently associated with a higher risk of thromboembolic events. We suggest that the interaction between thrombocytosis secondary to ID and genetic risk factors, even at heterozygous state, may trigger hypercoagulability.

B1841

NEW INSIGHT ON IRON STUDY IN MYELODYSPLASIA

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Background: Hepcidin plays a pivotal role in iron homeostasis. It is predominantly produced by hepatocytes and inhibits iron release from macrophages and iron uptake by intestinal epithelial cells. Competitive-ELISA is the current method of first choice for the quantification of serum hepcidin, because of its low limit of detection, low costs, and high throughput.
Aims: This study aims to discuss the role of hepcidin in iron overload pathogenesis in MDS cases recently diagnosed.
Methods: Methods: The study included 21 recently diagnosed MDS cases and 13 healthy control individuals.
We used ELISA technique for hepcidin assay in blood. In this work, we chose newly diagnosed MDS cases within 6 months of symptomatology to limit impact of over-transfusion on hepcidin level. And to abolish effect of acute transfusion of hepcidin expression, samples were drawn at least five days after last transfusion. All subjected to ferritin, hepcidin and soluble transferrin receptor assay using ELISA.
Results: This study included 21 MDS patients recently diagnosed in the Clinical Haematology unit of Cairo University (average 6 months since onset of symptoms) and 13 age and sex matched controls. Mean age was 56±10.2 years. Fifty seven percent were males and 43% were females. Mean HB level of MDS cases was (6.8 +/-4.8) gm/L. Mean blood transfusion was 6 units (3-9 units). The hepcidin mean in MDS was 55.8+/-21.5 ng/ml while in the control was 19.9+/- 2.6 ng/ml. Mean STF in MDS was 45.7+/-8.8 while the control was 31.1+/-5.6. Mean Ferritin in MDS was 539.14+/- 83.5 while in the control was 104.6+/- 42.9. Though the mean hepcidin, STF and ferritin were higher in MDS cases in comparison to the control, only ferritin was statistically significant among the two groups (P<0.005). Correlation analysis between hepcidin and other parameters was statistically significant with STF (r=0.45; P:0.039). No difference between males and females in hepcidin though it was less in males in comparison to females (47.9+/-27.6 vs 66.7+/-35.7) p value >0.05.
MDS patients were classified according to type of MDS; 8 cases with RCMD, 7 cases with hypoblastic MDS and 6 cases with RAEB. No differences among the three groups in hepcidin, STF or ferritin (p value>0.05). Mean hepcidin/ferritin ratio in MDS cases was higher than control (0.48+/ 1.2 vs 0.32+/-0.19) but statistically not significant (p value>0.6).
Summary / Conclusion: we can conclude that there is no correlation between hepcidin and serum ferritin in MDS cases and so hepcidin may not be a main player in iron overload in MDS.A possibility of peripheral unresponsiveness to hepcidin in MDS or failure of production may be the underlying cause but further studies are required.

B1842

PRESCRIPTION OF APPROPRIATE OPIATE ANALGESIA AND OTHER ESSENTIAL MEDICATION FOR PATIENTS WITH SICKLE CELL DISEASE PRESENTING WITH ACUTE VASOOCCLUSIVE CRISIS TO THE EMERGENCY DEPARTMENT.

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Background: Acute vasoocclusive crisis is the most common hospital presentation of patients with sickle cell disease (SCD). Prompt and adequate analgesia, often with parenteral opiates, is essential. At Homerton University Hospital we have a day unit dedicated to the acute management of patients with SCD. However, the day unit operates only during week days and from 9am to 7pm. Outside those hours and at weekends, sickle cell patients present to the emergency department (ED). Each of the patients known to the hospital has an individualised analgesia protocol in place to help guide ED and acute medical admitting teams on the ideal analgesic regimen.
Aims: To assess whether: 1) prescription of opiate analgesics is safe and appropriate and 2) other essential drugs are being prescribed correctly for patients with SCD being admitted to hospital from the ED. We also wanted to assess whether any initial mistakes or omissions were identified and corrected at the post-take medical ward round and after the haematology ward round.
Methods: We retrospectively examined the drug charts from the last 28 SCD admissions for vasoocclusive crisis that originally presented to the ED. We had initially aimed for the last 30 admissions, but 2 of the required drug charts were missing. Data were collected on what opiates at what doses and what combinations were prescribed, prescription of anti-emetics, anti-histamines, laxatives, folic acid, penicillin, simple analgesics, as well as hydroxyurea and chelating agents when applicable and regular prescription medications.
Results: Opiate prescription by the admitting junior doctor was not in accordance with the individualised protocol in 9 (32%) patients, and was potentially unsafe in 3 (11%). All three "unsafe" opiate prescriptions involved simultaneous prescription of a short acting opiate by two different routes of administration One unsafe combination of prescribed opiates (33%) was identified and corrected at the post-take medical ward round. 100% of opiate prescriptions could be considered safe after the patients were seen by the haematology team. Regular anti emetics, anti - histamines and laxatives were not prescribed by the admitting team for 8 (29%), 8 (29%) and 10 (35%) patients respectively. These were corrected in2, 1 and 5 cases respectively after the post-take medical ward round, and after the patients were seen by the haematology team there was only 1 patient with no prescribed anti-histamines and 1 without laxatives. Folic acid, hydroxyurea and penicillin V were appropriately prescribed by the admitting team in 26 (93%) of cases. However, other regular prescription medications were left off the drug chart in 6 (21%) of cases on admission. Missed drugs were boarded in 50% of cases at the post-take ward round and 100% of cases by the haematology team. None of the assessed patients was on chelation therapy.
Summary / Conclusion: Patients with SCD are often admitted to hospital for management of a vasoocclusive crisis after presenting to the ED. Individualised patient protocols devised by the haematoligsts are essential and should be made readily accessible to all relevant staff. Even with such protocols in place, the initial management differs and can sometimes even be unsafe. Furthermore, other essential medications to minimise opiate side effects are underprescribed. This is likely to result in sub-optimal management of a crisis, longer hospital stay and higher probability of related to opiate side effects.

B1843

TOLERABILITY, SAFETY AND EFFICACY OF RECOMBINANT ERYTHROPOIETIN TREATMENT IN PATIENTS WITH SICKLE CELL DISEASE: A SINGLE CENTRE EXPERIENCE.

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Background: Long-term erythropoietin (EPO) is being increasingly used in the management of patients with sickle cell disease (SCD). Its indications vary and can be used alone or in combination with a disease modifying therapy; hydroxyurea (HU) or transfusion. HU and EPO are more effective for treating patients with sickle cell disease who also have kidney disease or pulmonary hypertension.
Aims: To assess the tolerability, safety and efficacy of EPO in patients with SCD in our institution
Methods: This is a retrospective data analysis of patients with SCD treated with EPO at Homerton University Hospital
Results: Eight patients have received EPO to date (Table). The median age was 43.7 year-old (range 26-78) and the median level of endogenous EPO was 52.4 iu/L (9.8 – 75.1 iu/l). For 4 patients with secondary iron overload, the indication of EPO was to reduce the frequency of transfusion (one of them had chronic renal impairment); for 3 other patients, it was to gain the synergistic effect of combined therapy with HU and for the last patient, EPO was introduced because of his reluctance to start a formal transfusion programe or HU. The median dose of EPO was 30µg (30 – 40 µg). It was planned to administer 30µg EPO weekly at the initiation for all of them but for 4 patients, the dose was increased to 40µg and for 3 other patients, the frequency of administration was slowed down (2 to 3 weeks) due to high haemoglobin levels. Three patients stopped the treatment within 4 months (2 were having HU and one on transfusion programme) due to non-compliance of blood tests monitoring. There was no significant increase in the blood pressure or other side effects reported for any of the patients while on treatment with EPO. For 3 patients having transfusion, EPO allowed us to slow down the frequencies of transfusion from 4 weeks to 5 - 6 weeks. For the last patient with EPO alone, the advantage was marginal despite the fact that he was using it weekly. The median Hb level 90 days after the initiation of EPO was not significantly different (7.7 vs 8.2; P=0.5), which was expected because of a fix target of Hb levels in 4 patients on transfusion programme. Inversely, the median of HbF level was significantly higher after 90 days of EPO treatment (2.4 % vs 3.5 %; P<0.01), which tend to correlate with the fact that EPO is involved in HbF synthesis.
Table: EGFR = estimated glomerular filtration rate. * Normal range 3.7 – 31.5iu/l
Summary / Conclusion: In our experience, EPO appears to be safe in patients with SCD. We saw a modest improvement in some of the parameters measured but our sample is too small to draw any conclusions. Even though there were no side effects reported, some patients found it difficult to adhere to the administration schedule or its necessary monitoring, making poor compliance the main factor that would hinder its wider use.

B1844

THE 12 MONTHS FOLLOW-UP RESULTS FROM A COHORT OF PEDIATRIC PATIENTS WITH HEMOGLOBINOPATHY AND USING ORAL IRON CHELATOR FOR TRANSFUSIONAL HEMOSIDEROSIS IN TURKEY

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Background: Management of the transfusional iron overload (TIO) is still a challenge in treatment of hemoglobinopathies. The outcomes of chelation therapy for TIO show remarkable variation across geographical regions and socialeconomic status. The differences between efficacy, safety, tolerability and adherence profiles of chelators contribute the variation.
Aims: A nation-wide prospective, multi-center, non-interventional study in Turkey is still ongoing to evaluate the burden of TIO and its treatment with oral iron chelators (OIC) in patients with beta-thalassemia major (bTM) or sickle cell anemia (SCA). This abstract aims to represent 12-months follow-up results of the study.

	Baseline (n=476)	6. month (n=459)	12. month (n=420)	P value
Hemoglobin (g/dL)				
DFX (n=436) - mean (SD)	9.0 (1.2)	8.9 (1.1)	8.9 (1.1)	0.161
DFP (n=40) - mean (SD)	9.0 (1.2)	9.3 (1.2)	9.2 (1.0)	0.347
Serum ferritin (ng/mL)				
DFX (n=436) - mean (SD)	2070.0 (1318.5)	1940.9 (1412.4)	1746.4 (1213.5)	<0.001
DFP (n=40) - mean (SD)	2534.4 (1377.2)	2317.1 (1213.4)	2205.3 (1115.2)	0.697
ALT (U/L)				
DFX (n=436) - mean (SD)	34.8 (32.0)	35.9 (36.1)	32.1 (34.1)	0.341
DFP (n=40) - mean (SD)	39.4 (46.9)	37.0 (30.9)	30.8 (24.2)	0.651
Creatinine (mg/dL)				
DFX (n=436) - mean (SD)	0.41 (0.14)	0.41 (0.13)	0.41 (0.12)	0.614
DFP (n=40) - mean (SD)	0.40 (0.09)	0.43 (0.11)	0.44 (0.10)	<0.001

DFP: deferiprone, DFX: deferasirox, SD: standard deviation

Methods: The bTM or SCA patients with 2–18 years of age suffering from TIO and under OIC treatment have been included and are still being followed (planned follow-up time 3 years). The data about clinical and demographic characteristics of the patients, comorbidities, OIC details, laboratory and imaging results related to TIO, and safety are collected. All patients and/or their parents gave informed consent. The baseline characteristics of smaller population from study have been presented before.
Results: Of the 476 patient included from 30 centers, 452 (95.0%) (50.7% female, mean [standard deviation-SD-] age: 9.5 (4.2)) has been diagnosed as bTM whereas 24 (5.0%) (33.3% female, mean[SD] age: 10.1 (4.2)) as SCA. Of these patients; 420 (88.2%) completed 12-month follow-up. The mean hemoglobin levels of patients under deferasirox (DFX) and deferiprone (DFP) were similar and this could be sign of similar iron load and iron uptake in these patients. Hemoglobin levels of 233 (55.5%) patients at 12. month were ≤ 9 g/dL. On the other hand, serum ferritin levels decreased with all treatment, the decrease in serum ferritin over 12 months was significant with DFX but not significant with DFP. The ratio of patient with serum ferritin levels <1000 ng/mL increased from 80 (16.8%) at baseline to 107 (25.5%) at the 12. month. Similarly mean DFX dose increased from 26.3 (6.1) mg/kg/day to 28.5 (8.7) mg/kg/day at 12. month. As a parameter of liver function; ALT decreased with both treatments and only 10 (2.8) patients experienced 3 times increase of upper limit of normal (ULN) ALT level. Most of these patients also had relatively higher ALT level at the baseline. Mean creatinine level decreased with DFX while it increased with DFP, but creatinine level of no patients was over ULN.
Summary / Conclusion: The study is the largest cohort including bTM or SCA patients suffering from TIO and under OIC treatment and follow-up period is still ongoing.More than half of the patients were still under hemoglobin limit of 9 g/dL which reveals that such patients still do not receive optimal transfusion regimens. On the other hand, serum ferritin level decreased with oral chelators and the usage of DFX and DFP did not cause any significant change in liver and kidney functions. These findings show that oral iron chelator treatment at increased dose can provide improvement but many patients still need more intense treatments including active dose adjustment.
In the future, the results of the 24 and 36-month follow-up result will also be presented.

B1845

NON- IRON DEFICIENCY ANEMIA IN PATIENTS WITH INFANTILE RICKETS

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Background: Rickets and anemia are still prevalent in Egyptian infants in the age group 6 and 24 months respectively. Vitamin D receptors have widespread distribution on different body systems; not only on the skeleton, specially in the bone marrow; it is believed that they have a role in hematopoeisis.
Aims: We aimedto detect the prevalence of non iron deficiency anemia (Hb < 11 gm/dl with normal iron profile) as well as the iron status in infantile rickets.
Methods: A cross-sectional study included 100 infants (6months-2years) with rickets systematically recruited from the outpatient clinic, Ain-Shams University, Children `s hospital. Rickets was diagnosed according to clinical, biochemical and radiological criteria. Infants with either known chronic illness, healed rickets or stoss therapy for vitaminD were excluded. Dietary recall with calculation of iron, calcium and vitamin D according to food reference tables was performed. Biochemical analysis included serum Calcium (sCa), phosphorus(sPh), alkaline phosphatase(ALP), , iron profile and 25 Hydroxy Vitamin D level by Enzyme-Immuno-Assay . Vitamin D deficiency and insufficiency were considered at levels <30 and 30-75 ng/mL respectively. A complete blood picture and hemoglobin electrophoresis were done for all participants.
Results: Mean age±SD was 13.5 (6.1) months; 69.4% of them were males; 85.7% of patients had active rickets and 14.3% had healing rickets; their mean daily dietary vitamin D intake was 21.8±13.8 IU/day , calcium intake 466.4±172.5 mg/day and iron intake 2.8±1.89 mg/day. Two patients were further excluded as their anemia proved to be of hemolytic etiology. Mean sCa was 8.1±1.5mg/dl , sPh 3.5±1.1mg/dl , and ALP 937.8±626.3IU/L , while mean vitamin D level was 41.69 ±21.44ng/ml, hemoglobin level 9.96±1.60gm/dl , MCV 69.4 ±8.2 fl , MCH 22.1±3.6 pg , and reticulocytic count 0.7±0.6%. Mean serum ferritin was 21.1±24.7ng/ml , serum iron was 19.5±19.6 µg/dl , TIBC 521.4±127.4 µg/dl and transferrin saturation 5.3±8.8%. Out of the infants with rickets, 75.5% had anemia. Vitamin D deficiency was more frequent in anemic patients compared to non anemic patients (51.4% vs. 12.5%, P=0.001)). Vitamin D level was significantly lowerin infants with anemia (37.15±20.65ng/ml), compared to non-anemic 55.7±17.7ng/ml, P<0.001. Anemia among studied infantile rickets was IDA in 65 (87.8%) , while 9 (12.2%) were non IDA. A significantly low vitaminD level in infants with non IDA (21.2±16.5ng/ml) compared patients with IDA (39.4±20.3ng/ml) P=0.001.
Summary / Conclusion: Anemia was prevalent among infants with rickets ; a considerable group of them had non IDA which could be related to vitamin D deficiency. The role of vitamin D therapy in correcting non-iron deficiency anemia associated with rickets should be prospectively studied.

B1846

A COHORT STUDY TO ASSESS THE CONTRIBUTION OF PATIENT COMPLIANCE PROGRAM ON PERSISTENCE TO DEFERASIROX IN PATIENTS WITH CHRONIC IRON OVERLOAD IN TURKEY (EX-PAT PROGRAM)

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Background: Deferasirox is more efficacious and related a higher persistence than other treatment options in the treatment of chronic iron overload due to more convenient oral use. Moreover, higher persistence to chelating treatment is associated with improved mortality and morbidity.
Aims: It is highly recommended to educate the patients under iron chelating treatment about possible complication and usage of chelating agent. Exjade Patient Compliance Program (EX-PAT) was established to increase patients' knowledge about deferasirox usage. This abstract aimed to represent the results of the pilot EX-PAT program.
Methods: Patients using deferasirox were assigned to either the home-visit or control group. During February-June 2009; the home-visit group was educated and visited at home every 30 day, phoned every 15 days and texted daily by nurses, whereas the control group was only phoned and not educated. All procedures were performed after a written consent form had been signed by the patients. If a patient did not use deferasirox for ten days or less, he/she was accepted as persistent. All patients and/or their parents gave informed consent
Results: A total of 45 patients were in the home-visit group (58% male, median (inter-quartile range-IQR-) age: 135.8 (124.9) months), and 41 patients were in the control group (63% male) were followed up median (IQR) 83.0 (12.5) and 82.0 (25.5) days, respectively. Median (IQR) deferasirox dose-per-kilogram of patients with data was 25.0 (7.4) mg/kg. Based on 211.2 patient-months follow-up data; patients in home-visit and control groups did not use deferasirox mean (95% confidence interval-CI-) 1.6 (0.9-2.3) days and 5.5 (3.1-7.9) days in mean 81.1 days and 77.3 days follow-up duration, respectively (P=0.016). Persistency rates were 95.7% and 80.5% in home-visit and control groups, respectively (P=0.025). An exploratory covariance analysis with gender, follow-up duration and deferasirox dose as covariates revealed that a patient in home-visit group would use deferasirox mean (95%CI) 19 (6-32) days more than one in control group in one year (P=0.004).

Summary / Conclusion: EX-PAT program, including education and tight follow-up, increased persistence to deferasirox and similar nation-wide programs for patients and physician would improve correct usage of deferasirox.

B1847

EFFECT OF PATIENT EDUCATION ON IMPROVING KNOWLEDGE AND PRACTICES OF THALASSEMIC ADOLESCENTS AT ZAGAZIG UNIVERSITY HOSPITAL

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Background: Thalassemic adolescents need information about their disease in order to provide appropriate care, to decrease uncertainty, and to hold realistic expectations for themselves.
Aims: The present study was aimed to identify the effect of patient education on improving knowledge and practices of thalassemic adolescents at Zagazig university hospital.
Methods: A quasi experimental study was conducted on a sample of 50 thalassemic adolescents aged between 12- 18 years (30 male and 20 female) at the Pediatric Hematology Outpatient Clinic at Zagazig University Hospital in Sharkia Governorate, Egypt , in the period from February to October 2012.. Three tools were used in the present study. The first was a structured interview questionnaire to collect data about characteristics of the studied adolescents as well as their knowledge about β thalassemia major and chelation therapy. The second tool was a clinical checklist to evaluate adolescents during administration of deferoxamine subcutaneously with infusion pump. The third tool was health instructions to educate the studied adolescents about β thalassemia major and its management. Adolescents' knowledge about the disease and its care practices were assessed. Then, the health instructions were developed and implemented. Later, each adolescent was interviewed to reassess his knowledge and practices immediately after implementation of the health instructions and 2 months later.
Results: the present study revealed that the studied adolescents did not have satisfactory knowledge about their disease and its management before implementation of the health instructions. Our results showed that score of thalassemic adolescents' knowledge about the disease had changed from 4% to 100% and slightly decreased to 94% throughout the three phases of implementing health instructions, with highly statistically significant difference (P<0.01). No one of the studied adolescents had satisfactory knowledge score about disease's complications before the health instructions, but all (100%) had satisfactory score after the health instructions and 90% in follow up phase. Satisfactory total adolescents' knowledge score about their care had been changed from 42% to 100% and then to 96% respectively before, immediately after implementation of health instructions and after 2 months later (P<0.01).It was also found that 74% of the studied adolescents had satisfactory total practice score before the health instructions, compared to 100% of them after the health instructions. The percentage decreased to 86% in the follow up phase (P<0.01).
Summary / Conclusion: the health instructions had improved thalassemic adolescents' knowledge and practices. Based on the results of the present study continuous health instructions and educational programs should be conducted for thalassemic adolescents about the disease, its treatment regimen and care practices.

B1848

SUCCESSFUL LIVER TRANSPLANTATION IN SCD: CASE REPORT AND LITERATURE REVIEW

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Background: Sickle cell disease (SCD) is an escalating public health issue, with the potential for multi-organ damage. Little is known of the natural history of sickle hepatopathy; there are no management guidelines, and the role of liver transplantation in SCD remains to be elucidated.
Aims: Define the collective experience of liver transplantation in SCD
Methods: Case report and literature review.
Results: An African-Caribbean man was diagnosed with SCD (HbSS) as a child. He developed abnormal steady-state liver function at age 23 years, and regular blood transfusions were initiated to treat sickle-cell intra-hepatic cholestasis (SCIC). However, his baseline liver function deteriorated. Laboratory tests and liver imaging revealed the only other contributor to liver impairment was transfusion-related iron overload. Top-up transfusions were switched to exchange transfusions and iron chelation initiated with deferasirox. In early 2011, he developed decompensated liver failure, and was listed for liver transplantation. The patient was age 33 years when he received a liver transplant, from an 18-year-old heart-beating graft. The explanted liver was nodular and weighed 3700g; histology demonstrated sickle cells in sinusoids, sclerosing cholangitis, moderate siderosis, and a biliary abscess. Post-transplant, his liver enzymes have normalised and he has been maintained symptom-free on a monthly exchange blood transfusion programme with a HbS target of <30%.
This case adds to the 22 cases of liver transplantation in patients with SCD reported in the literature. Only six of these 22 cases report the primary indication for transplant as SCIC. The remaining 17 cases had the following indications: Hepatitis C (6), Hepatitis B (1), autoimmune hepatitis (3), biliary cirrhosis (1), sclerosing cholangitis (1), hepatic sequestration (1), iron overload (2), cryptogenic (2). We have full case details on five of the six patients with SCIC; four of the five had genotype HbSS, the fifth had HbSβ° thalassaemia; all five were maintained on an exchange transfusion programme post-transplant. Two cases were reported as successful. A third patient died 22 months post-transplant from a pulmonary embolus, with an intact graft. The fourth case had three liver transplants with early rejection, and died 6 months later. The final case died one month post-transplant of sepsis and multi-organ failure. Overall mortality rate was 58% in the wider cohort and 60% in the SCIC cohort
Summary / Conclusion: The collective experience demonstrates that liver transplantation can be successful as a treatment option in sickle patients with end-stage liver failure, but poses additional challenges. Particular attention must be given to maintaining a low HbS% with exchange transfusion rather than top-up transfusion both pre- *and* post-transplantation; minimisation of sickling pre-transplant reduces sickle-related damage of other organs, and post-transplant, it limits risk of recurrence. Surgery itself can pose problems for SCD patients: intra-operative events themselves can induce sickling (ischaemia, hypothermia and acidosis). Peri-liver transplantation issues of vascular problems and acute rejection may be confused with sickle-related crises. Risk of immunosuppression-related infections are higher in this population who have an underlying immunodeficiency.
There is a need to identify SCD patients at risk of developing SCIC early so that timely exchange blood transfusion can be initiated to ensure a successful liver transplantation if needed

B1849

ASSESSMENT OF ERYTHROCYTE PHOSPHATIDYLSERINE EXPOSURE IN B-THALASSEMIA

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Background: phospholipid asymmetry is well maintained in erythrocyte (RBC) membranes with phosphatidylserine (PS) exclusively present in the inner leaflet. The appearance of PS on the surface of the cell can have major physiologic consequences, including increased cell-cell interactions. Eryptosis, the suicidal death of erythrocytes, is characterized by cell shrinkage, membrane blebbing and cell membrane phospholipids scrambling with PS exposure at the cell surface.Erythrocytes exposing PS are recognized, bound, engulfed, and degraded by macrophages. Eryptosis thus fosters clearance of affected erythrocytes from circulating blood which may aggravate anemia in pathological conditions. Thalassemia patients are more sensitive to the eryptotic depletion and osmotic shock which may affect RBC membrane phospholipid asymmetry.
Aims: we aimed in this work to determine the erythrocyte PSexposure in splenectomized and nonsplenectomized β-thalassemia (β-TM) patients and correlate it with the clinical presentation and laboratory data.
Methods: fresh whole blood was simultaneously stained for annexin V (AV) to detect phosphatidylserine (PS) exposure in 46 patients with β-TM (27 splenectomized and 19 nonsplenectomized) in addition to 17 healthy subjects as a control group..
Results: we reported significant increase in erythrocytePSexposure in β-TM patients compared to control group (P=0.000). Erythrocyte PSexposure was significantly higher in splenectomized β-TM patients as compared with nonsplenectomized β-TM patients (P=0.001). No correlation was found between erythrocyte PSexposure and clinical or hematological data of β-TM patients but there was positive correlation between erythrocyte PSexposureand ferritin level in β-TM patients.
Summary / Conclusion: these findings suggest that β-TM patients have higher level of erythrocyte PSexposure and splenectomy was shown to aggravate erythrocyte PSexposure without aggravation of anemia

B1850

IRON OVERLOAD IN AN ADULT SICKLE CELL ANEMIA POPULATION

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Background: Iron overload is a significant source of complications in sickle cell anemia patients, especially in case of regular transfusions. Serum ferritin measurement shows a good correlation with the amount of liver iron as measured by MRI and can be used as screening test. Treatment aims to reduce the amount of body iron by the realization of exchange transfusions and the use

of chelating agents, three of which are commercially available (deferoxamine, deferiprone and deferasirox).
Aims: Data on iron overload in the adult sickle cell anemia population are more limited than for the children. We evaluated retrospectively this parameter in our cohort of patients regularly followed in a western public hospital without access restriction to care.
Methods: 51 adult sickle cell anemia patients were evaluated at the hematological consultation in 2011. The median age was 29 (17-48) and the M/F ratio 0.8. 48 patients exhibited a phenotype SS, 2 SC and 1 CC. Serum ferritin was measured in all but three of them.
Results: Serum ferritin median value was 244 ng/ml (27-3645). 8 patients (17%) had a serum ferritin value ≥ 1000 ng/ml; their mean age was 32,5 vs 28,7 years for the other patients. Mean hemoglobin concentration, white blood cells and platelets levels were identical in the two groups. Mean serum creatinin was more elevated in the heavily iron loaded patients: 4.35 vs 2.01 mg/dl (P=0.05). Liver enzymes (LDH, SGOT, SGPT, alkaline phosphatases) were comparable in two groups except for the alkaline phosphatases that were also more elevated in the patients with a serum ferritin ≥ 1000 ng/ml: 396 vs 255 IU (P=0.01). Measurements of cardiac tricuspid Vmax (2.4 vs 2.17 m/sec) and LVEF (58.8 vs 63.4%) were not statistically different. Major bone disease (e.g. hip or schoulder osteonecrosis) was also equally found in the two populations. Similarly, central neurological (MRI findings) and ophtalmologic complications were also evenly distributed in the two groups.
Summary / Conclusion: Patients with iron overload had a more compromised renal and liver function. but no increased need to be hospitalized for acute complications (vaso-occlusive crisis and/or acute chest syndrome). Interestingly, only three of these eight patients took initially part of a regular transfusion program, that was later changed for exchange transfusions. 6 of these patients received a chelating agent (5 deferoxamine and 1 deferasirox); the two others refused it. 3 patients with a serum ferritin < 1000 ng/ml were currently under erythrocyte exchanges, two of them without need for a chelating treatment.

B1851
LONGITUDINAL STUDY ON THYROID FUNCTION IN PREPUBERTAL AND PUBERTAL PATIENTS WITH THALASSEMIA MAJOR: HIGH INCIDENCE OF CENTRAL HYPOTHYROIDISM BY 18 YEARS
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Background: Primary hypothyroidism is one of the most frequent complications observed in patients suffering from thalassemia. We investigated and reviewed the thyroid function in all thalassemic patients attending the Pediatric Endocrine Clinic of Hamad Medical Center, Doha, Qatar during the last 10 years of follow up.
Aims: To investigate and reviewe the thyroid function in all thalassemic patients attending the Pediatric Endocrine Clinic of Hamad Medical Center, Doha, Qatar during the last 10 years of follow up.
Methods: 48 patients with ß-thalassemia major between 5 and 18 years of age. Thyroid dysfunction was defined as follows: overt hypothyroidism (low FT4 and increased TSH levels >5 µIU/ml); subclinical hypothyroidism (normal FT4, TSH between 5-10 µIU/ml) and central (secondary) hypothyroidism (low FT4 and normal or decreased TSH).
Results: 48 patients completed a 12 year-period of follow-up. During this period hypothyroidism was diagnosed in 17/48 (35%) of patients, 16 of them after the age of 10 years (94%). The prevalence of overt hypothyroidism had risen from 0 % at the age of 7 years to 35% at the age of 18 years. None of the patients had high anti-thyroperoxidase (TPO) antibody titers. Thirteen out of the 17 patients with hypothyroidism, had normal or low TSH level (not appropriately elevated) indicative of defective hypothalamic pituitary response to low FT4 (central hypothyroidism). Three patients (6.3%) had subclinical hypothyroidism (TSH between 5 and 10 uIU/ml and normal FT4). The general trend of free thyroxine level showed progressive decrease over the 12 years, whereas TSH levels did not show a corresponding increase. These data suggested defective hypothalamic pituitary thyroid axis involving both TSH and FT4 secretion in patients with TM over time. There was a significant negative correlation between serum ferritin and FT4 (r = - 0.39, P=0.007) but no correlation was found between ferritin and TSH.
Summary / Conclusion: Worsening of thyroid function was observed in 35 % of the studied thalassemic patients by the age of 18 years. The lack of proper increase of TSH in response to low circulating levels of FT4 in 13/17 (76%) of these patients indicates a relatively high incidence of defective pituitary thyrotrophic function in these patients.

B1852
AUTOIMMUNE THYROID DYSFUNCTION IN YOUNG EGYPTIAN TRANSFUSION-DEPENDENT B-THALASSEMIA PATIENTS
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Background: Although autoimmunity was not proved to be involved in tissue damage of B-Thalassemia, yet nonspecific triggering of autoimmunity by iron overload has been suggested. The prevalence of hepatitis C among Egyptian beta thalassemia patients is ranging from 60-80%. Hepatitis C virus infection associated autoimmune diseases are well reported, especially autoimmune thyroiditis .
Aims: This study aims to assess the prevalence of autoimmune thyroid disease among children and young adults with transfusion dependent beta thalassemia major ,especially in relation to iron overload and hepatitis C virus status.
Methods: The study is a cross sectional study done in the Pediatric Hematology Unit , Children's Hospital,Ain Shams University,Cairo,Egypt including 84 transfusion-dependent β-thalassemia patients compared with 40 age- and sex-matched healthy controls.Data were collected including: transfusion history, splenectomy, chelation/hydroxyurea therapy.Investigations included hematological profile ,serum ferritin,liver function tests ,ELISA testing for hepatitis C virus antibodies. Thyroid functions were tested by estimating free T4 and basal thyroid stimulating hormone (TSH), and antithyroperoxidase (anti-TPO) by enzyme linked immunosorbent assay (ELISA) was done as marker of autoimmunity.
Results: The patients age ranged from 10-25years (mean 14.96 ±4.40), with 1.2:1 male to female ratio. Thyroid dysfunction was found in 11 thalassemia patients (13.1%).Subclinical hypothyroidismwas found in 3 patients (3.6%) while overthypothyroidism in 1 patient (1.2%) . Five patients (5.9%) had subclinical hyperthyroidism and 2 patients (2.3%) had clinical hyperthyroidism. The male to female ratio was 1:3 in hypothyroid patients , and 1: 6 in hyperthyroid patients , with predominance of females ; but no age difference.Thalassemia patients with thyroid dysfunction (hypo- and hyperthyroidism) had insignificantly higher ferritin level, transfusion index than euthyroid patients (P=0.2 and P=0.25,respectively).The prevalence of hepatitis C antibodies was 82% in the whole studied patients and 90.9% in patients with thyroid dysfunction . Anti-TPO was significantly elevated in beta thalassemia patients compared with controls (P<0.001) ,values were relatively higher in patients with thyroid dysfunction in comparison to euthyroid patients (P=0.17). β-thalassemia patients with hypothyroidism had relatively higher anti-TPO levels than those with hyperthyroidism while the lowest anti-TPO levels were found in euthyroid patients. Patients with positive hepatitis C antibodies had higher yet insignificant AntiTPO level than those with negative antibodies (P=0.37). Anti-TPO positively correlated with ferritin level (P<0.001), but insignificantly with transfusion index(P=0.84).
Summary / Conclusion: The present results reveal that thyroid dysfunction in transfusion dependent thalassemia is related to iron overload,however thyroid autoimmunity in our cohort of Egyptian patients could be an associated contributing factor. The higher level of AntiTPO among our beta thalassemia patients compared to literature could reflect the combined effect of high prevalence of hepatitis C virus infection with higher degree of iron overload . Patients with hyperthyroidism might represent an early hyperthyroid phase of autoimmune thyroiditis before eventually becoming hypothyroid.

B1853
BIOLOGICAL DIAGNOSIS OF THROMBOTIC THROMBOCYTOPENIC PURPURA: NOTHING BUT EYES?
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Background: Thrombotic thrombocytopenic purpura (TTP) diagnosis is based on the association of mechanical haemolytic regenerative anemia with schistocytes, consumptive thrombocytopenia and typically includes neurological symptoms and end-organ damage. An optimal treatment with plasma exchange has decreased mortality rate from 90% to less than 20%. Fragmented red cell (%FRC) measurement has been described as a revolutionary automated laboratory method for shistocytes assay in the diagnosis of thrombotic microangiopathies, and thereby in therapeutic decision making. The measurement of %FRC is based on a double gating selection on the reticulocyte channel scattergram according to forward scatter and intensity of fluorescence. It combines rapidity, capacity to analyse numerous cells and huge reduction of inter-individual variability compared to reference method using microscopic examination. A %FRC below a threshold at 1% had an excellent negative predictive value especially in normocytic red blood cells and allowed biologists to exclude the presence of shistocytes without microscopic evaluation on blood smears.
Aims: However, we urge caution for the use of automated schistocytes measurement %FRC after patient's death of a typical TTP with several %FRC below the significant threshold.
Results: A 72-year old woman was brought to the hospital for sudden neurological disorders with right arm paresis and transient aphasia. In her clinical history, she was known to have untreated chronic hepatitis C with extrahepatic manifestations such as fatigue, cryoglobulinemia and distal sensory neuropathy. An angio-MRI realized in emergency to exclude stroke was normal. Admission laboratory investigation results revealed hemoglobin of 10.2g/dL, mean corpuscular volume 90.3 fL, reticulocyte count of 178 ×10^9cells/l, platelet count of 14×10^9cells/l, lactate dehydrogenase of 508 (normal range,84-246 U /L) and haptoglobin <0.1 g/l. The combination of haemolytic anemia, thrombocytopenia and neurological disorders evoked a TTP. From the first day to fifth day of

the hospitalization, six %FRC were done. During the first four days, %FRC results were between 0 and 0.16. At the fifth day, % FRC reached 0.7% but never rose 1%. The lack of significant automated schistocytosis delayed the patient's transfert in an intentive care unit and the setting up of plasma exchange. Unfortunately, the patient died of hypoxic cardiac arrest on the fifth day of evolution. Retrospective blood smears examination reveals schistocytosis ranging from 2 to 3.5%. Moreover, diagnosis of TTP was confirmed by the absence of ADAMTS13 activity (<3%) and the presence of anti-ADAMTS-13 antibodies to 40.5 U/ml (standard <15 U/ml). Our patient's case gives rise to several issues on the diagnostic value of automated %FRC. The cutoff value of %FRC for TTP of1% must be interpreted with caution especially in case of cryoglobulinemia. Known for erroneous white blood cells and/or platelet counts, cryoglobulinemia could hypothetically interfer with %FRC measurements. Furthermore, no quality control and standards are nowadays available for %FRC.
Summary / Conclusion: We argue for caution on management of patient with suspicion of TTP based on automated %FRC determination. Microscopic examination of blood smears, even though requires high skillness, should remain the reference method to eliminate shistocytosis. Finally, absence of schistocytosis should not overpass clinical expertise.

B1854
LIPOSOMIAL IRON HAS AN ANTI-INFLAMMATORY EFFECT AND IS BETTER THAN IRON SULFATE IN CORRECTION OF ANEMIA OF CHRONIC INFLAMMATORY DISEASE OF YOUNG WOMEN.
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Background: Liposome has a described anti-inflammatory effect and transports its content directly in blood, beyond gastric and enteric wall.
Aims: Aim of this study is to verify if liposomial iron is most effective than iron sulfate in correction of anemia of chronic inflammatory disease of young women.
Methods: In group A 9 patients (4 with systemic erythematous lupus, 3 with mixed connectivitis, 2 with rheumatic fibromyalgia), median age 32 years (R27-42), Hb 8.5 g/dl (R8-10), saturation of iron binding capacity < 20%, with a median ferritin level of 100 ng/ml (R90-250), ESR 35 mm/1st hour (R22-95), CRP 18 mg/l (R12-24), normal B12 and folate, received liposomal iron 60 mg/day orally for 3 months. In group B 12 patients (6 with systemic erythematous lupus, 3 with mixed connectivitis, 3 with rheumatic fibromyalgia), median age 38 years (R29-45), Hb 9 g/dl (R8-9.5), saturation of iron binding capacity < 20%, with a median ferritin level of 120 ng/ml (R80-190), ESR 33 mm/1st hour (R20-87), CRP 15 mg/l (R13-27), normal B12 and folate, received iron sulfate 210 mg/day orally for 3 months.
Results: After treatment, group A showed a median hemoglobin level of 11.5 g/dl (R10.5-12), a median ferritin level of 260 ng/ml (R 190-280), a ESR decrease to a median value of 8 mm/1st hour (R 3-10) and a median CRP 3 mg/l (R2-4). After treatment, group B showed a median hemoglobin level of 9.5 g/dl (R8-9.5), a median ferritin level of 100 ng/ml (R 90-180), and ESR and CRP don't showed any improvement. 4 patients showed hepygastralgia, 2 stipsis, 5 diarrohea.
Summary / Conclusion: Liposomial iron is most safe, effective, well tolerated, effective than iron sulfate in increase hemoglobin level and reduce inflammatory markers in correction of anemia of chronic inflammatory disease of young women.

B1855
ELEVATED SERUM FERRITIN LEVELS >3,000 MG/DL ARE HIGHLY ASSOCIATED WITH ENDOCRINOPATHIES IN PATIENTS WITH THALASSEMIA
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Background: Endocrinopathies are well recognized major complications in thalassemic patients with iron overload. Elevated serum ferritin levels reflect severity of iron overload and areassociated with adverse clinical outcomes. Increasedserum ferritin >2,500 µg/dl is associated with impaired cardiac function, but the predicting serum ferritin level for endocrinopathies has not been determined.
Aims: To evaluate the correlation between serum ferritin levels, non–transferrin binding iron (NTBI) and labile plasma iron (LPI) with endocrinopathies (diabetes, hypothyroidism and hypogonadism) in thalassemia patients.
Methods: All patients with thalassemia, age >18 years old were enrolled between August 2011 and December 2012. Fasting blood sugar, thyroid and gonadal functions were evaluated. Serum ferritin, NTBI and LPI were measured at the same time. The medical records were reviewed for age, sex, splenomegaly, history of splenectomy, transfusion requirement, serum ferritin levels, and iron chelation history.
Results: There were 118 patients with thalassemia [60% females] with a median age of 28 years (18-71). The majority of patients were β-thalassemia/hemoglobin E (49.2%), then homozygous β-thalassemia (28.8%) and hemoglobin H with variant (20.3%). Most patients (58.5%) underwent splenectomy while only one third (37.3%) were non-transfusion dependent thalassemia (NTDT). The mean values and maximum ferritin levels were2,379 µg/dl (279-9,817) and4,914 µg/dl (279-37,656), respectively.
The prevalence of diabetes mellitus, hypothyroidism, subclinical hypothyroidism and hypogonadism were 11.9%, 7% , 23.5% and 34.8%, respectively. Mean NTBI and LPI of these patients were 7.2 µM (0.05-30.3) and 4.3 µM (0.01-15.8). NTBI were correlated well with LPI, serum iron and transferring saturation. The maximum ferritin level of >3,000 µg/dl was independently associated with diabetes [OR 9.94 (95%CI 1.34-79.27), P=0.004], hypothyroidism [OR 3.73 (95%CI 1.51-9.20), P=0.003] and hypogonadism [OR 3.54 (95%CI 1.63-7.69), P=0.001].
Summary / Conclusion: High prevalence of endocrinopathies were found among patients with thalassemia. The maximum ferritin level of >3,000 µg/dl was a major risk factor of the development of diabetes, hypothyroidism and hypogonadism. These findings warrant the value of iron chelating therapy to maintain serum ferritin level below3,000 µg/dl for avoiding the development of endocrinopathies in patients with thalassemia.

B1856
MISDIAGNOSIS OF HEMOGLOBIN D-PUNJAB/BETA THALASSEMIA IS A POTENTIAL PITFALL IN HEMOGLOBINOPATHY SCREENING PROGRAMS: A CASE REPORT
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Background: Hemoglobinopathies are the most common single gene disorder in the United Arab Emirates (UAE); in particular, beta-thalassemia is a major public health problem in this country. A premarital screening program to prevent beta-thalassemia major has been in effect in the UAE since 2006. This is a compulsory procedure that utilizes complete blood count (CBC) and hemoglobin high-performance liquid chromatography (HPLC) as screening tools.
Aims: To emphasize that Compound heterozygous hemoglobin (Hb)D-Punjab/beta-thalassemia must be carefully differentiated from homozygous HbD-Punjab in premarital screening, especially when the partner has the beta-thalassemia trait.
Methods: A case of three months old baby with beta-thalassemia major, who was the product of a marriage between a mother with beta-thalassemia trait and a father with compound heterozygous Hb D-Punjab/beta-thalassemia, is presented. The father had been misdiagnosed with homozygous HbD-Punjab during premarital screening, even though the screening program utilized complete blood counts and high-performance liquid chromatography. On arrival at Dubai Thalassemia Centre the case was revaluated and reinvestigated. Consequently, both parents underwent CBC, iron study, HPLC, and alpha- and beta-globin gene DNA analyses.
Results: The baby's CBC showed hypochromic microcytic anemia and beta-globin molecular test showed homozygous beta-thalassemia IVS-1-5(G>C)/IVS-1-5(G>C), which was not consistent with the premarital HPLC results of her parents. While Reviewing parents' re-investigation, the father had again shown low Hb A_2 on HPLC but the molecular tests revealed that he was a compound heterozygous Hb D-Punjab/beta-thalassemia IVS I-5(G>C), not the homozygous HbD-Punjab disease that had been diagnosed by the premarital screening. Both the proband and her parents had a normal alpha globin gene.

TABLE 1 Laboratory Data of the Proband and Her Parents on Arrival at the Dubai Thalassemia Centre (normal ranges are in parentheses)

Parameters	Proband	Father	Mother
Ferritin (ng/mL)	–	141.3 (18.7-323.0)	32.2 (9.3-59.0)
Iron (mg/dL)	–	107.0 (59.0-158.0)	99.0 (37.0-145.0)
TIBC (mg/dL)	–	354.3 (250.0-450.0)	298.4 (250.0-450.0)
Hb (g/dL)	6.1 (11.1-14.1)	13.4 (13.0-18.0)	10.8 (11.0-15.0)
RBC (10^{12}/L)	3.13 (4.1-5.3)	7.11 (4.5-6.0)	5.68 (3.7-5.4)
MCV (fL)	77.6 (68.0-84.0)	60.8 (77.0-92.0)	61.5 (77.0-92.0)
MCH (pg)	19.6 (24.0-30.0)	18.9 (26.0-34.0)	19.1 (26.0-34.0)
MCHC (g/dL)	25.3 (30.0-36.0)	31.1 (32.0-36.0)	31.0 (32.0-36.0)
Hb A (%)	–	1.26 (90.0-95.0)	88.0 (90.0-95.0)
Hb A_2 (%)	–	1.19 (1.5-3.4)	5.09 (1.5-3.4)
Hb F (%)	–	0.03 (0.0-3.0)	1.2 (0.0-3.0)
Hb D-Punjab (%)	–	89.17	–
β Genotype	IVS-I-5(G>C)/ IVS-I-5(G>C)	IVS-I-5(G>C)/ codon 121(GAA>CAA)	IVS-I-5(G>C)/β^A
α Genotype	αα/αα	αα/αα	αα/αα

NB: The parent's premarital screening results (CBC and HPLC) were similar to those obtained on arrival at Dubai Thalassemia Centre

Summary / Conclusion: It is recommended that in cases of HbD-Punjab syndromes, the hemoglobin analysis results should be evaluated carefully in conjunction with a close examination of the mean corpuscular volume values. Moreover, molecular genetic studies should be performed in HbD syndromes if the partner is a beta-thalassemia carrier.

B1857

CHANGES IN HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN PATIENTS WITH SICKLE CELL DISEASE UNDERGOING AUTOMATED RED CELL EXCHANGE TRANSFUSIONS

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Background: Exchange transfusions are often used as a disease-modifying strategy in patients with sickle cell disease (SCD). Automated red cell exchange transfusions (ARCET) can rapidly and efficiently achieve low HbS levels whilst at the same reduce the risk of iron overload compared to top-up or manual exchange transfusions.
Aims: To assess the incidence and severity of biochemical and haematological imbalances occurring in patients with SCD during ARCET
Methods: At Homerton University Hospital we have a large number of patients with SCD on a regular exchange transfusion programme the vast majority of which are ARCETs. Pre- and post- transfusion samples are collected for all procedures and the results are recorded in a database. This is a retrospective analysis of the results in our database for a 12 month period (Jan2012 – Dec2012).
Results: There were 105 procedures involving 33 patients for the period 01/01/12 – 31/12/12. The mean reduction in HbS level was 80%, (median -82%). The mean rise in HbA was 114% (median +80%). The modest elevation in HbA reflects the fact that most of those patients were on a regular transfusion programme and their pre-transfusion HbA was already high. Both Hb and haematocrit rose by a mean 15% per procedure (range -14% to +81% and -17% to +83% respectively). There was a 28% mean reduction in the white cell count (median -35% and range -61% to +49%). We have previously reported a significant but transient reduction in the platelet count (BSH annual meeting 2013). Indeed we found a 67% mean reduction of platelets per procedure. Fibrinogen was also reduced ranging from -66% to 32% per procedure (mean -30%, median -31%). Many biochemical parameters were also found to be reduced post ARCET. Serum ferritin levels were reduced by a mean of 22% (range -73% to +123%). The reduction in albumin ranged from -38% to -3% (mean -25%, median -23%). LDH was reduced by a mean of 33% (range -70% to +80%). There was a 22% mean reduction in Alkaline Phosphatase (range -40% to +11%) and a 25% mean reduction in ALT per procedure (range -57% to +20%). Bilirubin was reduced by a mean 13% (range -54% to +93%) and serum Creatinine was only slightly reduced by a mean of 5% (range -33% to +40%). Hypocalcaemia is a known side effect of ARCET due to citrate toxicity. We found a reduction of calcium ranging from -24% to -4% (mean -13%, median -13%).
Summary / Conclusion: Automated red cell exchange transfusion is a quick, safe and efficient way of lowering HbS levels in SCD. Our results reveal a modest reduction in most biochemical parameters measured post ARCET which most likely occurs as a result of loss of plasma during the procedure.

B1858

HEMATOLOGICAL PROFILE BETWEEN PATIENTS WHO SUFFER FROM ISCHEMIC HEART DISEASE AND PATIENTS WITH DILATED CARDIOMYOPATHY

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Background: As we Know the relevant bibliography mentions that anaemia, definitely affects the evolution and the course of patients with congestive heart failure, yet the ESR is usually low in cases like these.
Aims: To investigate the variations of the hematocrit (Ht), the white blood cells (WBC), the 1st hour erythrocyte sedimentation rate (ESR), the C-reactive protein (CRP) and also of the fibrinogen (Fib) and the immunoglobulins found in blood, between patients who suffer from ischemic heart disease (IM) and patients with dilated cardiomyopathy (DCM) and to study the potential prognostic value in each case.
Methods: 106 patients, who have constituted the material for our study (81 males and 25 females), suffered from heart failure and presented left ventricular dilation and dysfunction, and ejection fraction (EF) <35%. 59 of them (44 males and 15 females) suffered from DCM and 47 (37 males and 10 females) from IM. For the patients who were under frequent observation at regular intervals for more than a year, specifications of Ht, WBC, ESR, CRP, fibrinogen and immunoglobulins were carried out, while previously, the coexistence of other pathologic situations such as malignity, hematological diseases, injuries, gynecological diseases, severe febrileinfections etc, which would affect the results of these tests was eliminated.
Results: Although the Ht, the White Blood Cells and the immunoglobulins were measured in higher values in DCM, in correlation with the IM, they did not present statistically significant difference between them. Also, even though the CRP and the fibrinogen presented higher values in IM, in correlation with the DCM, their difference wasn't statistically significant. On the contrary, regarding the ESR, it appeared substantially increased (P<0.001) in the case of IM (25,6+/-13,9), comparatively to DCM (10,6+/-9,6).
Summary / Conclusion: It is established then, that between IM and DCM, statistically, there is no significant difference in the laboratory parameters that were previously mentioned (which vary within normal ranges), with the exceptions of the ESR, who was presented with decreased values (<10) in DCM (but not with poor prognosis), and the Ht, in which its decreased value in IM, points undeniably an unfavorable prognostic factor, which must not escape from our attention.

B1859

FLOW CYTOMETRY QUANTITATIVE ANALYSIS OF HOWELL-JOLLY BODIES IN CHILDREN WITH CHRONIC HEMOLYTIC ANEMIA

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Background: In sickle cell disease functional asplenia is reported to develop early in life and has important clinical consequences, with quantitative measurement of splenic function reported using a flow cytometric method for quantitating of Howell-Jolly bodies (HJB) that isolates HJB-containing $CD71^+$ and $CD71^L$ erythrocytes.Analysis of these cell populations allows quantitative measurement of splenic filtrative function and possible chromosomal damage[1]. Splenic dysfunction has been described in Beta thalassemia major ,however quantitative measurement of splenic filtrative function by flow cytometry was not reported.
Aims: To evaluate the filtrative splenic functions using flow cytometry in patients with β thalassemia major (BTM)and thalassemia intermedia (TI) compared to patients with sickle cell anemia (SCA), sickle thalassemia (SCT),splenectomized BTM, and healthy controls.
Methods: The study is a cross sectional study done in the Pediatric Hematology Unit , Children's Hospital,Ain Shams University,Cairo,Egypt.It included 15 patients with sickle cell anemia (mean age 7.8±3.9 years),20 patients with sickle thalassemia, (mean age 12.5±4.9 years),30 non splenectomized β thalassemia major patients(mean age 9.8±4.8 years), 20 splenectomized patients with β thalassemia major, (mean age 14.0±2.7 years), and 32 patients with non spenectomized β thalassemia intermedia (mean age 12.5±3.7 years), compared to 30 age- and sex-matched healthy controls.Data were collected including: transfusion history, splenectomy, sickling crisis, thrombotic events, chelation/hydroxyurea therapy,as well the frequency of hospitalization with serious infections. Investigations included hematological profile ,hemoglobin electrophoresis and serum ferritin .Howell-Jolly Bodies (HJBs) were quantitated by flow cytometric method , identifying HJB-containing CD71- erythrocytes in peripheral blood (used to identify older erythrocytes containing micronuclei, indirectly measuring splenic function), and HJB-containing CD71+ reticulocytes, indicating young erythrocytes containing micronuclei (used as an index of cytogenetic damage and/ or ineffective erythropoiesis)
Results: Compared to healthy controls ,patients with SCA and SCT had higher HJB frequencies within CD71+ reticulocytes (P<0.05 and P< 0.01,respectively), and higher HJB frequencies within CD71- erythrocytes (P< 0.01 , for both). Patients with BTM (splenectomized) ,BTM (non-splenectomized) and TI had significantly higher HJB frequencies within CD71+ reticulocytes (P< 0.01,for the three groups) and CD71- erythrocytes (P< 0.01, P< 0.001, P< 0.01) compared to healthy controls . Comparing SCA patients with SCT, BTM (splenectomized) , BTM (non-splenectomized) and TI : there was no significant difference regarding the HJB frequencies in both CD71+ reticulocytes and CD71- erythrocytes (P>0.05). However, splenectomized BTM patients had higher frequencies of HJB in CD71+ reticulocytes in relation to non-splenectomized patients and TI (P=0.01 and P< 0.01). The mean level CD71+ reticulocytes containing HJB was higher in SCA and SCT patients on regular hydroxyurea therapy compared to non treated patients but not statistically significant (P>0.05).
Summary / Conclusion: Splenic hypofunction can be present in non splenectomized patients with β thalassemia major and thalassemia intermedia , and may contribute to the immune dysfuncion observed in this group of patients. However, splenic dysfunction in those patients can be to a lesser degree than splenectomized thalassemics and sickle cell disease patients. The use of hydroxyurea in SCD was associated with higher levels of CD71+ reticulocytes containing HJB, which represent an index of cytogenetic damage.

Reference

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B1860
RETROSPECTIVE STUDY OF IRON DEFICIENCY ANEMIA IN HEMATOLOGY CLINIC
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Background: Iron deficiency anemia (IDA) is the common nutritional deficiency worldwide and occurs in 3,5-5,5% of adult men and postmenopausal women. The studies concerning various causes of IDA in adult men are rare, although it is assumed that chronic gastrointestinal blood accounts for the majority.
Aims: *Aim* of the study is to evaluate retrospectively adult men with IDA.
Methods: One hundred and fifty-six pateients with IDA participated at this study from January 2005 to december 2012. Anemia was defined as Hg<13g/dL using the WHO criteria. IDA was considered present if serum ferritin was 15ng/mL combined with serum iron concentration <30ug/dL with a transferrin saturation of <10%. Complete physical examination and fecal occult blood test (FOBT) of three spontaneously passed stools was done in all patients. All patients had complete blood count, serum and total iron binding capacity, and a serum ferritin level. Mot patients underwent esophagogastroduodenoscopy (EGD). Colonoscopy was performed if lesion that caused IDA was not found, and/or FOBT was positive.The abdominal CT scan were performed according to clinician's recomandation.
Results: The median age was 56 (range 26 to 84) years old. 131 of 156 (83,9%) men with IDA had symptoms such as fatigue, dizzines, or digestive complaints. The history of prior gastrectomy, hemorrhoid, that probably had caused IDA were reported in 25 (16%), 33 (21,1%), patients, respectively. FOBT was positive in only 39 (25%) subjects. 140 (89,7%) patients underwent EGD. The most common findings from EGD were gastritis (36 patients) and peptic ulcer (27 patients). Forty-nine (31,4%) patients were found to have upper gastrointestinal disorders (9 patients with erosive gastritis, 13 gastric ulcer, 10 duodenal ulcer, 17 gastric cancer. Seventy-eight (50.7%) patients underwent colonoscopy. Evaluation with colonoscopy showed 37 clinically important lesions that probably caused IDA; colon cancer in 13 (8.3%) patients, colon polyp in 11 (7.05%) patients and hemorrhoid in 13 (8.3%) patients. Concerning malignant lesions which are responsible for IDA, 21 malignant lesions were found in patients older than 50 years accounting for 21.4% (21/98 patients) and patients younger than 50 years were 17.2 % (10/58 patients).
Summary / Conclusion: This study demonstrated that gastrointestinal blood loss is the main cause of IDA in adult men, and that there is a high rate of malignancy in men older than 50 years, emphasizing a complete and rigorous gastrointestinal examination in this group of patients.

B1861
DIAMOND-BLACKFAN ANEMIA: A CENTER EXPERIENCE
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Background: Diamond-Blackfan anemia (DBA) or congenital erythroblastopenia is a rare ribosomal disorder characterized by red cell aplasia, congenital anomalies and predisposition to develop malignancies. Several therapies have been tested, nevertheless only steroid have proven to be efficacious
Aims: Evaluate the clinical characteristics and treatment response of DBA in our medical center.
Methods: This is a retrospective observational study of patients diagnosed in our center DBA (n = 7). Patients medical records were reviewed by assessing the following parameters: age and Hb level at diagnosis, current Hb level, presence of other cytopenias, DBA associated genetic alterations, associated congenital anomalies, ferritin and current IST, type of treatment received, maximun dose, current dose and occurrence of side effects, current need of transfusions and secondary neoplasias.
Results: male: female ratio 4:3. Age at diagnosis was within the first 18 months of life in all cases with a median of 3 months (2-13 months). Medium current age: 15 years (6 months-36 years). Median hemoglobin (Hb) at diagnosis was 5.6 g / dL (2.1 to 6.6) with a current value of 9.9 (9.1 to 13.4). No patient had another cytopenia. The molecular study was conducted in five of them: two have shown mutations in the RPS19 gene regions analyzed; five patients are awaiting to complete the sequencing of the other 8 genes associated with DBA. The prevalence of congenital malformations in our series is 71%, mostly of the cardiovascular type. Prednisone was the main treatment in this series and 86% of patients achieved response. Highest median prednisone dose was 2 mg / kg / day (1.5 to 3); current median dose is 0.2 mg / kg / day (0.08 to 1.7). 71% of patients had therapy-related side effects, mild to moderate in all cases. Other adjuvant therapies: danazol in one case, with no objective response; hemotherapy support: 1 transfusion-dependent patient, iron overload in 3 patients and 2 patients are awaiting chelation. No patient developed secondary malignancies.
Summary / Conclusion: To date, there are few therapeutic options for the DBA; bone marrow transplantation remains as the only potentially curative therapy. As in most studies, in our series of patients corticosteroid therapy is the first line therapy. New therapeutic targets need to be identified urgently to improve the outcome of this ominous disease.

B1862
MACROCYTIC ANEMIA AND ITS CAUSES: A PROSPECTIVE COHORT STUDY
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Background: PAGAS, a prospective study concerning anemia and its causes, was started in February 2007 by the Albert Schweitzer hospital in Dordrecht, the Netherlands, in co-operation with 63 general practitioners. Between February 2007 and February 2013, 5833 patients who were diagnosed with anemia and did not exhibit anemia in the preceding two years, were included. Of these patients, 426 (7,3%) were found to have a raised MCV (reference values: 80-100 fl) and were diagnosed with macrocytic anemia.
Aims: An evaluation of the causes of macrocytic anemia found in these 426 patients.
Results: Additional testing demonstrated B12 deficiency in 48 (11,2%) of these 426 patients (reference values: 130-700 pmol/L), and folic acid deficiency in 24 (5,6%, reference values: >5). In addition, 122 patients (28,6%) were shown to have a raised LDH level (reference values: <450 E/L), of whom 52 patients (12,2%) showed raised reticulocytes (reference values: <2,5%), differential diagnosing haemolysis or myelodysplastic syndrome. Raised LDH levels, raised reticulocyte percentage and a raised bilirubine level (reference values: <17 µmol/L) was demonstrated in 9 (2,1%) patients, indicative for haemolytic anemia.
Summary / Conclusion: 179 patients (42%) did not demonstrate a raised reticulocyte count, bilirubin or LDH pointing to bone marrow diseases, e.g. myelodysplastic syndrome, of whom additional testing will be described.

B1863
TRANSCRANIAL DOPPLER IMAGING IN THE MANAGEMENT OF PEDIATRIC SICKLE CELL DISEASE PATIENTS: IMPACT OF HYDROXYUREA
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Background: Background: Abnormal interaction of sickle red cells with the vascular endothelium has been implicated in the changes affecting the large vessels of the central nervous system of sickle cell disease patients (SCD).
Aims: Aim: The purpose of this study was to evaluate these changes by transcranial Doppler imaging (TCD) and to assess the effect of hydroxyurea in ameliorating these abnormalities.
Methods: Methods: Fifty five SCD patients in the steady state of the disease (mean age of 10.48±4.44 years) were included in this study. Eleven β thalassemia patients (mean age 10.27±2.69 years) and 11 healthy candidates (mean age 10.38± 3.1 years) were selected as control groups. All patients and control groups were subjected to TCD of the middle cerebral (MCA) and the anterior cerebral arteries (ACA) at baseline, 6 months and one year.
Results: Results: Twenty four of the 55 SCD patients were on hydroxyurea therapy for one year. The mean values of MCA and ACA velocities were statistically higher in SCD patients compared to controls and β thalassemia patients (P<0.001) at baseline. At baseline, the time average peak systolic velocity (TAP) of the MCA was >200 cm/s (high risk) in two SCD patients. One patient was in the intermediate risk group (TAP =170-199 cm/s) and the rest of the patients (52 patients) were low risk (TAP<170 cm/s). After one year of follow up, the patients on hydroxyurea showed reduction of the peak systolic velocity (PSV) and TAP of the MCA when compared to those on conservative treatment. This drop only approached statistical significance (P=0.066& 0.060, respectively). There was a negative correlation between the PSV and TAP of MCA and the age of SCD patients (R = - 0.397 & - 0.348; P=0.003&0.009, respectively). A significant reduction in the number of transfused units of blood and vaso-occlusive crises was observed after hydroxyurea therapy (P=0.005& 0.017, respectively). The percentage of fetal hemoglobin significantly increased while the percentage of Hb S significantly decreased after treatment (P=0.003& 0.003, respectively).
Summary / Conclusion: Conclusion: TCD is a safe method for detection of CNS vasculopathy in SCD. Hydroxyurea can successfully decrease transfusion requirements and vaso-occlusive crises and lead to the reversal of the changes seen on TCD.

B1864

IRON AND VITAMIN D STATUS IN EXCLUSIVELY BREAST-FED INFANTS UNDER 24 MONTHS AND LACTATING MOTHERS

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Background: Breast milk is the best nutritional resource for infants. But breast milk cannot satisfy the requirements after 6 months. Since iron deficiency(ID) can adversely affect of neurodevelopment, lactating mothers must maintain sufficient Fe status. Vitamin D stores in the fetus depend on maternal vitamin D status and the breast-fed infants continue to dependent upon their mother afterwards. Vitamin D deficiency(VDD) in exclusively breast milk-fed (EBMF) infants and lactating mothers is prevalent in many parts of the world, especially in Asia. Concurrent deficit of micronutrients might result in wide spectrum of adverse effects in rapid growing infants.
Aims: To assess the Fe and 25-hydroxyvitamin D [25(OH)D] status in lactating mothers and their infants.
To find specific factors affecting both infant and mother to have iron deficiency anemia (IDA) and/or VDD.
To analyze the seasonality of Fe and 25(OH)D
Methods: The infants aged 4-24 months who were exclusively breast-fed were included from Mar 2012 to Feb 2013, who visited CHA Bundang Medical Center (Latitude 37.4°N). The data were collected by questionnaire and laboratory tests such as CBC, reticulocyte, ferritin, Fe, TIBC, CRP, Ca, P, ALP, 25(OH)D. The lactating mother was evaluated for the same laboratory tests a week later. Through the questionnaire, information about the maternal history, birth-related history, breast feeding and weaning pattern, etc.
The infants were divided into IDA, ID and Normal group according to Hb (<11 g/dL) and ferritin (<12 ng/mL). Vitamin D deficiency was defined as 25(OH)D < 20 ng/mL, vitamin D insufficiency as 20-30 ng/mL. The mother's Fe status was defined as ID if ferritin < 50 ng/mL. The subjects were divided into 2 group, winter/spring (November to April) and summer/autumn (May to October), according to the date of laboratory test. Winter/spring group (WS) was compared with summer/autumn group (SA).
Statistical analysis was done by SPSS (version 17.0) with P value < 0.05.
Results: There were 64 infants and lactating mothers who agreed to participate. Nine were excluded because they had previous Fe and/or vitamin D supplements. The average age of the infants was 10.2 months and the average age of the mothers at delivery was 32.2 years. IDA was found in 32 infants and VDD in 45. Maternal Hb was < 12 g/dL in 6 and VDD was found 51 mothers. Weaning food was started in 50 infants and 23 of their mother reported having feeding problem due to refusal. Thirteen of 56 mothers had previous history of IDA before pregnancy, 26 of them needed additional Fe supplementation during pregnancy and only 6 were taking multivitamin at present. The averages of 25(OH)D were subnormal in both infants and mothers (12.7, 12.9 ng/mL respectively). Maternal ferritin did not affect the Fe status of infants but mothers with 25(OH)D < 20 ng/mL had infants with lower 25(OH)D level (P<0.05).
If the mother had a history of IDA before pregnancy, there was a tendency to have maternal ferritin < 50 ng/mL at present (P<0.05), and maternal 25(OH)D < 12 ng/mL (P<0.05). If the mother started menstruation during lactation, they had a lower ferritin (t test, P<0.05). First born infants were 27. If the infant was 2nd child or after, there was a preference to have a lower maternal 25(OH)D and higher TIBC in infants but statistically not significant (P=0.61, 0.63 repectively).
The infants in WS group had significantly lower 25(OH)D (P<0.005), but no difference in Hb, MCV, reticulocyte and ferritin (Table 2). The mothers in WS group also had significantly lower 25(OH)D, but SA group had lower reticulocyte and ferritin (P<0.05) with no differences in Hb and WBC.

Table 2. Comparison between WS group and SA group*

	Infants			Mothers		
	WS	SA	p**	WS	SA	p**
Hb	10.4	10.1	ns	13.1	13.0	ns
MCV	72.1	69.5	ns	87.8	87.6	ns
RDW	14.8	14.9	ns	12.8	12.8	ns
Reti	1.51	0.82	ns	1.04	0.89	0.04
Ca	9.6	9.4	ns	9.0	8.9	ns
P	4.7	4.8	ns	3.7	3.8	ns
ALP	561	656	ns	226	237	ns
25(OH)D	9.6	15.7	0.003	11.5	14.2	0.02
Ferritin	41.4	31.6	ns	59.4	37.1	0.02

* All the data are in average,
WS: winter/spring, SA: summer/autumn
** Student's T test, ns: not significant

Summary / Conclusion: IDA was prevalent in EBMF infants but ID was not in lactating mothers because of amenorrhea. The average of vitamin D was subnormal both in infants and mothers. If the mother has a history of IDA before pregnancy, EBMF might result in concurrent deficiency of both Fe and vitamin D. Consecutive pregnancies might result in more profound deficits but research in needed. Seasonal variation of 25(OH)D emphasizes the concern about VDD, and ID might be prevalent among lactating mothers in summer and autumn in Korea. Supplementation of micronutrients such as Fe and vitamin D should be routinely practiced in EBMF infants and their mothers especially who starts menstruation during lactation.

B1865

MAY BE PREDICTED THE PATIENTS WITH IRON DEFICIENCY WITHOUT ANEMIA FROM PARAMETERS OF COMPLETE BLOOD COUNT?

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Background: Iron deficiency without anemia (IDNA) was three times more common from iron deficiency anemia (IDA) which is the most common nutritional deficiency in the world. Data from the Third National Health and Nutrition Examination Survey (NHANES III; 1988 to 1994) indicated that iron deficiency anemia was present in 1 to 2 percent of adults and IDNA was occurred up to 11 percent of women and 4 percent of men.
Aims: The aim of the study is to investigate whether or not differentiation of the cases with IDNA from healthy subjects using with parameters of complete blood count or related formulas.
Methods: This study was carried out on 37 IDA patients (35 female) ages ranging from 18 to 77 (mean±standard deviation: 39.46±13.29 years), 23 IDNA patients (22 female) ages ranging from 24 to 73 (37.74±11.20 years) and 40 healthy subjects (20 female) as control group ages ranging from 19 to 79 (42.27±13.35 years). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), plateletcrit (PCT), microcytic anemia factor (MAF) (=[hemoglobin x MCV]/100), volume hemoglobin/distribution factor (VHDWf) (=[MCV×hemoglobin]/[RDW×10]) and RDW x PCT were used for comparisons.
Results: There was statistically significance for indices of MAF and VHDWf between IDNA group and healthy subjects. The percents of sensitivity, specificity, accuracy and Youden's index belonging to cut-off values of these indices were shown in Table 1. The cut-off value of MAF <12.5 has high ratio from accuracy and Youden's index.
Summary / Conclusion: The cut-off value of MAF <12.5 may be warranted to differentiate the patients with IDNA from healthy subjects.

	Sensitivity	Specificity	Accuracy	Youden's indeks
MAF <11.21	60.9%	85.0%	76.2%	45.9%
MAF <11.50	65.2%	82.5%	76.2%	47.7%
MAF <12.00	65.2%	70.0%	68.3%	35.2%
MAF <12.50	91.3%	65.0%	74.6%	56.3%
MAF <12.81	95.6%	50.0%	66.7%	45.6%
VHDWf <7.86	56.5%	87.5%	76.2%	44.0%
VHDWf <8.00	56.5%	85.0%	74.6%	41.5%
VHDWf <8.50	65.2%	77.5%	73.0%	42.7%
VHDWf <9.00	82.6%	60.0%	68.3%	42.6%
VHDWf <9.37	87.0%	50.0%	63.5%	37.0%

Infectious diseases, supportive care

B1866

MANAGEMENT OF INVASIVE FUNGAL INFECTIONS IN PEDIATRIC ACUTE LEUKEMIA AND THE APPROPRIATE TIME FOR RESTARTING CHEMOTHERAPY

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Background: Invasive fungal infection (IFI) is a life threatening problem in patients with leukemia. Rapid and effective treatment is important for survival. The optimal time for restarting chemotherapy in these patients is not clear.
Aims: We aimed to describe variations regarding clinical features, treatment modalities, time of restarting chemotherapy (CT) and outcome in children with IFI and acute leukemia (AL).
Methods: The charts of all pediatric AL patients in our clinic between the years 2001 and 2013 were retrospectively reviewed and data of patients with IFI were analysed.
Results: IFI was defined in 25 (14%) of 174 AL patients. Of those, 17(68%) had ALL and 8(32%)had AML. The median age was 12 years (range 0.7-17.5 years). Nine (36%) of the patients were in induction, 14 (56%) of the patients were in the consolidation phase and 2 (8%) of the patients were in the maintenance phase of CT and overall 18 (72%) patients were in remission at the time of diagnosis of IFI. The median time between the leukemia diagnosis and the definition of IFI was 122 days (range 15-305 days). ANC was <500/mm3 in 86 % of patients. The median time for duration of neutropenia was 13 days (range 0-47 days). All of the patients were febrile at the time of diagnosis. Of the 25 AL patients with IFI, 23 patients had isolated pulmonary IFI, one patient had isolated orbitocerebral aspergillosis infection and one patient had both orbitocerebral and pulmonary mucor and aspergillosis infection. Those two patients with orbitocerebral IFI needed surgery . The most frequent finding on computed tomography was typical parenchymal nodules. Galactomannan was screened in 21 patients and of those, 9 (42%) had positive galactomannan antigenemia. The episodes were defined as proven in 2 (8%) patients, probable in 9 (36%) patients and possible in 14 (56%) patients. The median time for discontinuation of chemotherapy was 27 days (range 0-57 days). Chemotherapy was not restarted in three patients due to refractory/progressive primary disease. Out of 22 patient in whom chemotherapy was restarted, the duration for cessation of chemotherapy was 0-14 days in 5 (23%) patients and 15-28 days in 6 (27%) patients. Overall chemotherapy was restarted in 50% of the patients safely before 4 weeks, and none of those patients experienced relapse of IFI. IFI was treated succesfully in all patients with voriconazole, amphotericin B, caspofungin, posaconazole alone or in combination. All of them were given secondary prophylaxis with oral voriconazole, itraconazole, or posaconazole. The median time for secondary prophylaxis was 90 days (range 39-429 days). None of the patients died due to IFI.
Summary / Conclusion: Our data shows that rapid and effective antifungal therapy with rational treatment modalities may decrease the incidence of death in children with AL and IFI. Depending on the clinical status of the patient, restarting chemotherapy in several weeks may be safe and relapses of IFI may be prevented with secondary prophylaxis.

B1868

RISK FACTORS FOR ACQUIRED BLOODSTREAM INFECTION AMONG NEUTROPENIC PATIENTS.

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Background: Febrile Neutropenia is a major cause of mortality among hematological patients. It is estimated that about 10- 15% of patients with severe neutropenia will develop bloodstream infection during their hospitalization. Especially Gram negative -Gram(-)- bacteremia is associated with high mortality rates (25%) during the first 24- 48 hours of febrile episode onset
Aims: To estimate risk factors and incidence of bloodstream infection among neutropenic patients of our Hematology Department (10/2007- 9/2011) and to develop a risk score for Gram(-) bacteremia.
Methods: We evaluated 50 patients (25 male, 25 female), with a median age of 58.50 years, who underwent 106 episodes of febrile neutropenia. Bloodstream infection was diagnosed when a positive blood culture was in total compliance with clinical signs. Risk factors that were investigated were: Age, gender, malignancy type, diabetes, renal failure, insertion of central vein catheter (CVC), positive urine/ sputum culture, length of stay in the hospital, WBC/Neutrophil count, Serum Total Protein/ Albumin and prophylactic antibiotic administration. Multivariate logistic regression analysis (MLRA) was performed in order to determine independent risk factors of acquisition of blood stream infection. Covariates were included in the model if univariate analysis revealed that were significantly associated with bacteremia at a P<0.1. Independent risk factors in MLRA were used to build a predictive score for Gram(-) bloodstream infection
Results: Among 106 febrile episodes, 46 (43.4%) were attributed to bacteremia. In a univariate analysis, statistically significant risk factors for bloodstream infection were: insertion of CVC (P<0.0005), positive urine culture (P=0.03), Acute Myeloid Leukemia (AML) (P=0.009), Neutrophil Count (P<0.0005). A Multivariate Logistic Regression Analysis revealed neutrophil count (decrease of 10 degrees at the neutrophil count is associated to 4.9% higher probability of acquiring bloodstream infection) and presence of CVC (5 times higher risk) as independent risk factors. Gram(-) bacteremia in a univariate statistical analysis was significantly associated with neutrophil count (P=0.049), CVC line (P=0.01), AML (P=0.013) and positive urine culture (P=0.007). Multivariate Logistic Regression Analysis revealed positive urine culture (risk 8 times higher), presence of CVC (risk 5 times higher) and neutrophil count <250/μL (risk 5 times higher), as independent risk factors. A risk score for Gram(-) bloodstream infection was developed by weighting independent risk factors and the presence of AML (CVC+2, positive urine cuture+1.5, neutrophil count <250μL+1, AML+0.5). Diagnostic ability of our scoring system as reflected by the area under the curve of the receiver operating characteristic was 0.792 (95% CI: 0.705- 0.879). Performance of our scoring system and post test probabilities according to different prevalence rates of Gram(-) bacteremia (0.05, 0.1, 0.2) led us to propose a Gram(-) risk score >=2.5 as indicating high probability of Gram(-) infection. A detailed description of independent risk factors for acquiring bloodstream infection, and precisely Gram(-) bloodstream infection, and the performance of our Gram(-) score is shown on table attached.
Summary / Conclusion: Our scoring system identifies patients with high probability of Gram(-) bloodstream infection. If confirmed in a validation set, this score could be considered in the choice of first line antibiotics in febrile neutropenia.

B1869

POSACONAZOLE PROPHYLAXIS DURING INTENSIVE CHEMOTHERAPY FOR ACUTE MYELOID LEUKEMIA (AML): RESULTS OF A PROSPECTIVE AUDIT OF 102 PATIENT EPISODES

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Background: Aspergillus species are ubiquitous, but invasive pulmonary aspergillosis (IPA) only occurs in immunocompromised hosts. Posaconazole is a broad spectrum triazole, active against aspergillus species, which has been shown to be more effective than fluconazole or itraconazole at preventing IPA in patients receiving therapy for AML or high risk myelodysplastic syndrome (HR-MDS; N Engl J Med. 2007;356[4]:348).
Aims: This study was designed to look at the effectiveness of posaconazole prophylaxis in preventing IPA in patients receiving intensive chemotherapy for AML and HR-MDS.
Methods: Posaconazole 200mg tds was started when neutrophils were $0.8x10^9$/l and falling; and was stopped when neutrophils were $0.5x10^9$/l and rising. Data collected included length of stay, numbers of days of posaconazole and other antifungals, and whether posaconazole was started and stopped as per local protocol. Data collected included number of high resolution CT (HRCT) scans and their results including any follow up scans. We did not monitor galactomannans or perform bronchoscopy, and change in antifungal therapy was determined by radiological changes only. If an HRCT scan was positive for IPA, then caspofungin was commenced. This abstract looks at the outcomes of 102 patient episodes (57 patients) treated with prophylactic posaconazole.
Results: The mean patient age was 57.3y (range of 20-82) with median age of 61. Length of Stay mean was 21.9d (median 24, range 0-53) with 7 episodes where patients were not admitted. Mean length of posaconazole use was 22d (median 21, range 0-54). One patient did not become neutropenic and therefore did not receive prophylaxis. There was 1 dose of empirical caspofungin (given over a bank holiday weekend) and only 1 day of empirical liposomal amphotericin. Fifty five HRCT scans were performed in total (predominantly of chest, 2 of sinuses). There were 13 patient episodes where there was new radiological evidence of IPA. All but one of these was in patients during induction chemotherapy with proceeding neutropenia. One episode was in a patient during first consolidation phase that had entered a morphological but not cytogenetic remission. One patient died of haemoptysis due to IPA (*Hormographiella aspergillata* isolated at post mortem) following induction therapy but having obtained remission. Fifteen episodes received caspofungin for mean 8 days (range 1-14, median 7 days). Four episodes received liposomal amphotericin (mean 7.3d, range 1-16 days). Voriconazole was received in 5 episodes for mean of 10.2 days (range 7-16, Median 7 days).
Summary / Conclusion: No patient to date in complete cytogenetic and mor-

phological remission at the start of the chemotherapy cycle went onto develop clinical evidence of IPA. Of the 102 patient episodes in the study, there were 13 episodes where there was new radiological evidence of IPA. There was only one death from IPA in a patient who was in remission. Posaconazole prophylaxis appears effective in this situation.

B1870

PERIPHERALLY INSERTED CENTRAL CATHETERS IN PATIENTS WITH HEMATOLOGICAL DISEASES: A RETROSPECTIVE STUDY

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Background: Peripherally inserted central catheter (PICC) is an intravenous device inserted through a peripheral vein, under ultrasound guidance, and then advanced until its tip reaches the superior vein cava. There is a widespread use of PICCs in medical practice and especially in patients with hematological diseases, who usually face coagulation disorders or are at high risk for infection,and most of the times need long term central access. PICCs have eliminated the need for prolonged hospitalization, which was once necessitated for antibiotic and fluid administration, blood sampling or chemotherapy. This device is widely used due to its easy insertion, long term use and reduced risk of complications.
Aims: The present study aims to analyze the data available regarding the use of PICCs in hematological patients, using data collected from medical records of our hospital.
Methods: It is a retrospective study of 81 hematological patients, in whom a PICC was inserted in a 42-month-period, since January 2009 until June 2012. Complete data was available from the records for 72 patients. Definite catheter-related bloodstream infection was defined as the isolation of a pathological microorganism from the catheter segment and the blood drawn from the peripheral vein and/or the positive catheter tip's culture after its removal.
Results: A total of 72 patients were recruited, including 38 (52, 8%) males and 34 (47, 2%) females. In all patients PICC was inserted via a peripheral vein of the upper extremity. All insertions were successful and there were no major complications like hemorrhage or pneumothorax. The total duration with PICC use was 5504 patient days. The growth curve in the use of PICC was from 0, 75 per month in 2009 to3, 5 per month in 2012. Concerning the underlying condition of the patients data showed that: 30 patients (41, 7%) suffered from acute leukemia, 20 (27, 8%) from Hodgkin lymphoma, 14 (19, 4%) from non Hodgkin lymphoma, 3 (4, 2%) from chronic lymphocytic leukemia, 4 (5, 5%) from multiple myeloma and 1 (1, 4%) from severe iron deficiency anemia. The duration of PICC's use varied from a minimum of 1 day to a maximum of 306 days. The catheters that were routinely removed without any related complications were 39 (54, 2%), whereas those removed earlier than expected were 33 (45, 8%). Causes for earlier removal: catheter's migration in 2 cases (6, 1%), thrombosis in 2 cases (6, 1%), obstruction in 7 cases (21, 2%), localized inflammation in 3 cases (9, 1%), accidental removal in 5 cases (15, 2%), fever without definite catheter-related bloodstream infection in 8 cases (24, 2%) and definite catheter-related bloodstream infection in 6 cases (18,2%). Microorganisms isolated were: acinetobacter in 2 patients (33, 3%), serratia in 1 patient (16,7%), klebsiella in 1 patient (16, 7%), e.coli in 1 patient (16, 7%) and staphylococcus hominis in 1 case (16, 7%). In none of the patients who died, a catheter-related complication was the cause of the fatal outcome. According to our data, 9 patients died due to natural progression of their disease. The removal of the catheter in all cases was without the need of any special measures. All patients that carried the catheter on an outpatient basis received regular care by trained healthcare personnel.
Summary / Conclusion: Peripherally inserted central catheters have been used in clinical practice and seem to offer a reliable way of providing therapy and support. Their insertion and extraction is safe and simple. These catheters may be used in hematological patients, suffering from benign or malignant diseases that need long term therapy. Infectious complications, seem to be the same or even slightly better when a PICC is used versus a traditional central venous catheter. Larger comparative studies are needed in order to compare PICC and other central venous catheters concerning infectious and other complications.

B1871

ROLE OF SEDO-ANALGESIA IN EXECUTION OF BONE MARROW BIOPSIES

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Background: Both, morphological and architectural analysis of bone marrow (BM) represent one of the key aspects in the diagnosis and staging of hematological diseases. The former is obtained from the bone marrow aspiration, the latter from bone marrow biopsy (BMB). The BMB is one of the most painful procedures for hematological patients. It consists of an extraction of a boned-sized cylinder of 20x0.8 mm and it is executed on the posterior iliac crest. During the patient's medical history this procedure is often repeated several times. It generallyinvolves patients in a pediatric or old age with associated comorbidities. The BMB procedure is frequently performed in outpatients. For this reason, it has to be quick with an extremely reduced level of risk of complication. Being the procedure itself invasive, anesthesia has been introduced not to have negative phsychological effects on seriously ill patients. However, this procedure might encounter some difficulties because of the shortage of specialized personnel (anesthesiologist), lack of facilities for respiratory assistance, long standing and patient observation. It might increase clinical risk in patients with an severe comorbidity or mediastinal syndrome. In our Department, an observatory study has been carried out for the execution of BMB by using short sedation analgesia through pharmacological low posology.
Aims: The objectives of this study are: to verify the feasibility of a sedo-analgesia , to evaluate its clinical and operational advantages, to standardize the procedure, so that trained health personnel can perform it with the availability of the anesthesiologist.
Methods: From 10/11/2011 to 7/01/2012, 169 patients affected by oncohematology disease aged 12-78 years old (averaged 60 years old), 48% male and 52% female, were subjected to sedation to perform the BMB practice. Of these 169, 3 were classified in a pedriatric age, 42 were affected by cardiovascular diseases and 12 refearred neuropsychiatric diseases. Patient's medium weight was 56 kg (range 42-102 kg). In our court of patients, 85% was affected by lymphoproliferative disorders, 6% by myeloproliferative disorders, 9% by other affections. This procedure was performed morethan once in 33% of the patients. They were all monitored by ECG and by a digital oximeter. The induction was executed with 100 ug of Fentanyl push, whose quantity was reduced in pediatric and patients with acute heart disease. After 5 minutes it was followed by an administration of 7cc of Propofol at the 1% in 1-2 minutes. Reached the total sedation with spontaneous breathing with ventimask . When the BMB had started it was followed by a continuous infusion of 0,3 ml/min of Propofol as maintenance till the end of the procedure.
Results: The average duration of the sedation was 860 sec.(360-1800 sec), with a median administration of Profofol like 12cc (range 4-30cc); in 22% of the cases itwas necessary to re-induce it with 2cc of Popofol for an initial sedation at the time of the subluxation and the exportation of the bone cylinder, 3/134 (2%) had hiccups and in 1% the use of atropine was necessary. The wake up took about 110 seconds (35-390 sec.); just one patient required assisted ventilation for an acute desaturation caused by a regurgitation in a patient not completely compliant in the preparation. Any major cardiovascular side effect was verified. Discharge of patients took place about an hour after the end of sedation, no patients required hospitalization for late complications following the sedation. This one hour time comprised both, the quick wake up and the post-observation of the patient.
Summary / Conclusion: The above results have demonstrated that our protocol of sedation is effective, safe and simple to apply since it requires only the monitoring of anesthesia and the availability of the anesthesiologist.

B1872

EPIDEMIOLOGY OF BLOODSTREAM INFECTION IN FEBRILE ADULTS WITH HEMATOLOGICAL CANCER

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Background: The majority of patients with haematological malignancy are at high risk of infection during the period of neutropenia. Microbiologically documented infections, especially those with bacteriemia, remains a major concern in the treatment of hematological patients undergoing intensive cytotoxic chemotherapy. The study of the most frequent pathogens and their susceptibility must be continuosly used to guide the initial and empiric antimicrobial therapy and to develop strategies to minimize the emergence of resistant pathogens.
Aims: To describe the epidemiology of bacteriemia in adults patients with hematological cancer and febrile neutropenia (FN) in a terciary hospital.
Methods: A retrospective observational study of all positive blood cultures from June1, 2010 to January 31, 2013 from all consecutive oncohematologic patients >15 years old. The microorganism isolation was performed using BACTEC 9240 with classical identification and antibiogram disk by diffusion.
Results: A total of 193 episodes of FN corresponding to 94 patients, with 67 (34%) episodes of BSI. Male: 47 (70.14%), mean age 35.85 years (15-79); females: 20 (29.85%), mean age 32 years (16-79). Most common primary diseases associated with neutropenia were: acute lymphoblastic leukemia 29 (43%) episodes, acute myeloid leukemia 22 (32%) and non.Hodgkin´s lymphoma 8 (11%). Patients with disease relapse 18 (26%). An indwelling central catheter was present in 30 (44%) of them. We observed 36 (53%) Gram-neg-

ative bacteria infections, among *Escherichia coli* and *Klebsiella pneumoniae*, 5% and 16% respectively produced extended-spectrum β lactamases. Twenty-seven percent of *E. coli* and 25% of *K. Pneumoniae* were sensitive to cefepime, our first line empirical therapy. Twenty-nine (43%) Gram-positive infections were diagnosed (9 coagulase-negative *Staphylococcus* and 7 *Staphylococcus aureus*). Oxacilin resistance was present in 77% of CNS and in 14% of *Staphylococcus aureus*. Fungal represented 3% (2) of the isolates. Overall mortality was 14.92%, mortality caused by infection 10.44%.
Summary / Conclusion: The epidemiological profile of BSI and FN in our center and the necessity to continue the surveillance was described. Gram-negative bacteria are prevalent as cause of infection in our patients, followed by Gram-positive bacteria and fungal were uncommon.

B1873
HAND HYGIENE AUDIT AT THALASSEMIA CENTRE-DUBAI
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Background: The hands of staff are the most common vehicle by which microorganisms are transmitted between patients and staff. Hand hygiene is the single most important measure in preventing the transmission of infection and can be considered the entrance door to better infection control and safer patient care. Despite advances in infection control this simple message is not consistently translated into clinical practice. Clinical audit is identified by the World Health Organization (WHO) as one of the effective approaches to ensure compliance with hand hygiene in health care settings.

		Mean of compliance for all HCW	Mean of compliance for Nurses	Mean of compliance for Doctors
International Audit	NHS 1st Audit Feb 2007	68%	75%	50%
	NHS 2nd Audit Sep 2007	79%	84%	62%
	NHS 3rd Audit May 2008	88%	92%	75%
	NHS 4th Audit Aug 2008	90%	94%	80%
	NHS 5th Audit Nov 2008	93%	95%	84%
	NHS 1st Bimonthly Audit Mar 2009	92%	92%	86%
	NHS 4th Bimonthly Audit Sep 2009	92%	94%	88%
	NHS 8th Bimonthly Audit May 2010	95%	95%	90%
	NHS 12th Bimonthly Audit Feb 2011	95%	95%	92%
	NHS 13th Bimonthly Audit Apr 2011	95%	95%	90%
	NHS 18th Bimonthly Audit Feb 2012	95%	96%	89%
	NHS 22nd Bimonthly Audit Oct 2012	95%	96%	89%
Thalassemia Centre-Dubai	1st Audit May 2007		39%	
	2nd Audit Oct 2007		84%	
	3rd Audit July 2008	63%	85.5%	66.5%
	4th Audit May 2009	79%	83%	87.5%
	5th Audit Oct 2009	86.50%	91%	89%
	6th Audit May 2010	79%	80%	86.6%
	7th Audit Nov 2010	80.2%	83.3%	76.7%
	7th Audit May 2011	93.2%	93.6%	90%
	7th Audit Nov 2011	92.5%	94.4%	90%
	10th Audit May2012	90%	92.40%	87%
	11th Audit Nov 2012	92%	95%	88%

Table 1 Hand Hygiene Audit- mean compliance

Aims: To monitor compliance of health care providers (HCP) at thalassemia centre to hand hygiene guidelines and identify areas for improvement.
Methods: A prospective audit was conducted in 2007 at thalassemia centre through direct observationof nurses for compliance to hand hygiene guidelines before and after touching the patients. The audit was extended to involve all HCP in 2008. Thereafter, re-audits were performed twice annually (2009-2012). The grading of compliance was scored per RAG status as featured by Infection Control Nurses Association (ICNA) audit tool, used in NHS services audits; compliant(C)>85%, partially compliant (PC) 75-84% and minimally compliant (MC) <75%.
Results: The first audit conducted in May 2007 revealed 39% compliance (MC) which was improved to 84% (C) in Oct 2007 by identifying areas for improvement. This has included installment and thorough distribution of a new alcohol based hand rub that contained a moisturizer and vitamin E, in addition to continuous HCP education about the importance of their adherence to hand hygiene guidelines. In 2008, over all HCP compliance was 63% (PC) with nurses' compliance dropping to 65.5% (PC). Thereafter, compliance of all HCP could be maintained between PC to C (2009- 2010) by continuous regular education and by encouraging discussions among staff on how to improve hand hygiene. In 2011 and 2012, over all HCP compliance has improved to reach above90% (C) as shown in (Table 1). Compliance to hand hygiene after touching the patient was always better than before touching the patient. Our results are comparable to international results like NHS services and Niagara Health System.
Summary / Conclusion: Continuous re-auditing and targeted educational programs are effective approaches to improve compliance of HCP to Hand Hygiene guidelines as one of the infection control objectives.

B1874
A NOVEL FRAGILITY MARKER OF RECIPIENTS OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION : PERITRANSPLANT CMV VIREMIA
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Background: Cytomegalovirus (CMV) infection remains to be one of the major obstacles for allogeneic hematopoietic stem cell transplantation (allo-HSCT). CMV can cause infectious diseases in various organ, such as pneumonia, hepatitis, gastroenteritis, retinitis and encephalitis. In addition, the mechanism of CMV infection is complex with interaction with the immune system through several effects on NK cell function, HLA expression and cytokines production, which resulted in increased risk of transplant-related mortality. Multiple studies reported that because CMV replication is delicately balanced with host immune system, in such a situation of admitting an intensive care unit(ICU), CMV reactivation without overt disease occurred and had an association with adverse clinical outcomes.
Aims: By using realtime polymerase chain reaction (RQ-PCR), we have monitored weekly CMV viremia in allo-HSCT recipients and explored risk factors of peritransplant reactivation of CMV in allo-HSCT recipients and effect of peritransplant CMV reactivation on clinical outcomes of allo-HSCT.

Characteristics		Peritransplant CMV reactivation(+)	Peritransplant CMV reactivation(-)	p
Sex (F/M)		5 / 3	27 / 26	0.71
Age	Mean (Range)	51.5 (37-64)	48.0 (17-66)	*0.0385*
Underlying disease	Non-lymphoid AML/RAEB/RA/CMML/AA	1/0/0/1/0	24/3/1/2/1	*0.1351* (non-lymphoid vs lymphoid)
	Lymphoid NHL/ALL/Ph+ ALL/ATL/ Aggressive NK leukemia	2/2/1/1/ 0	9/6/4/2/ 1	
Donor source	BM / CB / PB	5/3/0	42/9/2	0.5031
Conditioning intensity	MAC/RIC	4/4	29/24	1
HLA matching	Match	3	23	0.4868
	Rj way: 1MM/2MM/3MM	1/2/0	16/5/3	
	GV way: 1MM/2MM/3MM	1/2/0	15/6/3	

Methods: We analyzed data for 61 patients who underwent allo-HSCT in our institution and whose CMV DNA levels were weekly monitored using blood samples by RQ-PCR method. We did not use measures for prophylaxis for CMV reactivation as routine, but used low-dose acyclovir (200mg 3-4times daily) as prophylaxis for VZV. Peritransplant CMV reactivation is defined as an event where plasma CMV DNA exceeds1,000 copies/mL during peritransplant period before engraftment. Engraftment is defined as emergence of more than 500 /μL of donor-derived neutrophils in peripheral blood.
Characteristics of patients with or without peritransplant CMV reactivation were compared using the Fisher's exact test. Predictors of peritransplant CMV reactivation was estimated in a multivariate logistic regression model. Multivariate analysis of potential prognostic factors for overall survival from stem cell transplantation was estimated in Cox proportional hazard model with time-dependent covariates of CMV reactivation.
Results: Consecutive sixty-one patients were included in the analysis. Median age was 49 (range, 17-66). All patients except 5 were CMV seropositive. Other patients characteristics is shown in Table. The blood CMV DNA exceeded1,000 copies/mL before engraftment in 8 patients (peritransplant CMV reactivation+). In multivariate logistic regression, predictors of peritransplant CMV reactivation was: previous CMV reactivation(OR: 3.87; 95% CI: 5.48- $1.18x10^3$; P=0.00258), lymphoid malignancy in non-remission state(OR: 3.14; 95% CI: 0.881- $1.15x10^3$; P=0.0656) and age of more than 50-years old(OR: 2.649; 95% CI: 1.45-$3.70x10^2$; P=0.0459). Previous chemotherapy regimen and use of immunosuppressant other than corticosteroid were not significant. The OS rate at 1 years in groups with and without peritransplant CMV reactivation were 41.7 % and 71.2%, respectively [HR: 3.23 (95% CI: 1.18-8.83), P=0.0224]. The causes of death in the peritransplant CMV reactivation group included infectious diseases not associated with CMV in 2(50%), multiorgan failure in 1(25%) and underlying disease in 1(25%).
Summary / Conclusion: Peritransplant CMV reactivation may adversely impact on clinical outcome after allo-HSCT despite of preemptive therapy for CMV same as in an ICU setting. The cause of death was not overtly associated with CMV infectious disease. For patients with risk factors of peritransplant CMV reactivation, more aggressive intervention might be warranted.

B1875

FEBRILE NEUTROPENIA EPISODES AND EVALUATION OF AVAILABLE BIOMARKERS AMONG CHILDREN WITH HEMATOLOGICAL MALIGNANCIES

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Background: Febrile neutropenia in children with hematological malignancies is related to substantial morbidity and mortality.
Aims: We prospectively monitored episodes of febrile neutropenia, complications and evaluated available biomarkers.
Methods: Prospective data collection on all episodes of febrile neutropenia (absolute neutrophil count<1000 cells/mm3) in children with hematological malignancies who were hospitalized in an 18-bed pediatric oncology department over one year period. Endpoints of this study were documentation of bacterial, viral or fungal infection and estimation of mortality. C-reactive protein (CRP) and procalcitonin were evaluated as predicting biomarkers.
Results: There were 423 admissions and 3189 bed-days during the studyperiod. A total of 78 episodes of febrile neutropenia (88% during induction chemotherapy) were identified in 37 children with median age 5 yeras old (range 1-14) and 67% girls. The majority of patients w (72%) were diagnosed with acute lymphoblastic leukemia. Documented bacterial infections were found in 22/78 episodes (19 bloodstream infections, 1 urinary tract infection and 2 other). The leading pathogen was Coagulase-Negative-Staphylococci (49%), followed by Escherichia coli (18%), Klebsiella spp (8%) and Pseudomona aeruginosa (8%). It is noteworthy that 4%% among Klebsiella spp and 39% among E.coli isolates were extended beta-lactamase producers (ESBL). There aws one documented CMV infection and 1 hepatosplenic candidiasis. A total of 3 deaths occured but only one was infection related. The median CRP in children with documented bacterial infection was 80.7, while in children without documented bacterial/viral or fungal infection was 29.3, respectively (P=0.019). Positive procalcitonin (>0.0 mcg/l) was significantly associated to proven bacterial infections (P=0.039)
Summary / Conclusion: Bloodstream infections remain the main complication of febrile neutropenia. Gram-positive bacteria have become more commonly isolated etiologic pathogens. Nevertheless, the significant increase of gram-negative episodes need special attention due to the growing incidence of antimicrobial resistance as a consequence of the widespread use of antibiotics. CRP and procalcitonin may provide useful and important assistance for guiding prompt treatment of episodes of febrile neutropenia in children with hematological malignancies.

B1876

PROCALCITONIN AND C-REACTIVE PROTEIN AS PREDICTIVE PARAMETERS FOR DEVELOPING SEVERE SEPSIS IN FEBRILE NEUTROPENIA.

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Background: The classical attitude towards treating febrile neutropenia (FN) is the intravenous administration of antibiotics, which in most cases, requires Hospital admission. In recent years a development of models that discriminate a subpopulation of patients with low-risk neutropenia may benefit from outpatient oral and / or a shorter duration of antibiotic treatment. This measure represents a significant impact on hospital resources and optimizes the quality of life of patients.
Inflammatory markers such as procalcitonin (PCT) and C-reactive protein (CRP) can help refine predictive models of low-risk febrile neutropenia validated in the literature (e.g. MASCC index).
Aims: Analyze the role of PCT and CRP as analytical predictors of clinical outcome in febrile neutropenia and its correlation with development of severe sepsis in patients with FN.
Methods: 84 consecutive episodes were collected in outpatients presenting with FN either to the Emergency Department or the Hematology Day Hospital. Values of PCT and CRP were measured on arrival, subsequently evaluating which of them developed sepsis during their clinical evolution.
Results: 41 patients (49%) had some hematologic malignant disease such as acute leukemia or myelodysplastic syndrome, 29 patients (35%) lymphoproliferative syndrome, 8 patients (10%) multiple myeloma and 6 patients (%) agranulocytosis.
24 patients (29%) developed sepsis, of whom 12 (14%) had a fatal outcome.
After having several cutoff values tested for PCT and CRP at the time of hospital care, and analyzing its correlation with development of sepsis, the optimal cut-off points based on negative predictive value (NPV) were the following:
· PCT 1 ng/ml:
  - · Sensitivity: 62.5% (42.7 to 78.8%)
  - · Specificity: 90% (from 79.9 to 95.3%)
  - · NPV: 85.7% (75 to 92.3%)
· CRP 10 mg/dL:
  - · Sensitivity: 70.8% (50.8 to 85.1%)
  - · Specificity: 70% (57.5 to 80.1%)
  - · NPV: 85.7% (73.3 to 92.9%)

Summary / Conclusion: The analytical determination of PCT and CRP is a valid tool for predicting clinical outcome in patients with FN, and can provide additional information to the predictive models of risk that already exist and therefore improve the management of these patients.
Cutoffs of 1 ng/ml for PCT and 10 mg/dL for CPR correspond to NPV around 85%.

B1877

BONE MARROW ALTERATIONS IN PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION IN THE HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) ERA, IN A HIGH COMPLEXITY UNIVERSITY HOSPITAL IN BOGOTA, COLOM

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Background: One of the most common complications of HIV is the presence of haematological alterations, that depend on different factors such as the levels of viral replication that rely upon the state of the disease, the opportunistic infections and antiretroviral therapy. Nevertheless, the physiopathological mechanisms that explain the hematological abnormalities remain uncertain. It is accurate to say that the detection of the cause of those haematological alterations would guide to an appropriate treatment.
Aims: To describe bone marrow findings in adult patients with HIV infection in the HAART era, which presented quantitative alterations in the different hematological cell lines during a four-week period.
Methods: A descriptive retrospective study of bone marrow findings in adult patients with HIV infection between 2009-2011
Results: Bone marrow findings of 52 patients were revised, the average age of the patients was 37 years±12.2, 43 patients were male (82,7%), average age in women was 38,8 years and in men 37,5 years; the time in months since the diagnosis of the retroviral infection and the bone marrow biopsy was 30.6±38.3 months; 100% of the patients was in stage C3; the average of CD4 count was 200 cells, the percentage of CD4 cells was 14% and the viral load was of 67.797 copies; 38 patients (73%) presented some sort of opportunistic infection; 27 patients were under other antibiotic treatment to control those opportunistic infections (51.9%), 41 patients received HAART (78.8%). The mean of the number of blasts in the myelogram was 1.2, promyelocytes 3.4, myelocytes 8.4, metamyelocytes 9.2, bands 10.8, neutrophils 23.8, eosinophils 3.8, lymphocytes 9.8, plasmocytes 4.8, histiocytes 1.7, monocytes 1.8 and erythroid series 23.5. In 26 patients (equivalent to 50%) hematopoiesis of the three cell lines without malignant evidence was found, myeloid hyperplasia was evidenced in 10 patients, megakaryocytic hyperplasia in 5 patients, lymphoma in 3 patients, hemophagocytosis in 3 patients, and in 5 patients epithelioid granuloma with augmentation of iron and reticular trama with unknown significance.The mean of the granulocytic line in the flow cytometry was 54,9, promyelocytes CD 13+/CD 11+4,4, myelocytes CD 13+/CD 11+ 23.4, immature neutrophils CD13+/CD11+37.5, monocytes 4.8, eosinophils 3.11, erithroid series 15.3, T lymphocytes 7.7, CD4 + 0.8, CD8 + 15,4, CD4-CD8- 0.09, CD4+CD8+ 0.05, B lymphocytes 1.84, Kappa 4.4, lambda 3.19, natural killers 0.92, plasmatic cells 1.1, myeloid CD34 1.05, lymphoid CD 34 0.3, B CD 34 precursors 0.2. There was no identified germen in the bone marrow culture in 31 patients, in 7 patients cryptococcosis was found, salmonellosis in 7 patients, tuberculosis in 5 patients and histoplasmosis in 2 patients.
Summary / Conclusion: Although 31 patients did not get any microbiological isolation is striking the high frequency of salmonellosis much higher than reported in the world literature, likewise by the conditions of our environment was often cryptococcal infection, mycobacterium tuberculosis and histoplasmosis. Additionally lymphoma was found in 3 patients, in 3 patients hemophagocytosis, and in 5 patients Granuloma with epithelioid augmentation of iron and reticular pattern with unknown significance.

B1878

EFFICACY OF POSACONAZOLE PROPYLAXIS IN AML INDUCTION THERAPY, SINGLE CENTER EXPERIENCE

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Background: Primary antifungal propylaxis with posaconazole in AML induction therapy was found to be effective in reducing infection related mortality in previous studies.
Aims: We aimed to validate the efficacy of primary posaconazole prophylaxis in our AML patients.
Methods: 45 patients with AML diagnosis who received 101 induction cycles (induction and relapse re-induction) in our center between 2009-2013 were investigated retrospectively. Patients who have received or not received primary posaconazole prophylaxis were compared with regard to further therapeutic

antifungal need and mortality.
Results: Median age was 51 (22-74), with 24 male (53.3 %) and 21 female (46.7%). Of 101 induction cycles, while 45 cycles consisted of antifungal propylaxis (36 primary and 9 secondary) 62 cycles did not. Therapeutic antifungal treatment was applied in 65 of 101 cycles. Of those, treatment was initiated as ampiric in 27, pre-emptive in 34 and evidence based in 4 cycles. In patients receiving posaconazole prophylaxis, antifungal treatment need decreased (56.6 vs 69.2 %) but was not statistically significant (P=0.169). Overall median antifungal administration period (including prophylaxis) was 28 days (15-54) in prophylaxis group and 16 days (3-42) in non-prophylaxis group and it was statiscially significant (P< 0.001). When infection related mortality was taken into account, while no mortality was observed in primary prophylaxis group, the non-prophylaxis group consisted of 13.8 % mortality with statistical significance (P=0.025).
Summary / Conclusion: Primary prophylaxis with posaconazole reduced infection related mortality in AML patients without a significant decrease in total antifungal need and administration duration.

B1879

THE RELATIONSHIP BETWEEN LEVELS OF SERUM MANNOSE-BINDING PROTEIN AND BACTERIEMIA/SEPSIS IN FEBRILE NEUTROPENIC CHILDREN WITH CANCERS

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Background: Cancer patients with febrile neutropenia after chemotherapy have a variable risk of bacteriemia/sepsis. Especially Gram-negative bacteremia is associated with high mortality and/or morbidity. Early diagnosis of patients with bacteriemia/sepsis at the onset of febrile neutropenia is potentially useful in tailoring therapy.
Aims: The main aim in this study was to determine the diagnostic relevance of mannose-binding protein (MBP) levels for predicting bacteriemia/sepsis in children with febrile neutropenia.
Methods: In a prospective study, 54 febrile neutropenic epizodes from 30 pediatric cancer patients whose age ranged from 15 to 191 months old (median age 62,5 months, 14 female and 16 male patients) were analysed. Samples were obtained in two different clinical periods: afebrile neutropenic period after chemotherapy and febrile neutropenic period (on the first day of fever). MBP levels were determined. Patients were divided into three groups: those with clinically or microbiologically documented infection, bacteriemia/sepsis, and fever of unknown origin.
Results: Twenty seven (50%) episodes were clinically or microbiologically documented infection, 17 (31,5%) episodes were fewer unknown origin, 10 (18,5%) episodes were bacteriemia/sepsis. The measured levels of MBP in febrile neutropenic period were not useful for identifying the types of febrile neutropenia. In all patients, significant differences were documented between MBP values of the afebrile neutropenic patients and the peak values of the febrile patients (P<0,001). In the bacteriemia/sepsis and fever of unknown groups, significant differences were also documented between MBP values of the afebrile neutropenic patients and the values of the febrile patients (P=0.001 and P=0,03). In clinically or microbiologically documented infection group, there was no significant difference between MBP values of the afebrile neutropenic patients and the values of the febrile patients.
Summary / Conclusion: An initial MBP level may not predict bacteremia/sepsis in cancer patients with febrile neutropenia.

B1880

COMPARISON OF THE SIDE EFFECTS OF ANTIBIOTICS IN PATIENTS WITH THE DIAGNOSIS OF FEBRILE NEUTROPENIA

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Background: Febrile neutropenia is a life-threatening complication that is common in patients with hematological malignancies.
Aims: Side effects of antibiotics used in febrile neutropenic patients were retrospectively reviewed, and in febrile neutropenic patients and to determine whether the effect of an increase in mortality and morbidity.
Methods: Patients with febrile neutropenia who were hospitalized and treated in the hematology clinic of our hospital between January 2005 and December 2011 were retrospectively evaluated.
Results: A total of 127 neutropenic fever episodes noted in 71 patients were evaluated. Of the fever episodes, 75 were noted in men and 52 were noted in women. The primary disease was acute leukemia in 94 (74%) episodes, Hodgkin's lymphoma in3, non-Hodgkin's lymphoma in8, agranulocytosis in3, hairy cell leukemia in3, chronic lymphocytic leukemia in3, blastic phase chronic myeloid leukemia in6, myelodysplastic syndrome in5, primary myelofibrosis in 1 and multiple myeloma in 1 episode. In this study, while the rate of change in drug combination for any reason was 27.3% in the imipenem + ciprofloxacin / amikacin (ICA) group, it was 60% in the P/T + ciprofloxacin/amikacin (PTCA) group; the difference between the groups was significant (P=0.006). The rate of drug change due to side effects was significantly higher in the PTCA group than the ICA group (P=0.03). The rate of reducing fever within 72 hours was significantly higher for imipenem combinations than P/T combinations (P=0.05).The rate of side effects was 19.3% for imipenem combinations while it was 30% for P/T combinations (P=0.29). The rate of grade 3–4 mucocytis was 9.2% in the ICA group and it was 5.3% in the PTCA group. However, the difference did not reach statistical significance. The mean duration of antibiotic use was 24.75 days in the ICA group and it was 27.82 days in the PTCA group. The difference did not reach statistical significance (P=0.5). The frequency of side effects was 28.8% in amikacin combination and it was 10.6% in ciprofloxacin combination (P=0.02). Gram-positive bacteria were the most common isolates. Consistent with the literature, coagulase-negative staphylococci were the most common pathogen isolated (36.5%), followed by E. coli (13%), Klebsiella spp. (9.7%), and Enterococcus faecalis (8.9%). E. coli were isolated in 16 episodes. The ratio of resistance to the number of antibiograms was 0/6, 6/8, 7/13, 0/15 for amikacin, P/T, ciprofloxacin and imipenem, respectively. Klebsiella spp. were isolated in 12 episodes. The ratio of resistance to the number of antibiograms was 1/12, 5/12, 6/12, 4/12 for amikacin, P/T, ciprofloxacin, and imipenem, respectively. Acinetobacter spp. were isolated in 11 episodes. The ratio of resistance to the number of antibiograms was 5/11, 6/11, 9/11, 7/11 for amikacin, P/T, ciprofloxacin, and imipenem, respectively. P/T resistance was more common, and the ICA group being more successful than the PTCA group was attributed to this finding.
Summary / Conclusion: In conclusion, in the treatment of neutropenic fever, every hospital should determine an empiric antimicrobial therapy regimen that is convenient with its microbial flora in accordance with the guidelines. We found that imipenem combinations were more effective as an empiric antimicrobial therapy in our hospital. In accordance with the literature, we found no significant difference between combination therapy and monotherapy in terms of efficacy. Side effects were more common in patients receiving amikacin.

B1881

FEBRILE NEUTROPENIA: INCIDENCE, FOCI OF INFECTION, AND UNDERLYING MICROBIOLOGICAL PATHOGENS

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Background: Patients with neutropenia have an increased risk for infections, especially bacterial and fungal infections. Febrile neutropenia is a potentially life-threatening complication.
Aims: The aim of our study was to identify the overall incidence of febrile neutropenia, specific foci of infection and the microbiological agents causing the infection.
Methods: Inpatients on hematological and infectiological wards with neutropenia were investigated on a regular basis. For practical reasons, neutropenia was supposed if leukocyte counts were below 1000/µl. Periods of neutropenia and febrile neutropenia were registered within a computer database.
Results: Investigation period was July 2012 until February 2013. 283 periods of neutropenia were included. Underlying diseases were: 1. Acute myeloid leukemia (N=115: 41%), 2. Non-Hodgkin-Lymphoma (N=52: 18%) and Hodgkins's disease (N=11: 4%), 3. Multiple myeloma (N=43: 15%), 4. MPS/MPN/Aplastic anemia/others (N=30: 11%), 5. Solid tumours (N=16: 6%), 6. Acute lymphoblastic leukemia(N=15: 5%). Others: 1 (Sarcoidosis). Febrile neutropenia occurred in 159 out of 283 periods (56%), 124 periods (44%) remained without fever during hospitalization. In the 159 febrile neutropenia episodes clinically documented infections could be detected in 116 cases (73%). Further evaluation revealed the following foci: Bacteremia (N=68: 34%), pulmonal infection (N=36: 18%), infection of central venous catheter (CVC) (N=35: 17%), abdominal infection (N=24: 12%), fungal infection (N=22: 11%), urological infection (N=15: 8%). Outcome of infections in cases with febrile neutropenia were cure (N= 136: 86%), improvement (N= 15: 9%), death (N=5: 3%), stable state (N=2: 1%), progress (N=1: 1%) during the febrile episode. Microbiologically documented infections could be detected in different materials: Blood culture (N=125: 52%), tip of CVC (N=31: 13%), urine (N=24: 10%), stool sample (N=20: 8%), wounds (smear) (N=13: 5%), anal-/rectal smear (N=10: 4%), sputum (N=8: 3%), bronchoalveolar lavage (N=4: 2%), serum (N=4: 2%), throat smear (N=3: 1%). The following pathogens were detected: Coagulase-negative staphylococci (N=89: 35%), E.coli (N=23: 9%), E. faecium (N=21: 8%), Enterobacteriaceae (N= 13: 5%), P. aeruginosa (N= 12: 5%), E. coli (ESBL): (N=12: 5%), Aspergillus spp.: (N=9: 4%), E. faecalis: (N=9: 4%), C. difficile: (N=8: 3%), Candida non-albicans: (N=7: 3%), Enterococcus spp.: (N=7: 3%), Streptococcus spp.: (N=7: 3%), S. aureus: (N=6: 2%), Candida albicans: (N=5: 2%), Others: (N=23: 9%).
Summary / Conclusion: Most patients in our cohort had underlying haematological malignancies. The incidence of febrile neutropenia in our cohort was

56%, and therefore lower than reported in the literature, which may be due to antimicrobial prophylaxis with a fluoroquinolone in neutropenia. Interestingly, clinically documented infections in those patients with febrile neutropenia could be observed in 73%, which is much higher than previously reported in the literature. The microbiologically documented infections showed coagulase-negative staphylococcus as the most common pathogen. Gramnegative organisms were detected in 24% of the microbiologically documented infections with a substantial amount of ESBL E. coli (5%). These observations strengthen the need for careful documentation and microbiological assessment of patients in neutropenia to evaluate the incidence, risk factors and trends for microbiological agents.

B1883

VISCERAL LEISHMANIASIS ASSOCIATED HAEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: A CAREFUL BALANCING ACT

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Background: The clinical course of visceral leishmaniasis (VL) and the haemophagocytic lymphohistiocytosis (HLH) have a significant degree of overlap, causing diagnostic confusion. This case illustrates that patients taking immunosuppression can be particularly vulnerable when travelling to endemic areas, and the potential favourable outcome if detected and treated promptly.
Aims: A 45 year old lady presented to the general medical take with a one month history of fever, night sweats and weight loss. She had a background history of orofacial granulomatosis, diagnosed 6 years previously, and was receiving maintenance azathioprine and low dose prednisolone. Ten months prior to this, she had been on holiday to southern Spain.
Methods: On admission, her full blood count showed pancytopenia with severe neutropenia, and raised inflammatory markers. She was treated with broad spectrum intravenous antibiotics and her immunosuppression was stopped. An initial bone marrow aspirate showed evidence of HLH, with no excess lymphoid cells. At this stage, her ferritin was elevated at 8700ng/mL, triglycerides were 3.6mmol/L and EBV DNA PCR was raised at 6162 copies per mL. She was treated with intravenous immunoglobulin, dexamethasone and ciclosporin as per the local HLH protocol, with the omission of etoposide. Her symptoms and blood counts improved significantly, and she was discharged with a steroid taper, maintenance ciclosporin and regular follow up. Unfortunately, she was readmitted after one week with a severe steroid induced proximal myopathy. Shortly after this, her pancytopenia worsened, and a repeat bone marrow was arranged. This showed ongoing HLH, but with widespread evidence of leishmania amastigotes. Her immunosuppressive therapy was rapidly reduced, and treatment was started with liposomal amphotericin.
Results: Following this, her symptoms and blood counts had a sustained improvement for several weeks. Despite the anti-leishmaniasis treatment, the patient became pancytopenic again. A further repeat bone marrow showed a marked decrease in leishmania amastigotes, but with significantly increased haemophagocytosis. Her case has subsequently been managed with a careful balance of immunosuppression, followed by further liposomal amphotericin. In addition to this, prophylactic monthly pentamadine infusions were commenced. To date, her symptoms have greatly improved, and her full blood count has normalised.
Summary / Conclusion: HLH associated VL is very rare, with only 8 cases reported in adults in a recent systematic review*. This is due to both the overlap of the clinical features and difficulty identifying amastigotes early in the course of the disease. The use of immunosuppression in HLH, though initially beneficial in dampening the inflammatory response, can lead to overwhelming VL. Therefore, a thorough travel history, and repeated bone marrow examination where there are concerns with diagnosis are essential for diagnosis. Treatment with liposomal amphotericin is effective, with possible benefits with intravenous immunoglobulin and low dose steroid as an adjunct.
*Rajagopala et al (2008) "Visceral leishmaniasis associated hemophagocytic lymphohistiocytosis – case report and systematic review" *Journal of Infection* **56**, 381-388

B1884

HEPATO-SPLENIC NECROTIC NODULES WITH NORMAL HEPATIC ENZYMES AND HEPATIC SYNTHETIC FUNCTION - AN UNUSUAL PRESENTATION OF ADENOVIRAL INFECTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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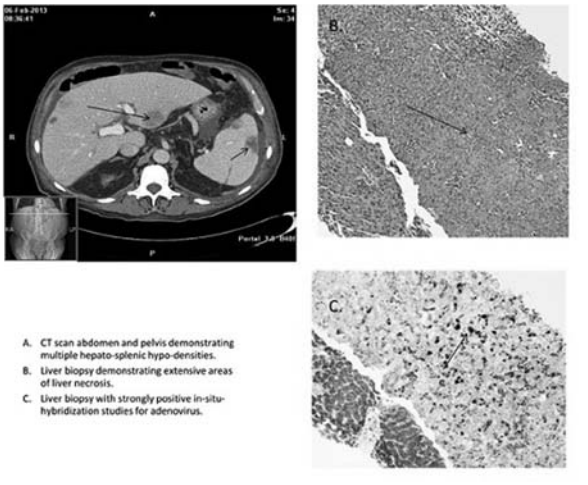
Background: Adenoviruses are non-enveloped double stranded DNA viruses belonging to the family Adenoviridae. Among the 52 identified serotypes, only a few are common clinically and include serotypes,1,2,3,5,7, 21, and 41. Majority of Adenoviral infections occur in the first decade of life, with both humoral and cell mediated immunity being critical in preventing and limiting infections. Adenoviral infections are also seen in patients that have undergone allogeneic stem cell transplants (SCT), with an estimated incidence of between 5-20%. Common presenting features include fevers, respiratory illness, pharyngo-conjunctival fever, hemorrhagic cystitis, gastroenteritis, with rare cases of disseminated adeno-viremia resulting in multi organ dysfunction, including acute hepatitis and hepatic necrosis.
Aims: To describe an unusual presentation of adenovirus infection with multiple hepato-splenic necrotic nodules in a patient who underwent allogeneic SCT.
Methods: The patient is 67 year old male, who underwent a reduced intensity conditioning (Fludarabine, BCNU and melphalan) 9/10 HLA mismatched unrelated donor allo SCT for transfusion dependant *JAK2*V617F mutated primary myelofibrosis. He received graft versus host disease (GVHD) prophylaxis with tacrolimus and methotrexate. Due to the development of seizures the tacrolimus was discontinued and he was started on sirolimus. At D+60 he developed grade II acute GI GVHD and was started on enteric coated budesonide and mycophenolate mofetil, with good response. He presented at D+125 with a self-limited prodrome consisting of; fevers, pharyngitis, nausea, vomiting, diarrhea and vague abdominal pain. A CT scan abdomen/pelvis demonstrated multiple hepato-splenic nodules (figure one A).



Results: His liver enzymes and hepatic synthetic function at presentation and through his clinical course remained normal. On admission, blood cultures and viral studies were drawn. An adenovirus PCR was positive, with a viral load of 500,000 copies/ml. He underwent an ultrasound guided biopsy of a liver nodule. This demonstrated extensive hepatic necrosis with strong in-situ-hybridization studies for adenovirus (figure one B & C). A transesophageal echocardiogram was negative for a cardioembolic source. His immunosuppression was tapered over the next 4 weeks and he received 2 doses of IV Cidofovir with an excellent response. At the time of writing this report his viral load was down to 20,000 copies/ml. He has had no recurrence in his GI GVHD and he is due to receive an additional two doses of cidofovir.
Summary / Conclusion: Adenovirus is not an uncommon opportunistic pathogen in immunocompromised hosts. While most cases present with limited respiratory or gastrointestinal manifestations, in a few patients hemorrhagic cystitis, keratoconjuctivitis, acute hepatic necrosis and multiorgan failure can occur. We describe a unique case of severe adenovirus associated hepatosplenic necrosis without alteration of liver enzymes or synthetic function. Withdrawal of immunosuppression and cidofovir are effective treatment strategies.

B1885

MICAFUNGIN FOR ANTIFUNGAL PROPHYLAXIS IN PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: Micafungin is an echinocandin antifungal approved for prophylaxis in patients undergoing hematopoietic stem cell transplantation. It has a broad spectrum against yeasts and filamentous fungi.
In our institution, we employ Fluconazole as first line option for prophylaxis in patients under autologous stem cell transplantation (ASCT), since the absolut neutrophils count (ANC) is less than 1000/mm^3, until two consecutive days above 1000/mm^3. For patients who are intolerant or resistant to azoles or have shown a previous Invasive Fungal Infection (IFI), we use Micafungin at doses of 50 mg daily as prophylaxis.
Aims: Analyzing the efficacy and safety of the use of Micafungin for antifungal phophylaxis in patients undergoing ASCT.
Methods: We have used the incidence of fungal infection and the proportion of patients requiring empirical treatment as measurement of the efficacy.
We have measured baseline levels of creatinine and total bilirubin to determine safety and we have measured the maximum value of these parameters under treatment with micafungin.
Between November 2011 and January 2013, 34 patients have received Micafungin at doses 50 mg daily. 23 of them were male. The mean age at transplantation was xx. Diagnoses were fifteen Non-Hodgkin Lymphoma (12 B and 3 T), six Hodgkin Disease, two Acute Myeloid Leukemia, nine Multiple Myeloma and 1 Case of Ewing Sarcoma. The median of days of granulocytes under 1000/mm^3 was 12 days (10-16). The mean baseline creatinine was 0.88 mg / dL (0.4-1.9) and the baseline level of total bilirrubin was 0.71 mg/dL (0.36-1.44). No patient had developed previous IFI.
Results: The median duration of treatment with micafungin was 15 days (11-23). Only two empirical treatments were initiated (6.06%) (with Voriconazole and Anidulafungin) and none of the cases developed IFI (0%). One case presented colonization by Candida Albicans and other one had persistent fever, isolated with Staphylococcus Aureus in blood culture later.
With regards to tolerance, there were no reports about any infusional reaction. The mean maximum creatinine was 1.23 with an average change of 0.34 mg / dL from baseline. Regarding hepatotoxicity, the maximum average number of total bilirubin was 1.34 with a variation of 0.59 mg / dL.
Summary / Conclusion: 1. Micafungin at doses of 50 mg/24 hours, is an effective and safe option for antifungal prophylaxis in patients undergoing ASCT. 2. In our series, there was no development of IFI and in most patients it was not necessary to use empirical treatment. 3. The safety of the treatment was excellent, without tolerance problems. The kidney and liver function was not altered by treatment with micafungin. 4.We will need to be stricter in the future in implementing the protocols in place, as the median of micafunina use (15 days) exceeds in three days the median of ANC phase below to 1000/mm^3 (12 days). 5. Retrospective studies are needed comparing these results with historical cohorts with itraconazole and fluconazole in our instituion, and cost-effectiveness studies to confirm these benefits.

B1886

REAL LIFE EXPERIENCE OF ANTI-FUNGAL PROPHYLAXIS WITH POSACONAZOLE IN PATIENTS WITH ACUTE LEUKEMIA AT A SINGLE CENTER LACKING HEPA-FILTER

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Background: Invasive fungal disease (IFD) is an important problem complicating the therapy of
patients with acute leukemia receiving induction chemotherapy. Both yeasts and molds cause serious infections. Candida is the most important yeast pathogen and rates of invasive Candida infection in patients with hematologic malignancies not receiving antifungal prophylaxis range between 8 to 24 percent. On the other hand, aspergillus is the most common mold pathogen and in patients with acute myelogenous leukemia , the incidence of invasive aspergillosis ranges between 5 to 10 percent. Recently, patients receiving induction chemotherapy for acute myeloid leukemia were shown to have fewer IFD and mortality attributable to IFD with posaconazole prophylaxis.
Aims: To document the real life experience with posaconazole on the incidence of IFD during the course of remission induction chemotherapy in patients with acute leukemia who were hospitalised in a single center without hepa-filter .
Methods: We conducted a retrospective review of patients with de novo or relapsed acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL) who were hospitalised for remission induction chemotherapy from December 2010 to December 2012. Our policy of anti-fungal prophylaxis was changed in June 2012 and we started to give posaconazole prohylaxis in AML patients. We compared our fungal disease incidence in AML patients before (group A) and during posaconazole period (group B).
Results: Fourty-seven treatment episodes for remission induction in 37 patients were included. Among these episodes, 22 were in ALL and 25 were in AML patients. A total of 28 (%59) episodes were noted to end up with the need for anti-fungal treatment , despite prophylaxis in 15 (%53) of them. Considering ALL, prophylaxis with fluconazole and posaconazole were given in 17 and 2 treatment periods respectively, and no prophylaxis was given in 3 episodes. A total of 8 (%36) episodes were ended up with anti-fungal treatment in ALL patients:1, 2 and 5 episodes for empiric, probable and possible IFD respectively. Regarding AML, there were 13 episodes in which remission induction was given without anti-fungal prophylaxis (group A) and among these 11 (%84) were ended up with need for anti-fungal treatment: the number of episodes for empiric therapy, probable and possible IFD were4, 1 and 6 respectively . There were 12 episodes after the initiation of policy for posaconazole prophylaxis (group B). In this group, despite posaconazole prophylaxis 9 (%75) patients needed anti-fungal treatment during neutropenia:3, 4 and 2 episodes for empiric, probable and possible IFD respectively. Analysis of the reasons to initiate anti-fungal treatment revealed 3 Candidial infections which were all in group A. There was no Candidial breakthrough in group B. Considering breakthrough with Aspergillus there was no difference between the two groups.
Summary / Conclusion: We found that in patients undergoing remission induction chemotherapy for AML posaconazole prophylaxis prevented Candidial infections, but it did not have any effect on Aspergilus infection. This may be due to the small number and retrospective nature of our study, since we were not able to evaluate the patients' drug competence during the prophylaxis period and posaconazole is known to be affected by meals and other drugs like proton pump inhibitors. It is necessary to evaluate the value of anti-fungal prophylaxis with posaconazole in the real life scenarios with prospective observational studies.

B1887

INCIDENCE, MORTALITY AND CAUSATIVE PATHOGENS OF SEPTIC SHOCK IN NEUTROPENIC PATIENTS AFTER INTENSIVE CHEMOTHERAPY

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Background: Chemotherapy used during induction and consolidation for acute leukemia causes severe agranulocytosis (ANC <500/mm3) lasting more than 10 days and patients are in a high risk of developing severe sepsis or septic shock.
Aims: Evaluation of septic shock in neutropenic patients.
Methods: 64 patients with diagnosis of acute leukemia (48 pt. AML and 16 pt. ALL) were treated with intensive chemotherapy at the University Hospital in Bratislava between September 2010 and December 2012. Together we have administered 149 cycles of chemotherapy (induction 64, second induction 21 and consolidation 64 times).
Results: Incidence of septic shock after intensive chemotherapy was 13,5% (20/149) and proven bloodstream infections (BSI) with positive blood culture was in 55% (11/20). Isolated patogens were gram-negative (7x) and gram-positive (4x) bacteria. Among gram-negative bacteria were isolated 2 carbapenem-resistant K.pneumoniae and 1 carbapenem-resistant P.aeruginosa, resulting in irreversible shock. Mortality rate from septic shock in neutropenic patients after chemoterapy was 30% (6/20).
Summary / Conclusion: Sepsis remains a leading cause of mortality in neutropenic patients. Despite of a great improvement in managment of septic shock, we stand in front of the problem, what to do with increasing number of carbapenem-resistant bacteria.

B1888

THE DEFEAT OF THE COLON BY HEMOBLASTOSES: CLINICAL, ENDOSCOPIC, IMMUNOHISTOCHEMICAL FEATURES AND OPTIMIZATION OF TREATMENT

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Background: Conducting polychemotherapy (PCT) in patients with hematological malignancies in 20-40% of cases accompanied by a lesion of the colonic mucosa (CM).Mechanisms of epithelial lesions of the colonic mucosa in patients receiving chemotherapy, not sufficiently studied, and the successful treatment of colorectal disease largely determines the success of treatment of the underlying disease.Thus, improving supportive care lesions of the colon in patients with hematological malignancies is actual scientific task.
Aims: To assess the effectiveness of dibikorum in the treatment and prevention of colorectal pathology in patients with hematological malignancies, based on studies of apoptosis index and cellular regulation molecules (Ki-67 and TLP).
Methods: Total 135 patients were examined: 120 patients with hematological malignancies receiving polychemotherapy (PCT) and 15 healthy people. All

the patients were divided into 2 equal groups, depending on the severity of lesions of colorectal colon: Division 60 patients with first degree mucositis and 60 patients with the second degree mucositis. Patients in each group are 2 equal subgroups: in the first subgroups conducted therapy from linex end enterol; in the second conducted therapy from enteral, linex and dibikorum. Patients were surveyed in dynamics to treat colorectal disorders and 4 weeks later. All patients were sigmoskopy, General morphological study, determined by the index of apopotosis (Iapt) colon cells, immunohistochemical studies conducted using monoclonal murine antibodies to marker of proliferating cells-Ki-67 (1: 100, Novocastra, USA) and transterritin-like protein (TLP) (1: 100, Novocastra, USA).
Results: Application of dibikorum in the treatment of colorectal pathology in patients with hematological malignancies receiving PCT, to achieve the best clinical outcomes compared to patients without dibikorum. In patients receiving dibikorum, is significantly more severe clinical symptoms early colorectal disorders (bloating, diarrhoea), accompanied by the restoration of the mucous membrane of the large intestine and the improvement of parameters of cellular homeostasis (decreased Iapt, increased expression of Ki-67 and TLP).
Summary / Conclusion: Application of dibikorum in the treatment of patients with hematological malignancies receiving PCT, improves the process of cellular homeostasis colon cells in terms of reducing the Iapt, increase of the expression of Ki-67 and TLP, accompanied by early termination clinical symtoms intestinal disorders, normalization of macroscopic and microscopic structure of mucous membranes of the colon.

B1889

THE EFFICACY OF PALONOSETRON IN MANAGING SYMPTOMS INDUCED BY HIGH AND MODERATE EMETOGENIC CHEMOTHERAPY-THE EXPERIENCE OF FUNDENI CLINIC OF HEMATOLOGY

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Background: Antiemetic agents represent an useful tool for supportive care in patients with hematological malignancies treated with high and moderate emetogenic chemotherapy.Their role is directed through controlling nausea and emesis induced by chemotherapy both in acute (0-24 hours from the first dose of chemotherapy) and late phases(24-120 hours). The antagonists of $5HT_3$ receptors represent the basis of antiemetic therapy. Palonosetron is a major antagonist of $5HT_3$ receptors with an unique profile of action due to high receptor's affinity, long lasting action and high efficacy in both acute and delayed phases of emesis; it is preferred to be administered in high emetogenic chemotherapy and in multi day schedules.
Aims: assessment of complete response (no emesis, no nausea) in acute and late phases after moderate or high emetogenic chemotherapy and safety profile of Palonosetron
Methods: an unicentric, retrospective, clinic-epidemiological, observational study(march-september 2012) including 57 patients treated in Fundeni Clinic of Hematology using moderate and high emetogenic chemotherapy and multiday schedules of treatment . Palonosetron was administered as first intention or second line antiemetic therapy. We included patients with a large broad of hematological malignancies (31 patients with chronic lymphoproliferative syndroms, 10 patients with Hodgkin's lymphoma, 1 patient with chronic myelogenous leukemia treated with Imatinib 600 mg/day, 10 patients with acute myeloblastic leukemias and 5 patients with acute lymphoblastic leukemias). There was a female preponderence (40 female patients - 68,9%) . 22 patients were treated with Palonosetron as first line antiemetic therapy, all of them associated to high emetogenic chemotherapy (ABVD,DHAP, multiday regimens in acute leukemias). 5 patients from those who were previously treated with first generation $5HT_3$ R inhibitors experienced anticipatory emesis
Results: in acute phase (first day of chemotherapy) 47patients (81%) had complete response (no emesis, no nausea) and 56 patients (96,5%)with complete response during late phase (24-120 hours). We identified as risk factors for anticipatory emesis the female sex and younger ages. The repeated administration of Palonosetron in day 3 and 5 of a multiday regimen was associated with complete response both in acute and late phases of emesis. The most frequent adverse reactions were constipation and headache.
Summary / Conclusion: Palonosetron is efficient not only in controlling symptoms induced by moderate and high emetogenic chemotherapy regimens but also for anticipatory emesis. Adverse reactions were easily manageable.

B1890

COMPARISON OF ANTIFUNGAL AGENTS EFFECTS AND SIDE EFFECTS IN NEUTROPENIC FEVER ATTACKS

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Background: Febrile neutropenia (FEN) is a life-threatening complication that is common in patients with hematological malignancies. Invasive fungal infections (IFI) are serious cases of mortality in neutropenic patients. In order to treat these infections early and right time are preferred instead of targeted antifungal therapy.
Aims: The aim of this retrospective study was to evaluate efficiency of antifungal treatment and side effect of antifungal agents in patients with high-risk febrile neutropenia (the expected duration of neutropenia was more than 7 days and PNL count <100/mm^3)
Methods: Between January 2005 and March 2012, 78 febrile neutropenic patients was retrospectively analyzed in Manisa Celal Bayar University, Department of Hematology.
Results: 78 patients **(**43 (55%) male and 35 (45%) female and 46.7±15.06 years) were included in the study. The distribution of disease was AML, ALL, chronic leukemia, lymphoma and other hematologic malignancies and ratios were 50%, 12%, 7%, 15%, 6% respectively. In 78 patients, 135 FEN attacks were evaluated. 90 FEN attacks were received antifungal agents. Empirical antifungal therapy was given early and changed by clinical, microbiological and serologic results for the high efficacy. 45 FEN attack were not needed any antifungal treatment. The efficacy of first-line antifungal therapy was 72% (65/90 FEN). The efficacy of second-line antifungal therapy (i.e. refractory to (25/32) or intolerant of prior antifungal therapy) was 97% (30/32 FEN). The side effect of first line antifungal therapy was 48% (43/90 antifungal used FEN). The side effect of second-line antifungal therapy was 42% (13/32). First and second-line antifungal therapy were included conventional amphotericin B, liposomal amphotericin B (LAmB), caspofungin, vorikonazol and flukonazol. The side effects of first and second-line antifungal therapy were infusion related, metabolic and visual toxicity, hepatotoxicity, rash, diarrhea and their numbers and ratios were 3(5%), 49(86%), 2(3,6%), 1(1,8%), 1(1,8%), 1(1,8%) respectively. Conventional amphotericin B was used in three FEN attack and infusion-related side effects were seen. Within these severe anaphylaxis reaction was observed in one of them. Metabolic side effects such as hypokalemia, hypomagnesemia were observed in LAmB used patients. These side effects were less in patients who used voriconasol. Voriconazole-induced transient visual disturbance were observed in FEN attack. And also hepatotoxicity was observed in LAmB used patients. The another side effects such as rash and diarrhea were observed in fluconazol used patients. In this study 27 fungal pathogens (27/90) were isolated microbiologically and/or histopathologically in 27 FEN. C.albicans, C.tropicalis, C.glabrata, C.crusei, Aspergillus, yeast species were found in 12(44%), 3(11%), 2(8%), 1(4%), 3(11%), 6(22%) respectively. The most isolated fungal pathogen was C.albicas (P=0.001). After the isolation of fungal pathogens antifungal treatment were given in accordance with the guidelines of antifungal treatment to these patients (7 patients; liposomal amphoterisin, 12 patients; caspofungin, 2 patients; vorikonazol, 5 patients flukonazol and 1 patient; posakonazol)
Summary / Conclusion: Most common observed side effect was metabolic side effect in our study but treated without mortality. Future studies with larger number of patients are needed to confirm these results.

Transfusion medicine

B1891

EFFECT OF BLOOD DONATIONS ON IRON STORES OF BLOOD DONORS

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Background: A blood donor looses approximately about 200 to 250 mg of iron per donation which corresponds to a loss of four to ten percent of total iron. Loss of iron replaces quickly by mobilizing iron stores, followed by filling the iron stores if diet is adequate. The situation, however, is different for donors with high frequency of blood donations. For men who donating six or more times in the last two years; serum ferritin level has began to decrease within time. In the absence of iron replacement this can lead to emptying of iron stores. We undertook this study to evaluate the effect of frequent blood donations on iron stores of regular male blood donors in Turkey.
Aims: To evaluate the effect of frequent blood donations on iron stores of blood donors in Turkey.
Methods: This study was planned prospectively with randomization of blood donors into two groups. The first group was 'frequent donation group' that comprised of donors who had donated more than three times in the last year and more than 6 times in the last two years. The second group was 'infrequent donation group' that comprised of donors who was donated one or two times in the last year. Serum hemogram, serum iron, total iron binding capacity, ferritin, peripheral blood smear and monthly income of all cases were evaluated.
Results: 169 cases were recruited to study. 52/169 (30%) of cases were classified in 'frequent donation group'. Any of the cases in this group was not deferred from blood donation because of low haemoglobin level. The other 117 were classified in 'infrequent donation group'. There was no statistically significant differences in hemoglobin, serum iron, total iron binding capacity, mean corpuscular volume, peripheral blood smear findings and monthly income between two groups. Mean serum ferritin level was 45 ng/ml in frequent donation group and 55 ng/ml in infrequent donation group and these results were statistically significant (P<0,05).
Summary / Conclusion: Being a frequent blood donor is a condition that could lead to the development of iron deficiency in blood donors. Monitoring ferritin levels yearly for frequent blood donors and education of the donors about iron supplementation and diets are highly recommended.

B1892

CERTIFICATION AND EDUCATION PROGRAM. HOW TO IMPROVE QUALITY MARKERS OF TRANSFUSION THERAPY IN HOSPITAL

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Background: The regular assessement of blood bank activity allows us to know changes over time and influence clinical practice at point "weak" system. Actions taken in order to modify and standarize transfusions procols and transfusion indications in hospital practice are difficult and complex due to staff resistance to change.
Aims: We analyse de dates in order to know if certain mesures are be able to change clinical practice and improve quality markers of transfusion therapy specially those related to the percentage of fresh frozen plasma transfusion
Methods: We examine whether the actions carried out: 1.-active committee of transfusion with publishs update monthly statistics, 2.-transfusion guide, 3.-joint meetings with all hospital sections and 4.-a program of clinical medical education (CME) and 5.-Process of certification by an external agent (CAT)achieved in april 2010; have had a real impact on the comsumption of blood products. We analyse the blood products transfused and quality markers specially those related with FFP transfusión.
Results: During the past 17 years we have transfused a total of 33.106 blood products (1947 per year). ther is no clear trend in terms of global transfusion and erythrocyte transfusion, but there is a significant decrease in the case of fresh frozen plasma and platelets concentrates, specially in the case of FFP in year 2000 and in the periode from year 2009 to 2012, a sistematically CME program was developed in both cases previously. The improvement of packed erythrocytes/fresh frozen plasma ratio reached was 11,1 in year 2000 and 38,6 in year 2012. This is very important because we can product other products: inmunoglobulins, albumin, factor VIII concentrates with this raw material.
Summary / Conclusion: 1.-Ther is a stability in transfused blood products over time. Global average ratio: blood products/hospital admissions: 0,31.
2.-Ther is a statistically significant decrease transfusion of FFP from 2009 to 2012 that produces an improvement in packed erythrocytes/fresh frozen plasma ratio= 38,6 higher to that found after the development of a law about plasma safety in year 2000. Our problem today is plasma expiry date, so we have only a short stock of blood group plasma AB and A.
3.-The CME "campaigns" on transfusion therapy are effecive but lose its effect, so it is very important to maintain a "continue" CME program in transfusion therapy.

B1893

EVALUATION OF APOPTOSIS MARKER AT THE MEMBRANE SURFACE OF RESIDUAL LYMPHOCYTES IN GAMMA-IRRADIATED PLATELET CONCENTRATES

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Background: Gamma irradiation (GI) of blood components, including platelet concentrates (PC), is used for inactivation of allogenic T-lymphocytes to prevent transfusion-associated graft-versus-host disease. The accepted dose of GI in Russia is 25 Gy. Damaging of T-lymphocytes DNA leads to their death by apoptosis way.
Aims: The aim of this study was to evaluate apoptosis marker exposure at the allogenic lymphocytes membrane surface after gamma-irradiation of PC during the storage.
Methods: To perform investigation of residual lymphocytes, we used an experimental model of PC (not for clinical using) in the form of pooled random-donor PC.16 PC were obtained by the buffy coat method (five buffy coats were pooled) with centrifugation. We studied four groups of samples obtained from each PC: 16 samples after GI on Day 1 and 16 - on Day 5 of storage, 16 untreated samples on Day 1 and 16 - on Day 5 of storage. PC were irradiated with a gamma-source cesium-137 in dose of 25 Gray during the first twenty-four hours after collection. All samples were investigated by flow cytometry. Lymphocytes were identified by the binding of the PE Cy-7-conjugated anti-CD45 antibodies and lymphocytes apoptosis was measured by phosphatidylserine (PS) exposure with FITC-labeled Annexin V. During the investigation we analyzed more than 2000 events in the lymphocytes region, however the total number of all events ranged from 200000 to 600000. Further the number of lymphocytes was expressed as percentage of all analyzed events.
Results: The percentage of lymphocytes in untreated samples on Day 1 was1,44±0,92%. Gamma-irradiation of PC was not lead to statistically significant changes (P=0,77) in the number of CD45+ cells on Day 1: the percentage of CD45+ cells was1,5±0,95%. The same number of CD45+ cells (P=0,26) was detected in untreated samples on Day 5:1,27±0,99%.The investigation of gamma-irradiated samples stored for 5 days has shown significantly (p=0,04) reduction of lymphocytes: the percentage of CD45+ was1,18±1,0%. PS exposure at the lymphocytes membrane surface of untreated samples on Day 1 didn't exceed 10,32±3,79%. The degree of Annexin V binding didn't change significantly (p=0,94) after GI on Day 1 (10,34±3,95%). The percentage of apoptoticlike changed lymphocytes in untreated samples of PC remained unchanged (p=0,51) after 5 days of storage and PS exposure was detected on 10,84±2,81% of CD45+ cells. PS externalization at the membrane surface of lymphocytesin gamma-irradiated PC was detected on 13,22±3,16% of CD45+ cells on Day 5. This level of PS expression was statistically significant increased in compared with Day 1 after GI of PC samples (p=0,007) and in compared with untreated PC on Day 5 (p=0,02) and on Day 1 (p=0,011) of storage.
Summary / Conclusion: There was no found destruction of residual lymphocytes in untreated PC within 5 days of PC storage. While GI of PC with 25 Gy leads to reduction of lymphocytes by apoptosis on Day 5 of PC storage.

B1894

ACUTE EFFECTS OF BLOOD TRANSFUSION ON LEFT CARDIAC FUNCTIONS AND CARDIAC MARKERS IN WELL-TREATED THALASSEMIA MAJOR PATIENTS

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Background: Cardiac complications are still the leading causes of mortality and morbidity in thalassemia major patients.
Aims: This study was planned to investigate acute effects of erythrocyte transfusion on left cardiac functions and to assess changes in serum levels of cardiac troponin I, creatine kinase, creatine kinase-MB due to transfusion in a group of patients with thalassemia major.
Methods: Twenty-five thalassemia major patients (14 male, 11female) aged between 3 and 24 years (mean age 12 ±5,49 years) were included in the study. Patients with impaired left ventricul functions, any congenital or acquired heart disease were not included in the study. We recorded pre-transfusional hemoglobin levels, serum CK, CK-MB, troponin levels which were studied from the blood obtained before tranfusion. These measurements were repeated after 5 days as post-transfusional evaluation. Pre and post transfusional echocardiographic indices for left ventricular functions were compared.
Written informed consent was obtained from adult patients or parents of children.
Results: Mean serum ferritin level at the time of the study was 1359,20±477,76 ng/ml (ranged between 560 and 2190 ng/ml). Mean pre-transfusional hemo-

globin concentration was7,85±0,98 gr/dl while mean post-transfusional hemoglobin concentration was 10,24±1,28 gr/dl. Pre-transfusional CK,CK-MB, troponin I levels were not statistically different than post-transfusional values. Pre-transfusional EF, FS, LVIDd, LVIDs, aort diameter, left atrium diameter did not differ than the post-transfusional measurements, the differences were not statistically significant for these parameters, P>0,05 for all.
Pre-transfusional aort valve peak gradient, aort valve mean gradient, pulmonary valve peak gradient, pulmonary valve mean gradient measurements were not statistically different than values obtained after transfusion (P>0,05)
Pre and postransfusional EF, FS values of patients with ferritin levels <1000 ng/ml and those with serum ferritin levels ≥1000 ng/ml did not differ statistically (p›0,05).
There was not any correlation between CK, CK-MB, cardiac troponin I and LVIDd, LVIDs, EF, FS (P>0,05). Pre and post transfusional hemoglobin concentrations did not show any correlation with LVIDd, LVIDs, EF, FS (P>0,05). Hemoglobin concentration did not show any correlation with troponin, CK and CK-MB levels either.
Summary / Conclusion: The present study demonstrates thatcardiac troponin I, creatine kinase and creatine kinase-MB are not effected from hematological profile of thalassemia major patients. Echocardiographic measurements are not effected from acute changes in hemoglobin concentration in well-treated patients. Erythrocyte transfusion does not lead to any acute change in cardiac parameters

B1895

AUDIT AND REAUDIT OF WEARING IDENTIFICATION WRISTBAND DURING BLOOD TRANSFUSION AT DUBAI THALASSEMIA CENTER

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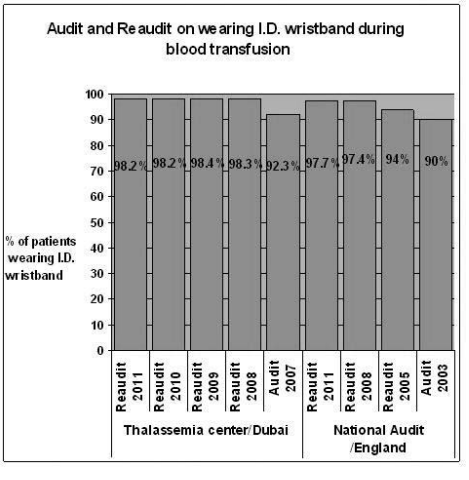
Background: Mistransfusion is the term used to describe an episode where "the wrong patient receives the wrong blood" and is considered by most authorities to be the leading cause of transfusion-related morbidity and mortality. Positive patient identification is essential at all stages of blood transfusion process to prevent such a serious adverse event. Dubai thalassemia centre daycare unit serves patients with hemoglobinopathy requiring chronic blood transfusion. Nearly7,000 patient visits are received every year.
Aims: The purpose of this audit was three folded. Firstly, assess compliance to wearing the correct identification (I.D) wristband all the time during blood transfusion cycle. Secondly, identify the reasons for not wearing I.D wristband. Thirdly, evaluate the impact of process standardization and staff and patients' education through re-auditing.
Methods: Five prospective audits were performed from 2007 to 2011 using the same methodology. Each audit was conducted prospectively on all patients admitted to thalassemia centre day care unit for blood transfusion over one month period. Patients were inspected twice daily for wearing I.D wristband that has the correct five core identifiers. The reasons for not wearing were recorded.
Results: In June 2007, 495 patients were subjected to auditing, 457(92.32%) patients were wearing the correct I.D. wristband during blood transfusion. 1.7% refused to wear their I. D. wristband because they are known to all staffs, 1.4% clipped their band to the IV dressing, 1.0% was removed by the child patient himself accidentally, 0.2% had ID band allergy and the remaining 3.4% were others. Following staff and patients' intensive education, 490 patients were subjected to re-auditing in Jan 2008 which revealed compliance improvement to 98%. 1.8% continued to refuse to wear I.D wristband because they are known to all staffs and 0.2% were allergic to the band. The same level of compliance to I.D wristband was maintained in the subsequent reaudits (2009, 2010 and 2011) and the reasons for not wearing the I.D wristband remained the same.Our results were comparable to other international audits' results (Graph1).



Summary / Conclusion: Auditing and continuing staff and patients' education about the importance of wearing the correct identification band all the time during blood transfusion are useful tools to improve compliance to identification guidelines.

B1896

POSTTRANSFUSION PLATELET RESPONSE IN HEMATOLOGICAL INPATIENTS

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Background: Thrombocytopenia is a common feature in hemato-oncological diseases, mainly in acute leukemia patients. Platelet transfusion (PT) may be used to prevent or treat associated hemorrhagic events and diminishes morbidity and mortality. Unfortunately, PT is not always effective on increasing the platelet counts, and the specific cause is identified in only a minority of cases.
Aims: To evaluate posttransfusion platelet response and its contributing factors.
Methods: From 13/04 to 13/06/2012, a prospective observational study of 354 consecutive transfusions in 50 inpatients (26 men; median age 58 years; acute myeloid leukemia as predominant pathology) was performed. Posttransfusional platelet response was evaluated using the 1 hour and 24 hour corrected count increment (CCI). Good responses were defined as 1h-CCI ≥ 7.500/µL or 24h-CCI ≥ 4.500/µL. Platelet quality, clinical status, laboratory results (renal and hepatic function, coagulation, complete blood count, LDH, PCR) and prescribed medications in the 24 hour interval before and after transfusion were evaluated.
Results: The median 1h and 24h-CCI were 6.500/µL (interquartile range, IQR, 2.632 - 11.985) and 2.146/µL (IQR -715 - 8.123), respectively; 44% of the PT had a good response after 1 hour, while only 39% after 24 hours. There was no significant correlation between 1h and 24h-CCI (rho 0.39). The platelets had acceptable quality concerning pH and lactate. The pretransfusion platelet count, (median 9.000/µL, IQR 6.000 – 15.000), did not influence the 1h-CCI. Male and older patients had significantly poorer responses. Also, the presence of fever and splenomegaly negatively affected the CCI. None of the laboratory results correlate with the CCI. Patients who were given piperacillin-tazobactam, potassium chloride, cyclophosphamide or omeprazol had significantly better responses, while etoposide, acetaminophen and bromazepam negatively influenced the posttransfusional response.
Summary / Conclusion: We found a low posttransfusional response among our hematological patients, which is in agreement with other similar institutions. The identification of manageable contributing factors for a deficient response may help optimizing the way we transfuse these patients, and it would probably improve efficacy and reduce costs. These factors should be further investigated in clinical trials.

B1897

CLINICAL EXPERIENCE OF GRANULOCYTE TRANSFUSION IN THE MANAGEMENT OF PEDIATRIC PATIENTS WITH FEBRILE NEUTOPENIA: A RETROSPECTIVE STUDY FROM A REFERENCE CENTER IN TURKEY

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Background: Despite intensive chemotherapy with improved supportive care for neutropenia contribute to the recent advances in treatment outcome in children with cancer.Febrile neutropenia is a still major risk factor for severe infections and mortality in malignancies. Granulocyte transfusion (GT) therapy is recommended to treat infections.
Aims: We retrospectively analyzed the clinical characteristics of patients with malignancies receiving GT therapy.
Methods: A total of 50 patients (30 boys, 20 girls) with a median age of 9 years (range: 2 months-21 years) were included in the study. Out of these 50 patients, 15 suffered from relapsed acute lymphoblastic leukemia, 11 acute myeloblastic leukemia, 10 aplastic anemia, 3 familial hemophagocytic lymphohistiocytosis, 6 lymphoma, 4 neuroblastoma and 1 germ cell tumor. All patients had persistent fever and prolonged neutropenia (>7 days). GT therapy was given because of fungal (13), viral (CMV 27, herpes virus 1) and bacterial (gram-negative11, gram-positive 20) infections.
Results: All patients received a median of 5 tranfusions (1-15) and appropriate antibacterial therapy combined with or without antifungal agents. G-CSF and addition to GT therapy was given to all patients except 8 with AML. The controlling of persistent fever and clinical signs of infection were achieved in all patients, but 12 patients (24 %) died due to subsequently developed uncontrolled infections.
Summary / Conclusion: A substantial proportion of severely ill pediatric patients with complicated febrile neutropenia benefited from GT therapy.

B1898

PLATELET EXPENDITURE IN HEMATOLOGY PATIENTS

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Background: Thrombocytopenia is a major cause of morbidity and mortality in patients receiving chemotherapy for malignancy as well as for other specific hematologic disorders that also require frequent platelet (PLT) transfusions
Aims: To report on the expenditure of platelets (PLTs) aimed for the patients at the University Clinic of Hematology in relation to the total annual number of released PLTs for clinical use
Methods: A retrospective three - year survey (from January 2010 through December 2012) has been done, using data from the blood component production and apheresis department at the National Institute for Transfusion Medicine (NITM) – Skopje.
Results: The total annual number of whole blood units aimed for component preparation varied from 21 488 to 28 443 blood units. In 2010, 81.9% (12 666) of the produced PLTs by mainly the platelet- rich plasma (PRP) – method, were released for clinical use. 80.7% of these PLTs were intended for hematologic patients. These percentage has slightly decreased in 2011 showing 77, 5 % PLT release for these patients. Further reduction of usage of PRP- derived PLTs was noticed the next year (64.4%). Regarding this issue, the apheresis department has increased its performance by increasing the production of PLTS (from 46 in 2010; 65 in 2011 and 80 apheresis procedures in 2012). Most of these single-donor apheresis PLTs were aimed for hematologic patients (78.3% in 2010; 75.4% in 2011 and 55% of the collected PLTs were released in 2012).
Summary / Conclusion: The number of PLT transfusions for the purpose of treating hematologic patients has slightly decreased. This may be due to a more stringent PLT transfusion policy. However, these patients still remain most transfused ones when considering PLT transfusions. Replacing prophylactic transfusions by a therapeutic transfusion strategy following the latest guidelines as well as introducing new therapeutic modules, the number of PLT transfusions may further be reduced.

B1899

USE OF RECOMBINANT FACTOR VIIA (RFVIIA) IN ACUTE LIFE THREATENING PRIMARY POSTPARTUM HAEMORRHAGE: A CASE REPORT

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Background: Primary Postpartum Haemorrhage (PPH), although falling in recent years, is one of the leading causes of maternal mortality and morbidity in the western world with a maternal mortality rate of 8.4% of all direct maternal deaths in the United Kingdom (CEMACH 2011). Massive PPH is defined as a cumulative blood loss >1,500mls of blood or ongoing severe bleeding. The treatment for massive PPH is a combination of blood and blood product transfusion, uteronics, surgical and pharmacological agents such as Tranexamic Acid and recombinant FVIIa (rFVIIa). RFVIIa is a prohaemostatic drug that activates Factor X on activated platelets and on tissue factor to promote thrombin generation at the site of injury with the formation of a stable fibrin clot. RFVIIa is used in the treatment of haemophilia and FVII deficiency. Outside this, use of rFVIIa is very controversial, in particular in the field of Obstetrics where it is not licenced for the treatment of obstetric haemorrhage due to severe risk of thrombosis in an already prothrombotic patient.
Aims: To review the efficacy of rFVIIa post major post-partum haemorrhage and to review the clinical outcome of the patient post obstetric haemorrhage.
Methods: This was a retrospective individual case report. 39 year old, Para3, (LSCS x3, cord prolapse, breech presentation and elective section). Antenatal care was uneventful; however ultrasound at 33 weeks noted placenta acretia. Planned caesarean section and tubal ligation planned for 38 weeks gestation.
Results: Delivery: Laparotomy, high transverse caesarean section, ligation internal iliac artery, sub total hysterectomy and left salpingo-oopherectomy. Liveborn female infant, 3710g, Apgars $7^{1}9^{5}$. Maternal estimated blood loss (EBL) = 19570 mls. Hb decreased from 12.0 to 4.0 g/dl, received 31 RCC, 4 Pools of platelets, 21 Plasma units, and 11g of fibrinogen. The patient continued to bleed. The patient then received recombinant Factor VIIa under Consultant Haematologist instruction (Fibrinogen = 1.63g/l and PT = 13.4 seconds). The bleeding arrested with no further requirement for red cell transfusion. A top-up platelet transfusion was required post event.
Summary / Conclusion: This case demonstrates that rFVIIa was effective in arresting an acute life threatening primary post partum haemorrhage with no thrombotic adverse effects seen it the aftermath. In the absence of randomized controlled trials on the use of rFVIIa in obstetrics, the use of independent case reports and review articles published lend support to the weak evidence that is currently available on the use of rFVIIa for obstetric haemorrhage.While some studies support the use of rFVIIa as a safe and efficacious treatment for massive obstetric haemorrhage, other studies do not support its routine use. Until randomised controlled trials take place in obstetrics with established protocols for its use, rFVIIa should only be prescribed by a Consultant Haematologist.

B1900

PHENOTYPE FREQUENCIES OF BLOOD GROUP SYSTEMS AND ALLOANTIBODIES TO RED BLOOD CELLS IN BLOOD RECIPIENTS IN CENTRAL ANATOLIA OF TURKEY

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Background: In routine practice, appropriate blood group for ABO and Rh blood group system should be given for a safe and effective erythrocyte transfusion. A total of 308 RBC antigens are now recognized by the International Society of Blood Transfusion (ISBT), 270 of which are clustered in 30 blood group systems, 9 of which (ABO, Rh, Kell, Kidd, Duffy, MNS, P, Lewis and Lutheran) are considered to be major blood group systems. Alloimmunization caused by the immunogenic reactions is a serious problem especially in patients receiving multiple transfusions.
Alloantibody data bank can be obtained by determining the antibody profile of the community by the screening of red cell antigen phenotype. Thus, potential development of alloimmune transfusion reactions can be prevented in patients. In literature, except for the frequency of ABO and Rh antigens, there is no study available about the frequency of red cell antigens in Turkey.
Aims: Routine erythrocyte antibody screening to prevent alloimmunization reactions in erythrocyte suspension recipients has been going on in Erciyes University in Kayseri, which is a city in the central Anatolia in Turkey, since January 2008. The present study is the first report detecting the frequencies of the RBC antigen and phenotypes of different blood groups in Kayseri, a city in middle Anatolia in Turkey.
Methods: A total of 48750 blood recipients typed for ABO, Rh system and other blood groups were retrieved from January 2009 to July 2011. The patients in whom antibodies were detected were also screened for the presence of autoantibodies. ABO group types and Rh specifities of the blood samples are screened and identified by DiaMed–ID Micro Typing System (Diamed AG, Switzerland) by gel centrifugation technique. The screened blood group antigens in recipients were Kidd (Jk^a, Jk^b), Kell (K, kp^a, kp^6), (Duffy Fy^a, Fy^b), MNS (M, N, S, s), Lewis (Le^a, Le^b), P (P), Lutheran (Lu^a, Lu^b) and Xg (Xg^a). ID card antigen profile-I (P_1, Le^a, Le^b, Lu^a, Lu^b), ID card antigen profile–II (K, k, kp^a, Kp^b, Jk^a, Jk^b) and ID K typing cards were used with a red cell suspension (5 %) for phenotyping. ID card antigen profile–III (M, N, S, s, Fy^a, Fy^b) was used with a red cell suspension prepared in 0.8 % LISS.
Results: The retrospective analysis of transfusion history and medical records of 48750 patients who have received at least one transfusion found that 196 (0.4 %) patients had developed antibodies. Of the patients whose antibody test results were positive, 28 (14 %) were found to have autoantibodies. In the Kell blood group system, 22 % (43/196) recipients were typed as K antigen positive. This rate was found to be 0.08 % (43/48750), in the screened population. A rare Kp^a phenotype was found with a frequency of 2.04 % (4/196) in recipients. For the Kidd and Duffy blood group system, Jka rate was 2.5 % (5/196), Jkb was 1.02% (2/196); and Fy (a+) was 2.5 % (5/196). Neither Luteran nor Xg^a phenotypes were observed in this study population. M + and S + were the most common phenotypes observed in the MNS blood group system (3.6 % and 1.02%, respectively). According to the Lewis blood group system, Le^a and Le^b antigens were observed with a frequency of 1.5 % and 0.5 % respectively, whereas frequency of P_1 antigen was 0.5 %.
Summary / Conclusion: Multiple antigen detection is an important problem in blood banking. In patients undergoing chronic transfusion, if hemoglobin levels drop suddenly and need for transfusion frequency increases without any other cause, the probability of multiple antibodies should be considered. Also transfusions applied in different centers increases the antibody rate.
In conclusion, we think that logical alloantibody screening can prevent transfusion reactions in patients undergoing multiple transfusions, if a data bank can be created. Antibody screening tests particularly in recipients would avoid both time and the financial loss.

B1901

CORRELATION BETWEEN ABO BLOOD TYPE AND THE SITE OF CANCER

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Background: The relation between ABO blood type and various malignancies has been an issue of research since the 1950's, but only in the late 2000's a certain association between A blood group and pancreatic cancer was established. The existence of pancreatic cancer loci in the ABO gene and the ele-

vated plasma markers of inflammation in these patients suggested a link between chronic inflammation, A blood type and pancreatic cancer, according to which, blood group antigens may alter the systemic inflammatory response that leads to this specific type of neoplasia.

Table 1: ABO distribution (cancer patients- general greek population)

CANCER SITE	N(%)	A (%)	B (%)	AB (%)	O (%)	P-value
BREAST	312(23,5)	127(40,7)	48(15,4)	16(5,1)	121(38,8)	0,989
COLORECTAL	199(14,2)	79(41,8)	24(12,7)	13(6,9)	73(38,6)	0,546
LUNG	164(12,3)	70(42,7)	12(7,3)	7(4,3)	75(45,7)	0,022
PANCREAS	71(5,3)	40(56,3)	7(9,9)	2(2,8)	22(31)	0,049
UTERUS	63(4,7)	27(42,9)	8(12,7)	3(4,8)	25(39,7)	0,488
STOMACH	63(4,7)	31(49,2)	4(6,3)	3(4,8)	25(39,7)	0,489
OVARY	53(4)	25(47,2)	6(11,3)	2(3,8)	20(37,7)	0,655
BLADDER	44(3,3)	17(38,6)	12(27,3)	0(0)	15(34,1)	0,535
OTHER	370(27,8)	145(39,2)	53(14,3)	18(4,8)	154(41,6)	
TOTAL	1329(100)	561(42,2)	174(13,1)	64(4,8)	530(39,9)	0,172
GENERAL GR. POPULATION	1105(100)	441(39,9)	178(16,1)	58(5,2)	428(38,7)	

Table 2: ABO distribution in respiratory and pancreatic cancer (recent results)

RESPIRATORY CANCER		PANCREATIC CANCER	
PATIENTS (N=198)	GENERAL GREEK POPULATION (N=1105)	PATIENTS (N=75)	GENERAL GREEK POPULATION (N=1105)
Blood Type B: N=15 (7,7%)	Blood Type B: N=178 (16,1%)	Blood Type A: N=41 (54,7%)	Blood Type A: N=441 (39,9%)
Blood Type non-B: N=183 (93,2%)	Blood Type non-B: N=927 (83,9%)	Blood Type non-A: N=34 (45,3%)	Blood Type non-A: N=664 (60,1%)
P-value: 0.002		P-value: 0.012	

Aims: To check the distribution of the ABO blood type among the Greek cancer patients and detect the existence of any correlation between ABO and the site of cancer.
Methods: From the archives of the Blood Bank Department in our Oncology Hospital we had recorded 1329 cases of cancer patients, their ABO blood type and the site of cancer. The ABO distribution was studied in the whole cancer-population and also among various sites of cancer-categories, and it was compared to the distribution in the general Greek population. The results had revealed the already known association between pancreatic cancer and A blood type, and also a newly mentioned association between respiratory cancer and non-B blood-type. Now, having expanded further the database (1518 cases), we are particularly focusing in the prevalence of A and non-B blood type among the pancreatic and the respiratory cancer patients respectively, by comparing the A and non-A and the B and non-B distribution in the general Greek population to the distributions in the pancreatic cancer group and the respiratory cancer group respectively (x^2test).
Results: The ABO distribution in the general Greek population, in the whole cancer population and in each cancer site-oriented group studied, as well as the levels of statistic significance testing the O versus non-O prevalence in each site of cancer, are shown in table 1. The results regarding the A/non-A distribution among the pancreatic cancer patients and the B/non-B distribution among the respiratory cancer patients are shown in table 2 (the calculations in these two categories were done on the updated data).
Summary / Conclusion: 1. The ABO distribution among cancer patients is not different when compared to that in the general Greek population and this is in accordance to the national bibliography. 2. Blood group A is associated with pancreatic cancer, according to the national bibliography. 3. Non-B blood group is associated with respiratory cancer.
Gathering further data, especially on patients with respiratory cancer, would enforce the level of statistic significance of these results

B1902
CLINICAL OUTCOMES OF ABO INCOMPATIBLE RBC TRANSFUSIONS
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Background: ABO mismatch blood transfusion is one of seriousness events related to blood components administration.
Aims: we performed a retrospective review of ABO-incompatible RBC transfusions from our institution during four years (2007-2011).
Results: Within the period of observation, 20 ABO mismatches were detected (effective transfusions and near miss events). 12 ABO mismatches were detected when blood samples are studied for pre-transfusions tests (mistake in sample collection or identification and one case of usurpation of identity). Of 8 patients who received more than 50 ml of incompatible blood, 4 (50%) manifested signs or symptoms related to the incompatible transfusion but none died. Hypotension, hemoglobinuria, postoperative bleeding was detected in survivors. In the half of our cases (4 patients), there were no associated signs or symptoms due to the incompatible transfusions.
Summary / Conclusion: ABO incompatible RBC transfusion does not inevitably mean death or even occurrence of symptoms. Awareness of staff at the bedside and laboratory of the potential for errors is one of the most effective tools for detecting and preventing transfusion errors.

B1903
COMBINATION OF DEFERASIROX AND DEFEROXAMINE IN MANAGEMENT OF IRON OVERLOAD IN MYELODYSPLASTIC SYNDROMES (MDS): AN UPDATE IN A HEPATOPATIC PATIENT
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Background: Although other treatments are now available, the standard treatment for many MDS patients remains supportive care. Most MDS patients eventually become red blood cell (RBC) transfusion dependent, risking iron overload, which may lead to cardiac, hepatic, and endocrine dysfunction.
Aims: Iron-chelation is recommended in guidelines in MDS when there is at least an evidence of iron overload: elevated serum ferritin, iron related organ dysfunction, or chronic RBC transfusions. Deferasirox is a well tolerated oral iron chelator drug that produces relevant benefits but, because of its potential hepatotoxicity, it is usually not recommended for patient with known hepatic diseases.
Methods: A 62-year-old man, with Refractory Anaemia, affected by HCV positive cirrhosis, started recombinant Erythropoietin therapy without results, and then he went in RBC transfusion program, with 2 blood package/month. At a ferritin serum concentration 700 ng/mL iron chelation therapy with deferoxamine was started in consideration of pre-existing hepatic disease. The patient was absolutely not compliant to this therapy, and transfusion need increased until to 2 blood package/week while serum ferritin concentration was, after 12 months, more than 6000 ng/mL. Since high levels of ferritin correlate with a dangerous condition for hepatic cells, deferasirox was started at low dosage, 10 mg/kg/die. Before treatment was started, an accurate study of hepatic, renal and cardiac functions was performed. After three months, serum ferritin was not modified as well as other biochemical parameters, and so deferasirox dosage could be gradually increased, reaching 30 mg/kg/die after two months without any liver damage. After five months of therapy with deferasirox at full dosage, serum ferritin concentration was more than 5098 ng/mL.
Results: Then, considering all risks related to secondary hemochromatosis, a combined iron chelation therapy with deferasirox (30 mg/kg/die) and deferoxamine (2 g/day for 5 days/week) was established : after 3 months serum ferritin concentration decreased to 3000 ng/mL. In that period, Haemoglobin concentration decreased significantly, so that the patient had to receive 2 RBC package/week and, after two years of combined therapy, serum ferritin concentration was at a stable level under 3000 ng/mL. During treatment, a regular monitoring of hepatic, renal and cardiac functions was performed and there was no alteration. Then, the patient, after a 4-years period of transfusion and related iron-chelation therapy, died from septic shock.
Summary / Conclusion: In conclusion, management of iron overload with deferasirox and deferoxamine as combined therapy can be considered as a safe and useful therapeutic choice in critical transfusion-dependent iron overload in MDS patients with pre-existing hepatic disease, even if these preliminary observations need to be validated by controlled studies enrolling larger number of patients.

B1904
COMPARATIVE STUDY OF DEFERRED BLOOD DONORS CHARACTERISTICS- HOW THEY CORRELATE WITH WHERE THE BLOOD DONATION TOOK PLACE (HOSPITAL OR MOBILE UNITS)
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Background: A study of the questionnaires that the possible blood donors fill in was performed.
Aims: To record and study the characteristics of the possible blood donors who were deferred and to comparatively study this data according to the place of blood collection.
Methods: During the last six years, 16580 possible blood donors presented. 13974 of them gave blood, while 2606 (15.7%) were deferred. In the hospital presented 14241 possible donors and 11922 of them gave blood, while 2319 possible donors where deferred (16.3%). In case of collecting blood with a mobile unit, 2339 possible donors presented, 2052 of them gave blood and 287 (12.3%) were deferred.
Results: You can see the result at the Table below:

	MEN	WOMEN	VOLUNTARY	FAMILY	OLD	NEW REPLACEMENT
IN HOSPITAL	56,2%	43,8%	17,7%	82,3%	63,6%	37,4%
MOBILE UNITS	38,6%	61,4%	78,2%	21,8%	51,7%	48,3%

Summary / Conclusion: he result's are readily explained by the fact that in case of mobile unit blood giving, all of the donors are voluntary, non remunerated, and most of them are frequent donors, so they usually are better informed and prepared, compared to the mixed population of voluntary and family replacement donors we deal with at the hospital. In both cases, the deferral rate for women is higher, mostly due to the lower Hb levels. Another fact that needs to be emphasized is the large number of young people (age<35 years) who present to donate blood. This is particularly important in our country, where the problem of ageing population is getting bigger every year.

Granulocytes

B1905
EOSINOPHILIC FASCIITIS
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Background: Eosinophilic fasciitis is characterized by edema, painful induration, and progressive muscle weakness. It is more frequently seen in men, and though not an absolute necessity, eosinophilia can be observed in this disease with partially revealed etiology. Scarce number of cases have been reported so far.
Aims: We wanted to present this case considering its scarcity, and critical role in the differential diagnosis of eosinophilic disorders.

Methods: A 12-year old male patient started to suffer from itching, and skin rash all over his body 3 weeks before his admission. Eruptions were as small as pinheads. Later on swellings, and painful skin rashes had emerged on his wrists, elbows, knees, and ankles. His personal, and familial medical history was not remarkable. His physical examination demonstrated edematous eyelids, diffuse subcutaneous nodules all over his body, more numerous on articular areas, and 1-2 mm-sized nodules on his back. In addition a 7 x8 mm-skin lesion with an orange peel appearance was seen on his back. Peripheral eosinophilia (17 %), and a higher eosinophilic cationic protein (ECP) level (42.000 mg/L; normal: 0.00-20.00 mg/L) were detected. Surface ultrasound revealed a minimal amount of subcutaneous edema. On MRI, in sagittal plane a hyperintense lesion measuring 80 x 8 mm localized on the posterior aspect of the left elbow was observed. On T2A-weighted, and also fat-suppressed sequences, this hyperintense lesion was consistent with fasciitis. Histopathologic examination of the biopsy material revealed a nodular lesion characterized with increased collagenous tissue, and irregular contours which infiltrated into subcutaneous adipose tissue almost completely starting from dermal layer, and also fascial layer covering the inferior aspect of the adipose tissue. This local collagenous tissue had patchy areas of myxoid tissue, but it mostly demonstrated a fibrinoid degeneration. This collagenous tissue contained numerous lymphocytes, and occasionally eosinophils.
Results: Methylprednisolone therapy (60 mg/m^2/d) was initiated. One week later any regression of his complaints, and subcutaneous nodules was not observed. Oral methotrexate (20 mg/m^2) therapy was started. A week later his complaints, and subcutaneous nodules disappeared. With weekly dose-tapering protocol, at 5. week of his therapy, his methylprednisolone, and methotrexate doses were reduced to 20 mg/m^2, and 14.58 mg/m^2, respectively which resulted in reappearance of previously regressed lesions on the anterior aspect of the chest. At the 12. week, doses of methylprednisolone, and methotrexate were increased to 25.6 mg/m^2, and 20 mg/m^2 which again brought lesions under control, and his complaints resolved.
Summary / Conclusion: In cases with eosinophilia, underlying causes should be looked for. Eosinophilic fasciitis should be considered among etiological factors of eosinophilia.

B1906
NEUTROPAENIA FOUND ON ROUTINE LABORATORY TESTING – IS FOLLOW-UP REQUIRED?
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Background: In our teaching hospital laboratory, all full blood count results that demonstrate a neutrophil count of less than 1.0 x 10^9/L are highlighted for the attention of a consultant haematologist. There is little published evidence suggesting what investigation and follow up is required.

Aims: We aimed to follow up all patients identified as neutropaenic during a 3 month period, to build up a picture of patient characteristics and outcomes.
Methods: Between November 2010 and January 2011, 171 patients were identifed as neutropaenic by our laboratory. We performed a retrospective observational study following up each patient over a 2 year time period. We used a combination of hospital case notes, primary care medical histories and our hospital electronic laboratory results system to establish patient outcomes.
Results: Full blood counts came from general hospital wards, outpatient departments and Accident and Emergency (50%), primary care (25%), oncology (19%) and haematology (7%).
Causes of neutropaenia included chemotherapy (24%), infection or subsequent antibiotics (27%), haematological (15%), and drugs (4%). A cause was not identified in 21% of cases; the majority of these were primary care patients who were not further investigated. Excluding patients post-chemotherapy, 31% had a previous diagnosis explaining their neutropaenia. Of the remaining hospital patients, almost all had an attributable cause, most commonly infection; the 10 patients with no obvious cause were either followed up by haematology or were young children who were well, but should have had a repeat full blood count to check resolution of neutropaenia. Of the primary care patients without a previous diagnosis, 59% had a persistent/recurrent mild neutropaenia with no proven aetiology and have remained well. Several of those with new neutropaenia did not have sufficient repeat full blood counts.
There were 35 deaths. 33 patients had an underlying cause for their neutropaenia. There were only 2 deaths in those without a known cause of neutropaenia, both primary care patients in their mid-80s with a persistent mild neutropaenia; cause of death is unclear from the records but we suspect that further investigation would not have altered management.
A significant number (33%) of patients, excluding those post-chemotherapy, had a transient neutropaenia, mostly due to infection or subsequent antibiotics. In around 20% of patients, duration of neutropaenia is unknown. A number were lost to follow up as they moved out of area or were under the care of another hospital; the remainder were assumed to have a transient or benign neutropaenia but did not have sufficient repeat full blood counts to confirm this.
Summary / Conclusion: Almost all hospital patients had either an established reason for neutropaenia, or an obvious attributable cause. Very few needed to be further investigated and those who did require follow up were appropriately referred to haematology.
It is much more difficult to establish causality in primary care patients, due to lack of accessible documentation. However, patients without a diagnosis appear to have a benign cause for their neutropaenia since they remain well.
A small number of patients in both primary and secondary care were assumed to have a transient or benign neutropaenia, but did not have sufficient repeat full blood counts to confirm this. We will ensure that all future neutropaenia blood film reports include a recommendation to repeat full blood count afer around 4 weeks.
Our results suggest that patients with neutropaenia are being followed up appropriately; those who remain without a diagnosis appear to have a benign cause and may not need further investigation in the absence of other signs or symptoms.

B1907
THE CLINICAL FEATURES AND GENETIC MUTATIONS OF CHRONIC GRANULOMATOUS DISEASE: RESULTS FROM A REFERENCE CENTRE AT MIDDLE ANATOLIA
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Background: Chronic granulomatous disease (CGD) is a rare innate immune deficiency with neutrophil function disorder.
Aims: In this retrospective study, we aimed to evaluate the clinical features of the patients with CGD.
Methods: We presented eight patients (6 boys, 2 girls) with CGD which were evaluated at Erciyes University Medical Faculty hospital between 1996 and 2012. The initial complaints, age at diagnosis, consanguinity of the parents, similar disease history or death of the siblings, physical examination, diagnostic tests, clinical courses, and geneticcharacteristics were analyzed.
Results: The initial complaints were started before the age of one in four patients; whereas only two patients diagnosed before the first birth day. Lymphadenomegally, suppurative infections, pneumonia, diarrhea were the most noted initial complaints. All parents were consanguineous. The clinical features were mild; and the ages of diagnosis were late in patients with p47 and p67 defect. The patient with X linked CGD was diagnosed when he was 3 months old; his clinical course was complicated with chronic otitis media, zygomatic abscess, lung abscess, and facial paralysis. The patient with p22 defect was diagnosed at two months of age; and gastric wall granuloma, inflammation in proximal femur was detected.
Summary / Conclusion: The awareness of the clinicians about CGD will result in early diagnosis and consequently reduce the mortality and morbidity of this disease.

B1908
THE COINCIDENCE OF FAMILIAL MEDITERRANEAN FEVER AND HYPEREOSINOPHILIA IN A PATIENT WITH HEREDITARY ELLIPTOCYTOSIS
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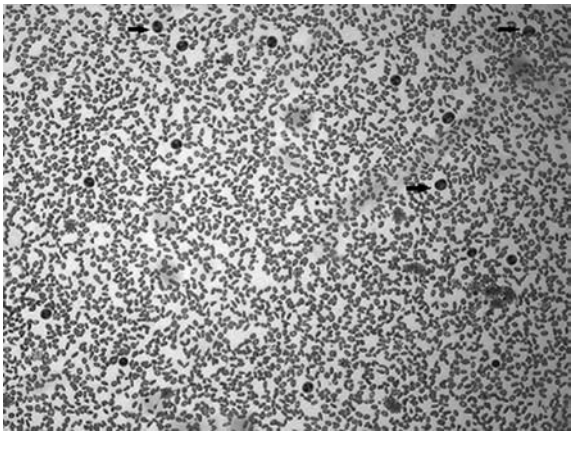
Background: Familial Mediterranean fever (FMF) is a genetic disease with autosomal inheritance characterized by recurrent fever, abdominal pain, and serositis attacks. It is relatively common in the races and ethnical groups around Mediterranean Sea (Sephardic Jews, Armenians, Turks and Arabians).Hereditary elliptocytosis (HE) is common genetic defect of the red blood cell membrane skeleton. In the present study, we reported a HE case accompanied by FMF and hypereosinophilia, as it hasn't been reported in the literature so far.
Aims: This case report describes a Turkish female HE patient who presented

with FMF and hypereosinophilia. The causes of hypereosinophilia and it was considered as reactive hypereosinophilia. Genetic analysis revealed heterozygous mutation in exon 10 of the *MEFV* gene (V726A). The patient was successfully treated with colchicine and steroid treatment with 3 months follow-up. To the best of our knowledge, this is the first report of association between FMF, HE, and hypereosinophilia.

Methods: A 27 years old female patient visited emergency room of Erciyes University for fever, abdominal pain and vomiting. It was found that anemia and splenomegaly was found and she was diagnosed as HE by peripheral blood smear in pediatrics clinic; followed by splenectomy. She had no past another medical history, family history and history of tobacco, alcohol, illegal drug use, and travel. She had been suffering from recurrent periodic fever, abdominal pain, and vomiting every two months since 6 months ago. She complained of abdominal discomfort for three days and fever for two days followed along with whole abdominal pain and vomiting, and then the sympoms resolved spontaneously. Her vital signs were blood pressure 110/70 mmHg, pulse 88/min, respiratory rate 23/min, and body temperature 38.7^0C on admission. In abdominal examination, there was generalized tenderness but not defense or rebound tenderness. Initial laboratory results showed that leukocyte was 35,860/μL (neutrophil28,330/μL, eosinophil5,080/μL), hemoglobin 15.5 g/dL, platelet 521,000/μL, C-reactive protein 53.68 mg/L, and the erytrocyte sedimentation rate 40 mm/h. There were diffuse elliptocytes and increased eosinophils on the peripheral blood smear.In blood sample molecular analysis of patient, the PDGFRA-FIP1L1 was found negative.Her symptoms lasted for around 2 days and then were spontaneously subsided. We performed MEFV gene test for definite diagnosis. Mutational analysis detected gene mutation on one allele in patient, and one missense mutation was detected in the exon 10 of the MEFV gene, V726A in heterozygous. Diagnosis of FMF was determined according to Tel Hashomer criteria for FMF.

Results: The patient is current under treatment with colchicine 1.2 mg/day, oral prednisolone 1mg/kg and hydroxiurea 3 tablet of 500 mg a day. The progress is being followed up with gradually improved severity and duration of the symptoms. Eosinophil count reduced to 0,370/μL at the week 3 after initiation of therapy. Steroid treatment was stopped after 1 month and the patient is now, 3 months after diagnosis, in normal health.

Summary / Conclusion: In the present study, we discussed FMF with adult onset and hypereosinophilia in a patient with HE, and causes of this situation. FMF can be diagnosed based on characteristic clinical patterns, response to treatment for colchicines, and gene test results.In conclusion, this case is considered to be important as it is first case with association of HE, FMF and hypereosinophilia.



B1909

HYPEREOSINOPHILIC SYNDROME: CASE REPORT

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Background: Idiopathic hypereosinophilic syndrome (IHES) is characterized by prolonged eosinophilia in peripheral blood and bone marrow and multiple organ dysfunction. Although the pathogenesis of the disease is not known exactly, its believed that the enzymes released by eosinophils.

Aims: We report a case with cough and urticaria, presented with frequent relapses of clinical signs and idiopathic hypereosinophilia, without known cause. Our patient is admitted to hospital with frequent relapses of pulmonary and skin involvements, which are treated with steroids successfully.

Results: A 4-year old male was referred to our clinic with complaints of fever, cough, wheezing and rash. Five months before the current admission he was treated for allergic dermatitis due to pruritic wheals and edema in his arms and feet. At admission, the child had fever, rash and angioedema. Auscultation of chest revealed bilateral ronchi. The spleen and the liver were palpable 3 cm below the costal margin. Complete blood count revealed an abnormal total leukocyte count of 41.08/μL (neutrophils 18%; lymphocytes 20% and eosinophils 62%), normal hemoglobin level (12.9 g/dL) and normal platelet count (342x103/ μL). The erythrocyte sedimentation rate was 35 mm/h. Serology for hepatitis, HIV, fasciolasis, toxocariasis, syphilis and echinococcosis were negative. Skin prick test were negative. Bone marrow aspiration aspiration revealed a hypercellular bone marrow with the percentage of eosinophils to be 55% among other cells seen. Cytogenetic examination of marrow was normal.We do not detected FIP1L1-PDGFRα fusion gene in our patient. Because of the patient's long lasting peripheral, the multiple-organ involvement and the exclusion of other causes of eosinophilia, a diagnosis of idopathic hypereosinophilic syndrome (IHES) was made. He was treated again successfully with prednisolone.

Summary / Conclusion: Pulmonary involvement can be seen in 40% of cases. The most common respiratory symptom is chronic, persistent nocturnal cough. In our case, the patient had cough and pruritic lesions lasted for more than 18 months. But we could not find any infiltration in chest radiography and thorax tomography. First line therapy for IHES are generally steroids. In our case, we used prednisolone treatments in each admission with clinical and laboratory improvement. In conclusion, our case highlights the importance of IHES, that can be complicated with vital organ involvements and malignities. The disesase is seen rarely but can be admitted to hospital with frequent relapses.

Platelets and thrombocytopenia

B1910

TYPE I GAUCHER´S DISEASE: CLINICAL, EVOLUTIVE AND THERAPEUTIC FEATURES

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Background: Gaucher disease (GD), the most common lysosomal storage disorder is a heterogeneous multisystem condition. Patients with non-neuronopathic (type 1) GD may suffer from hepatomegaly, splenomegaly, thrombocytopenia, bleeding tendency, anaemia, skeletal pathology, growth delay and decreased quality of life (QoL). GD is one of the few inherited metabolic disorders that can be treated by enzyme replacement therapy (ERT). In a majority of series there is a delay of 4 to 10 years between beginning of symptoms and diagnosis.
Aims: To analyze the clinical features, course and treatment response of a group of patients with type I GD.
Methods: Retrospective analysis of nine patients with diagnosis of GD between 1986 and 2012 in a Central Hospital in the North of Portugal. QoL was assessed by SF-36 modified inquiry.
Results: Nine patients with GD were identified (five female and four male) with a median follow-up time of 17 years (range: 5-36 years). The median age of diagnosis was 45 years and the first symptoms appeared around 38 years old. Seven were diagnosed in adulthood and two in childhood. Prior to diagnosis, the main symptoms were anorexia (78%), fatigue (67%), bone pain (44%), abdominal distension (22%), bleeding tendency (22%), and growth delay (22%). Diagnosis was confirmed by low leucocyte acid beta-glucocerebrosidase activity. Morphologic documentation of GD disease in bone marrow aspirate and biopsy was obtained in 44% of the patients. The most frequent genotype was N370S/L444P (56%). At diagnosis, 44% patients were anemic, 66% had moderate to severe thrombocytopenia, 66% moderate to severe splenomegaly and 78% moderate to severe hepatomegaly. Abnormal liver function tests were found in 11% of patients. Bone abnormalities were present in 78%, which included bone marrow infarction (56%), Erlenmeyer flask deformities (44%), osteoporosis (44%), bone pain (44%) and pathologic fractures (22%). ERT with Imiglucerase was performed in eight cases. The median time of Imiglucerase treatment was 10 years. The median time to achieve hematological and visceral therapeutic goals was 24 months. There was an improvement in median hemoglobin concentration from 12g/dL (range: 8.8-14.4 g/dL) to 14.6 g/dL (range: 9.8-16.1 g/dL). Platelet count improved from a median $73X10^9$/L (range: $33X10^9$/L-$236X10^9$/L) at diagnosis to a median $140X10^9$/L (range: $76X10^9$/L-$247X10^9$/L). At the end of follow-up, only 1 (12.5%) patient had moderate thrombocytopenia and 88% anemic patients at diagnosis had normal hemoglobin levels. Only 1 (12.5%) patient had hepatomegaly and 2 (25%) patients had splenomegaly. The majority of patients (87.5%) had stable or poor skeletal response. Women had the worst skeletal response. Self-evaluation of health showed large improvements, 87.5% of patients referred subjective improvement. None of the patients had good QoL prior to treatment, but at the end of follow-up, 4 (50%) patients had good QoL and 3 satisfactory QoL (37.5%). Psychological disturbances were present in 37.5% of patients. Median SSI (Severity Score Index) before and after treatment was 9 (range: 1-14) and 3 (range: 2-9), respectively.
Summary / Conclusion: The hematologic and visceral disorders were the most serious and prevalent manifestations at diagnosis, but also the most changeable with treatment. Despite the poor bone response, patients had clear improvement in QoL and a better SSI score with ERT. Because early treatment can prevent development of irreversible complications, early diagnosis is crucial to improving ultimate outcome. Delay diagnosis was seven years.

B1911

THE CLINICAL SIGNIFICANCE OF PLATELET INDICES IN PATIENTS WITH SEPSIS

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Background: Platelet activation has both beneficial and potentially harmful effects. Platelet aggregation at sites of inflammation may be responsible for impairing microcirculatory flow in patients with sepsis.
Aims: To investigate the clinical significance of the platelet indices-platelet count (shorted for PC),mean platelet volume (shorted for MPV), platelet distribution width(shorted for PDW), and platelet large cell ratio (shorted for P-LCR) in patients with sepsis.
Methods: Ninety four patients with sepsis were recruited in this retrospective study. All patients were divided into Survivors(n = 71) and non-survivors (n = 23) according to the prognosis□the platelet count was detected andAcute Physiology And Chronic Health Evaluation II(shorted forAPACHE□)score was calculated the day on admission to ICU. All patients were divided into Group A (APACHE □ score <10 points, n = 12), Group B (APACHE□ score 1 to 19 points, n = 39) and Group C (APACHE □ score ≥ 20 points, n = 43) according to the APACHE□ score, the platelet count and mortality were analyzed among the three groups. In addition, all patients were divided into thrombocytopenia group (PC < 100 x 109 / L, n = 39) and non-thrombocytopenia group (PC≥100 x 109 / L, n = 55) according to the platelet count , the incidence of septic shock, APACHE□ score, mortality, days in ICU, MPV, PDW and P - LCR were analyzed between these two groups. All the results were statistically analyzed.
Results: 1)The platelet count was higher in survivors than that in non-survivors□P<0.01□,the APACHE□ score was lower in survivors than that in non-survivors(P<0.01).2) The higher the APACHE □ score was, the lower the platelet count was□and the higher the mortality was□(P<0.01).3) The incidence of septic shock, APACHE □ score, days in ICU, MPV, PDW and P-LCR were higher in thrombocytopenia group than those in non-thrombocytopenia group□P<0.01□. 4) Platelet count and its indices: MPV , PDW and P - LCR were detected dynamicly, the relationship between MPV, PDW, P-LCR and platelet count was negatively correlated.
Summary / Conclusion: Thrombocytopenia could be used as an early warning indicator of severity and prognosis in sepsis. The change of MPV , PDW and P – LCRcould be an indirect sign of bone marrow compensatory hyperplasia ,disturbance in platelet production and activity ,and the prognosis in patients with sepsis, be indicative of the reovery of the platelet count in sepsis with thrombocytopenia.

B1912

RITUXIMAB THERAPY IN REFRACTORY AND RELAPSED THROMBOTIC THROMBOCYTOPENIC PURPURA; A RETROSPECTIVE ANALYSIS OF A SINGLE CENTRE IN QATAR

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Background: Thrombotic thrombocytopenic purpura (TTP) is a life threatening disease that occurs mainly in young adults. Acquired cases are usually a result of antibodies directed against ADAMTS 13, a protease that cleaves the Von Willibrand factor multimers .Plasma exchange is the standard treatment, however some acute severe forms are refractory to plasma exchange and the incidence of relapse is high up to 35% .The mortality remains at 12-20%. The treatment options for refractory and relapsing cases are limited and often unsuccessful. Rituximab , a chimeric monoclonal antibody against CD20 has been recognized as a useful therapy for antibody mediated autoimmune diseases.
Aims: We studied the clinical response to rituximab in 8 cases with refractory or relapsed TTP treated in our center.
Methods: Retrospective analysis of 8 patients diagnosed as refractory or relapsed TTP between 2008 and 2012 and recieved rituximab as a second line therapy. 6 patients were females and 2 were males.7 patients were refractory and 1 patient was a relapse case. The median age of patients was 36 years (17-48).TTP was idiopathic in 5 cases, secondary to pregnancy in 2 cases, and associated with CML in one case. All patients were initially treated with plasma exchange and steroids. Refractory cases were considered after failing a median of 12 sessions of plasma exchange (5-23), the relapsed cases presented after 21 months of complete response. All patients received rituximab 375mg/m2 weekly (4-6 cycles) in combination with the initial therapy
Results: All patients achieved complete response(CR), 2 cases relapsed after 12 and 28 months respectively and responded well to subsequent rituximab therapy, the other 6 are still in CR with a median follow-up of 29 months.
Summary / Conclusion: Rituximab represents an effective second line treatment option for refractory and relapsing TTP, all patients need to be closely monitored for relapse with extended follow up plans.

B1913

A STUDY OF 100 EGYPTIAN CHILDREN WITH INHERITED PLATELET DISORDERS:AN UNDERESTIMATED AND UNDERDIAGNOSED PROBLEM

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Background: The true incidence of inherited platelet disorders is not known. Though Glanzmann(GZ) thrombasthenia and Bernard Soulier (BS) syndrome, the commonest platelet function disorders are reported to represent 12% of rare coagulation disorders yet represent 7% in the Egyptian National Registry with 20 cases with GZ. Also, platelet function testing is limited to specialized centers so diagnosis is missed in some children.
Aims: to determine prevalence and baseline epidemiological data of inherited thromasthenias in the biggest tertiary pediatric hospital in Egypt for better understanding of the problem.
Methods: A retrospective study conducted in Hematology outpatient clinic, Cairo University Pediatric Hospita.Records were reviewed and data document-

ed of all children aged 1 to 18 years with diagnosed platelet function disorders over the past ten years.This included age, sex,family history, bleeding spectrum, hospital admissions, treatment recieved and specific diagnosis if known
Results: Inherited thrombasthenias represented 17% of rare bleeding disorders.Patients' mean age was 9.8 years, 60% males and 56% were of consanguineous marriage.Subcutaneous bleeding was initial bleeding symptom in 24%, mucous membrane bleeding in 52% and combined bleeding in 24%. Five patients recieved platelet transfusion, 20 blood transfusion and 5 required hospitalization. Specific diagnosis was reached in 52 children, 39 GZ and 13 BS whilst not reached in remaining 48 children.
Summary / Conclusion: Prevalence of Inherited thrombasthenias is relatively common in Egyptian children though underestimated at the National level so more awareness is needed and better facilities and interantional collaborations to reach specific diagnosis.

B1914
SINGLE CENTRE EXPERIENCE OF THROMBOTIC EVENTS IN PATIENTS TREATED WITH ROMIPLOSTIM
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Background: Romiplostim is a thrombopoietin analog approved for increasing platelet count in selected patients with chronic immune thrombocitopenia (ITP)
Aims: To assess thrombotic occurrence in patients under romiplostin long term treatment for ITP and myelodisplastic syndromes (MDS)
Methods: Twenty-four patients consecutively treated with romiplostim in our unit between July 2009 and January 2013 were retrospectively reviewed.
Results: The mean age was 65 years (range: 28-87). The indications for treatment were: 17 with ITP, 6 patients with MDS and 1 with bone marrow aplasia. Seven thromboembolic events in 4 patients were identified (16%). The patients were treated with romiplostim during a mean time of 530 days (range: 31-1187) The total follow up was 34.6 patients-year. The incidence of thromboembolic complications was 20% patients-year. Six out of 7 events (86%) were arterial thrombosis (5 strokes and 1 coronary artery occlusion). Only one venous event, a pulmonary embolism in a patient with a previous history of deep vein thrombosis, was observed. All patients with a thrombotic event had ITP. The median platelet count at the moment when the event occurred was 419x109 (99-1453). The mean time since the beginning of treatment with romiplostim until the thrombotic event occurred was 491 days (30-750). Of all patients, 58% had cardiovascular risk factors. All patients that developed thrombosis had at least one cardiovascular risk factor
Summary / Conclusion: In our revision we had a superior incidence of events compared to a phase 3 extent randomized study (Gernsheimer et al. reports an incidence of 2% patients-year). In our series, no patients with MDS developed thrombosis. Our study limitations are: the low number of patients, the follow up time and being a retrospective analysis. Despite the recommended standard control during the treatment, some of the patients that developed a thromboembolic complication had a platelet count higher than >400x x109 at the time of the event. Therefore we think it would be useful to perform a more frequent platelet count control, especially in patients with cardiovascular risk factors. Further studies are needed to establish if romiplostim may increase the incidence of thromboembolic events.

B1915
RETROSPECTIVE EVALUATION OF CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA PATIENTS
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Background: Immune thrombocytopenic purpura (ITP) is an acquired and autoimmune disease characterized by low platelet count and generally mucocutaneous bleedings. In adult, chronic form is usually seen, which spontaneous remission is rare and most often need treatment. Corticosteroids, intravenous immunoglobulin (IVIg) and anti-D therapies are generally considered as first-line therapy in patients with ITP. The patients who fail to respond to those therapies undergo splenectomy. For the patients who don't respond or are not eligible for splenectomy, although rituximab and thrombopoietin receptor agonists (TPO-RAs) have been usually preferred nowadays, immunosuppressive therapy and/or danazole stillare the other treatment alternatives.
Aims: In this study we evaluated the responses to the treatments in the patients diagnosed and/or followed as ITP in our center between 2003-2010 years. In those years, TPO-RAs was not available in our country and rituximab was on used for the treatment of 2 patients in last 2 years of the study.
Methods: In this retrospective study the file of 150 patients diagnosed and/or followed as ITP in our center between 2003-2010 years were reviewed. All patients had chronic ITP, which was defined as a platelet count <150,000/mm3 that had been present for at least 3 months with no clinical or laboratory findings that could account for it. The patients with a platelet count below 30,000/mm^3 and/or bleeding were given therapy. Post-treatment response was considered as complete response (CR) with a platelet count ≥150,000/mm^3 lasting ≥ 4 weeks, as partial response (PR) with a platelet count of 50,000 – 150,000/mm^{3}lasting ≥ 4 weeks and no response (NR) with a platelet count of ≤50,000/mm^3.As first-line treatments, steroids based treatment including standard dose steroids (1 mg/kg/day) and high-dose steroids (30 mg/kg/day steroids for 3 days or 40 mg dexametazon for 4 days) and IVIg were used as medical therapy. For the patients refractory to first-line treatment and the relapsed patients who had NR to a second course steroids treatment splenectomy was done. Immunosuppressive therapy (azathioprine, cyclosporine and rituximab)and/or danazole were given to the patients refractory to splenectomy and steroids treatments
Results: In our study, the mean age of the patients was 44 years (range: 18-91). Female-male ratio was2,57. The median follow-up of 150 patients was 15 months (range 2-83 months). Thrombocytopenia was incidentally detected in 51 (34%) of the cases. During the study period, 21 (14%) the patients were followed up without treatment. As a first line treatment, medical therapy were given to 129 patients. CR were seen in 93 (72%) patients, PR in 14 (11%) patients and NR 22 (17%) patients. High dose steroids therapy had no significant benefit over the standard dose therapy (P=0,59). Of the 107 patients who had response to the treatment, relapse were observed in 48 (45%) of the patients in2,5 years. Fifty-six % of the patients had relapse in one year. Eight patients had platelet counts >30,000/mm^3 and no bleeding, and was followed up without treatment. A second course of steroids based medical treatments were given to 40 relapsed patients. Of 40 patients, there were CR in 15 (38%) patients, PR in 9 (22%) patients and NR in 16 (40%) patients. Splenectomy was performed in 38 patients. Of 38 patients, 22 patients were refractory to the first-line treatment, and 16 patients who had relapsed and had NR to second course medical therapy. CR was achieved in 32 (84%) patients, PR in 2 (%5) patients and NR in 4 (%16) patients. Relapse was observed in 12 (35%) patients of 34 patients responding to splenectomy. Of 12 patients, 2 patients had achieved CR after steroids treatment and 1 patient had platelet count >30,000/mm^3 and no bleeding, and was followed up without treatment.
Severe thrombocytopenia persisted despite steroids therapy and splenectomy in 13 patients. Eleven of 13 patients received azathioprine and 2 patients cyclosporine. Response to azathioprine was seen in 6 patients. Three of 6 patients had platelet count >100,000/mm^3, 1 patient platelet count >50,000/mm^3 and 2 patients platelet counts >30,000/mm^3. Two of 5 patients who had no response to azathioprine achieved PR after cyclosporine plus danazole treatment. One of 5 patients had platelet count >30,000/mm^3 after rituximab. The other 2 patients died from intracranial bleeding.
One of 2 patients given cyclosporine had response (platelet count >30,000/mm^3), and the other had no response, but, had PR after rituximab.
Summary / Conclusion: This study showed that steroids based treatment and splenectomy are very effective treatment in ITP patients. Only 13 (%8.6) patients in our study needed further treatment.

B1916
ATYPICAL PRESENTATION OF UPSHAW-SCHULMAN SYNDROME IN A PREGNANT PATIENT WITH OMOZYGOUS FOR V LEIDEN MUTATION
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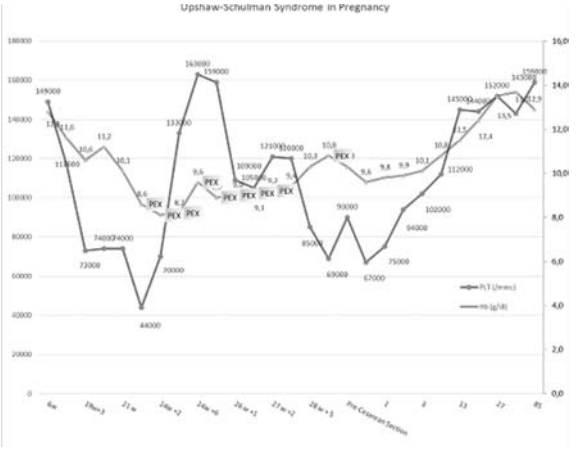
Background: Upshaw-Schulman Syndrome (USS) is a rarely congenital form of thrombotic thrombocytopenic purpura (TTP) that results from mutations in ADAMTS13 gene. Pregnancy can be the trigger event for approximately 5-25% TTP cases (late-onset USS forms or acute acquired TTP).
Aims: During pregnancy TTP may be particularly challenging because of difficult differential diagnosis with other thrombotic microangiopathies (TMAs), such as preeclampsia and HELPP syndrome, and its dramatic consequence on the fetus and its specific therapeutic management.
Methods: ADAMTS13 activity was measured using the residual collagen binding assay (CBA). The detection limit of the assay is 6%. The inhibitor titer (BU/ml) was measured using a procedure based on the Bethesda method (values >1 BU/ml were considered high titer).
Results: We report a case of a 28-year old white pregnant woman, gravida 2 para 0 at 20 weeks of gestation, who presented at our Department for worsening fatigue in the last week. Two years before she underwent an urgent cesarean section at 29 weeks of gestation for an atypical form of HELLP syndrome with perinatal death of a growth restricted fetus. Thrombophilia evaluation revealed omozygous for V Leiden mutation. In the actual pregnancy thromboprophylaxis with LMWH was adopted from the late first trimester. At hospital admission at 20 weeks laboratory data were: Hb 10.7 g/dl, platelets 73 x 10^9/l, schystocyte 12/1000 and LDH 265 U/L. Serum haptoglobin levels, DAT, coagulation test, transaminase, creatinine and blood pressure were normal. The patient was found to have an ADAMTS13 activity of < 6% with the presence of a weakly positivity for antibodies anti-ADAMTS13, confirmed in two following samples. She started oral prednisolone. No clinical sign of TTP or fetal compromise were noted, whereas platelets slowly decreased. Plasma ex-change (PEX) was initiated at 24 weeks' gestation for progressive worsening of laboratory data (Hb 9.5 g/dl, platelets 37 x 10^9/l, schystocyte 65/1000), obtaining a prompt increased of platelet count. A cesarean section was performed without complications at 30 weeks' gestation, after nine PEXs (Hb 9.3 g/dl, platelets 90 x 10^9/l) for progressive onset of allergic reactions to the procedures. A female

neonate of 1440 grams (Apgar score 5) was born in good health condition. After the delivery, a spontaneous progressive normalization of the blood count of the patient was observed. LMWH administering was continued for six weeks postpartum. Nowadays she is in good health condition with a normal blood count. Repeated analysis of ADAMTS13 confirmed a level < 6% with no antibodies. We performed mutational analysis of the ADAMTS13 gene and Upshaw-Schulman syndrome was diagnosed.

Summary / Conclusion: Diagnosis of TTP can be difficult during pregnancy for the potential overlap with other TMAs. The clinical manifestation of Upshaw-Schulman syndrome reported appeared unusual for the weakly positivity of ADAMTS13 antibodies, probably due to pregnancy-related alteration of the immunity system. Furthermore, we supposed that the atypical form of HELLP syndrome reported in her first pregnancy could be a manifestation of the Upshaw-Schulman Syndrome and its prompt recognition and treatment could have been able to avoid the perinatal death that had occurred. It is therefore critical to create a multidisciplinary team involving haematologists, obstetricians, apheresis and genetic counselling physicians to follow patients with thrombotic microangiopathies (TMAs) and to prevent unnecessary mortality both of the mother and the baby.



B1917

AN INVESTIGATION OF PLATELET DISORDERS IN PATIENTS WITH SUBCLINICAL HYPOTHYROIDISM

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Background: To examine the potential platelet disorders in patients with subclinical hypothyroidism (patients with mild rise of thyrotropin-TSH levels and normal values of FT3 and FT4 hormones), as an effect of the abovementioned abnormality on the coagulation system and possibly on platelet activity has been referred, resulting in an elevation of the risk of thromboembolic-cardiovascular disease.

Aims: A total of 82 participants were enrolled to our study; of those 37 were diagnosed with subclinical thypothyroidism (female/male: 24/13, mean age: 45.8) (Group A) and 45 were healthy subjects (female/male: 28/17, age-matched) (Group B) who consisted the control group.

Methods: In all the participants, the levels of TSH, FT3 and FT4 hormones were measured with chemiluminescent immunoassay. Whole blood parameters such as platelet count (PLT), mean platelet volume (MPV) and platelet distribution width (PDW) were determined with the use of *automated haematology analyser*. The statistical analysis of the results (t-test) was performed with the use of SPSS software.

Results: The results of the performed tests are presented at the table below:
Table: Average (mean±SD) values of PLT, MPV and PDW parameters in patients with subclinical hypothyroidism (Group A) and healthy controls (Group B).

	P	LT (K/μL)	MPV (fL)
PDW (%)			
Group A	262±111	10.84±0.82	12.94±1.68
Group B	271±65	10.23±0.41	11.34±2.48

Summary / Conclusion: The results suggest that in patients with subclinical hypothyroidism, changes in platelet indices confirming the theory of their activation are observed, as both MPV and PDW values were significantly higher when compared to the control group (P=0.05). Platelet parameters were not found to be correlated with thyroid autoantibodies (anti-TPO and anti-TG) in cases to which the relevant data were available.

B1918

EVALUATION OF AFFECTING FACTORS FOR PLATELET FUNCTION SCREENING TEST

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Background: Platelet function screening studies using commercial analyzer are useful and make objective interpretation for the result. With in-vitro measures, principle of the instrument is that anticoagulated whole blood is flowed under high shear force through a narrow hole punched out of a membrane coated with collagen and epinephrine (EPI) or ADP. The combination of biophysical shear and chemical stimulation initially promotes platelet adhesion to the outer edges of the cut membrane, subsequently, platelet-platelet aggregation leads to full occlusion of the channel, which is recorded as closure time. However, platelet function screening test using this commercial analyzer can be affected by hemoglobin level, platelet counts, blood type, venipuncture time, and so on. Several specimen's or patient's factors without these known factors can also affect.

Aims: We investigated the influences of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and mean platelet volume (MPV) about EPI result of platelet function screening test using commercial analyzer.

Methods: Two-hundred seventy-four results among total 874 specimens requested for platelet function screening test during 1 year were selected for this evaluation. Patients' specimens with prolonged prothrombin time or activated partial thromboplastin time, platelet inhibitor treatment, low hematocrit (<30%), low platelet count (<70x10^9/L), and results over maximum closure time were rejected.

Results: Means and standard deviations of each variable were 140.79 and 45.49 for EPI, 22.83 and 21.10 for ESR, 2.58 and 4.86 for CRP, and 7.46 and 0.82 for MPV. Significance values of Pearson correlations were 0.254 for EPI and ESR, 0.001 for EPI and CRP, and 0.33 for EPI and MPV.

Summary / Conclusion: EPI closure time was affected by MPV and ESR values. ESR would affect to EPI results more than MPV, because width of MPV results was lesser than ESR. ESR was considered one of important affecting factor for EPI closure time with hemoglobin level, platelet counts, blood type, venipuncture time, platelet inhibitor drugs, and so on. Therefore, whether increased ESR results should be considered when platelet function screening test do interpret.

B1919

TREATMENT OF REFRACTORY KASABACH-MERRITT PHENOMENON WITH SIROLIMUS

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Background: Kasabach-Merritt phenomenon (KMP) is a rare disease, usually in infants, which is characterised by profound thrombocytopenia, haemolytic microangiopathy, a consumptive coagulopathy and an enlarging vascular lesion (either a kaposiform haemangioendothelioma (KHE), or a tufted angioma, or a mixture of both).

Aims: We report a case of sirolimus successfully treating refractory Kasabach-Merritt phenomenon. A male infant of a secundigravida was born at term, and noted to have a large neck mass. Platelet count at birth was 76 x10^9/L, but fell to 27 in 72 hours. Coagulation screen was initially normal. MRI and biopsy of the lesion confirmed the diagnosis of mixed KHE and tufted angioma.

Methods: Treatment, under the supervision of haematology and dermatology, initially with prednisolone was unsuccessful. During the initial phase of treatment, support with platelets and cryoprecipitate was required, but need lessened with the addition of weekly vincristine. Intervention with selective embolisation was required on two occasions within the first year of life. Blood product support was only required for procedural intervention after six months of life. Vincristine frequency varied over a three year period as per clinical need (platelet count, coagulopathy, myofascial pain and tumour size).

Results: Decision was made to commence sirolimus as the thrombocytopenia became more refractory to vincristine. Dose was titrated slowly. The refractory thrombocytopenia responded excellently and has normalised since initiation of therapy.

Summary / Conclusion: There are only a few reported cases of KHE/tufted angioma with refractory KMP responsive to sirolimus therapy worldwide. mTOR inhibitors may prove to be a very important and potent treatment modality in selected cases of refractory Kasabach-Merritt phenomenon.

B1920

VENOUS THROMBOEMBOLIC EVENTS IN PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) PATIENTS AFTER SPLENECTOMY OR DURING THROMBOPOIETIN RECEPTOR AGONISTS (TPORA) THERAPY

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Background: Recent epidemiological evidences suggest that chronic ITP patients could have an higher risk of venous thromboembolism (VTE) in comparison to general population.
Between second-line approaches to manage adults with chronic ITP, splenectomy and TPOra, are regarded as the most suitable treatment choices.
Although laparoscopic approach have reduced short- and long- term complications of splenectomy, VTE may occur. Equally, occurrence of VTE was demonstrated in registrative and extend trials with TPOra, raising the question on the potential thrombophilic risk of these new agents. No direct comparisons between splenectomy and TPOra are available in clinical trials.
Aims: A retrospective, monocentric study was carried out to evaluate the rate of VTE in ITP patients after splenectomy and during treatment with TPOra.

Methods: We retrospectively evaluated 37 ITP patients who underwent splenectomy (20 patients) or TPOra (17 patients) between 2008 and 2012 at our Institution.
Among splenectomized patients, 8 (40%) were male and 12 (60%) female, with a median age at diagnosis of 40 years (11-68) and a median age at splenctomy of 45 years (16-78). At splenectomy,14 (70%) patients had a chronic ITP, 5 (25%) a persistent ITP and 1 (5%) patient a newly diagnosed ITP. Median time from diagnosis to splenectomy was 19 months (1-250). Laparoscopy approach was used in all patients.
Among TPOra-treated patients, 6 (35%) were male and 11 female (65%), with a median age at diagnosis of 58 years (11-86) and a median age at the start of TPOra treatment of 64 years (28-89). 5 patients received TPOra because of refractory, splenectomized ITP, while 12 patients were in chronic or persistent phase and not suitable for splenectomy. Median time from diagnosisto TPOra therapy was 84 months (5-246).
The median observation time was 15 months in both groups (range 1-59 and 3-45 respectively).
Results: Two thromboembolic events were recorded in the splenectomized group (cumulative incidence 10%). A splanchnic vein thrombosis (SPT), occurred in a 38-years old male with persistent ITP, while a VTE (pulmonary embolism and deep vein thrombosis at leg) occurred in a 70-years-old female with chronic ITP. Both events occurred after few days from splenectomy, despite peri-surgery thromboprophylaxis with low molecular weight heparin (LMWH). Both patients were treated with full dose of LMWH (for one month then with warfarin for 12 months because of complete remission of ITP in the first case; for 4 months. with suspensions due to relapse of ITP in the second case).
Among TPOra-treated patients, two SPT was recorded (cumulative incidence 11.7%). The events occurred in a 55-years-old female with refractory ITP and in 62-years-old female with chronic ITP, after one month and two years from the beginning of TPOra, respectively. TPOra was interrupted and LMWH started in both cases. In the first case LMWH was temporary interrupted for relapse of ITP, treated with immunoglobulins; in the second patient LMWH was definitively stopped 4-months-later for relapse of thrombocytopenia, refractory to specific therapies; a diagnosis of criptogenetic cirrhosis was made.
Summary / Conclusion: In this small, retrospective study, a similar cumulative incidence of VTE, in a same follow-up period, was observed in two group of chronic ITP patients after splenectomy and during TPOra treatment; 3/4 VTE were SVT, an event quite rare in ITP. In a patient a known risk factor for SVT (hepatic cirrhosis) was diagnosed. However, in absence of a well-designed randomized clinical is not possible to evaluate any difference in thrombogenic risk of splenectomy and TPOra therapy.

B1921

PERSISTENT REMISSION OF CHRONIC IMMUNE THROMBOCYTOPENIA AFTER ROMIPLOSTIM DISCONTINUATION

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Background: Primary immune thrombocytopenia (ITP) is an immune-mediated condition characterized by isolated thrombocytopenia, with peripheral blood platelet count of < 100x 10^9/L in the absence of an identifiable underlying cause of thrombocytopenia. Increased platelet destruction by antiplateled autoantibodies plays a key role in the pathogenesis and may also impair megakaryocyte platelet production; thrombopoietin levels are normal or only slightly elevated in patients with ITP, suggesting that the lack of compensatory stimulation of megakaryocytes may contribute to impaired platelet production. Clinical studies in patients with ITP demonstrated that thrombopoietin (TPO) mimetics increase platelet production and can outpace platelet destruction. Romiplostim is a Fc-peptide fusion protein (peptibody) that has been shown to produce a dose-dependent increase in platelet counts in individuals and improve platelet counts during both short- and long-term use in patients with chronic ITP. TPO-receptor agonists are currently used for patients at risk of bleeding, who relapse after splenectomy or who have a contraindication to splenectomy and who have already failed at least one other therapy. As far as we know an interruption of treatment with TPO mimetics' is not feasible. In our study we evaluated the feasibility of stopping treatment with Romiplostim.
Aims: In our study we evaluated the feasibility of stopping treatment with Romiplostim.
Methods: We evaluated treatment course with Romiplostim in 27 patients with chronic ITP referred to our institution between 2008 and 2013. Diagnosis of ITP was made according to established guidelines. Median age was 72 years (range 52-93 years), 13 were male and 14 female. Prior starting treatment with TPO mimetic, all patients demonstrated severe ITP and platelet counts were below 20 × 10^9/µl. Romiplostim was started at 1 µg/kg per week and the dose was adjusted to a maximum of 10 µg/kg per week to reach a target platelet-count range of 50-250 × 10^9/L.
Results: At the last follow-up, 22 patients (81%) showed a clinical benefit consisting in achieving a platelet count ≥ 50 × 10^9/L. 4 of 27 (15%) patients were able to maintain a stable platelet response when Romiplostim was stopped. Two patients with a stable platelet count > 250 x 10^9/L discontinued Romiplostim without no dose reduction. At time of interruption one patient was currently treated with 1 µg/kg and the other one with 3 µg/kg per week. With a follow up of 17 and 3 months respectively, they are still off-treatment. 2 patients discontinued treatment after a progressive dose reduction and they showed a stable platelet count > 100 x 10^9/L at 22 and 8 months of follow-up respectively.
Summary / Conclusion: In our limited experience we were able to discontinue Romiplostim in selected patients observing stable platelet counts > 100 × 10^9/µl during the period of observation. Romiplostim seems a very promising therapy for the treatment of refractory forms of ITP; further investigations and specific clinical trials are warranted to explore the feasibility of stopping treatment .

B1922

A CASE OF MYH9 DISORDER WITH KIDNEY DYSFUNCTION SUCCESSFULLY TREATED BY ELTROMBOPAG FOR THE INHERITED THROMBOCYTOPENIA

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Background: MYH9 disorder is one of the most frequent inherited forms of thrombocytopenia. It is transmitted in an autosomal dominant fashion and derives from mutation of MYH9, the gene for the heavy chain of non-muscle myosin IIA (NMMHC-IIA). Patients present with congenital macrothrombocytopenia with mild bleeding tendency and may develop kidney dysfunction, deafness and cataracts in later life. Here we present a male patient diagnosed as MYH9 disorder with the manifestation of kidney dysfunction nine years after the recognition of macrothrombocytes in the peripheral blood smear. His platelet count normalized with Eltrombopag therapy. We confirmed his MYH9 disorder by using immunofluorescence staining with anti-NMMHC-IIA localization and genetic analysis *MYH9* using PCR and direct DNA sequencing.
Aims: A 58 year-old male patient was transferred because of progressive worsening of kidney function and hypertension (HT). At the initial visit to our hospital, urinalysis showed protein 3+, and hematiuria.2+. Serum creatinine and BUN were elevated 1.91mg/dl and 22.5 mg/dl, respectively. He had a history of diabetes mellitus (DM) and HT for 10 years and had been well controlled. From 7 months before presentation, his blood pressure became uncontrolled with an associate increasing level of serum creatinine. Renal biopsy had been planned, but it was postponed by his low platelet count as 22,000/µl. Past medical history revealed that he had large platelets in the peripheral blood smear 9 years previously. Bone marrow aspirate revealed normocellular marrow, and megakaryocytic hyperplasia, which is compatible with the diagnosis of idiopathic thrombocytopenia (ITP).
Methods: The patient was treated by prednisolone for ITP but his glycaemia control worsened. Reduced dose of prednisolone with Eletrombopag restarted and the platelet count increased to 138,000 /µl which was sufficient for performing a renal biopsy. This was performed 8 months after his initial visit, when the serum creatinine level was 2.7mg/dl. At that time his daughter was referred to the hematology department because of asymptomatic thrombocytopenia during surveillance check-up for her pregnancy. Her peripheral blood smear stained with May-Giemsa, which confirmed the presence of large platelets and granulocyte inclusion bodies that resemble DÖhle-like bodies which suggested the diagnosis of May-Hegglin anomaly. Additional medical history from his sister was revealed the diagnosis of May-Hegglin anomaly (data are not available). To further characterize this, we analyzed his blood sample with immunofluorescence staining against anti-NMMHC-IIA localization in granulocytes and direct DNA sequencing of *MYH9*, after informed consent for genetic analysis was obtained from the patient.
Results: The findings of light microscopy revealed destruction of one third of the kidney gromeruli and the mesangial cell proliferation. His kidney function deteriorated progressively and at the age of 59 years he was diagnosed as chronic kidney disease stage 4 in association to sensorineural hearing loss. Immunofluorescence analysis showed abnormal NMMHC-IIA accumulation.

Direct sequencing analysis of the MYH9 gene revealed a heterozygous E 1841 mutation.
Summary / Conclusion: The term May-Hegglin Anomaly, encompasses four autosomal-dominant thrombocytopenias that were previously described as distinct disorders into four categories, namely the May-Hegglin Anomaly, Sebastian, Fechtner and Epstein syndromes. However, they are not distinct entities, but represent a variable expression of a single illness. Although the variable expressions of the immunofluorescence test for NMMHC-IIA distribution within the granulocyte are detected, these four categories are all derived from mutations of the *MYH9* gene. Regarding the genotype-phenotype correlation, the MYH9 disorder with E 1841K amino acid mutation generally shows a mild to intermediate risk of developing renal impairment. In our case, the onset of renal impairment was relatively late, in his sixth decades, but the progression of dysfunction was very rapid.
It is necessary to maintain high surveillance in order to recognize cases to therefore investigate the genotype-phenotype correlation in the MYH9 disorder.

B1923
THE EFFECTIVNES AND SAFETY OF THE THROMOPOETIN(TPO) RESEPTOR AGONISTS IN THE TREATMENT OF ADULTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA(ITP) REFRACTORY TO OTHER TREATMENTS
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Background: ITP is an acquired autoimmune disorder characterized by low platelet counts (increased platelet destruction and suboptimal platelet production) and an increased risk of bleeding. The goal of treatment is to eliminate symptoms of bleeding, and to maintain a platelet count ≥50 x 10^9/L. Initial therapy consists of corticosteroids or intravenous immunoglobulin (IVIg). For most children, ITP is a self-limiting disease; however, for some children and most adults, thrombocytopenia can become chronic. Patients with chronic refractory ITP (CRITP) might undergo splenectomy. Adults with ITP fail to respond to conventional therapies in almost 30% of cases, developing CRITP. Newer therapies for ITP include rituximab and TPO receptor agonists. Rituximab is a useful second-line therapy and may be splenectomy-sparing. TPO agonists have demonstrated large treatment effects in many randomized studies with respect to increasing platelet levels. In 2008, the FDA approved the use of two new TPO mimetic agents, romiplostim and eltrombopag. Both medications are registered in Russian Federation.
Aims: To evaluate the efficacy and safety of romiplostim in adult CRITP patients from a single institution.
Methods: Adult patients (n=27) were prospectively treated with romiplostim. 25/27 (93%) patients were women and had a mean age of 53 years (range, 30 – 71), with a median time from their initial diagnosis being 13 years (range, 1.5 – 47). All the patients entered into the study had insufficient response to previous therapies. The most common first-line treatments were corticosteroids and IVIg (17 patients, 63%), 10 patients (37%) were treated with second-line therapies including splenectomy (4 patients). Six patients (22%) were receiving concurrent corticosteroid therapy (prednisolone with daily dose ranged from 5 to 30 mg) at the start of romiplostim. Baseline platelet count was 18x10^9/L (range, 1–47x10^9/L). Romiplostim was administered as a subcutaneous injection weekly. The initial dose was 1 mcg/kg/wk, with adjustments up to 10 mcg/kg/wk to maintain platelet counts ≥50x10^9/L. The primary end point was a durable platelet response (≥50x10^9/L). Response was classified as sustained (SR) when it was stable for a minimum of 6 months. No other immunosuppressive drugs were used.
Results: The median duration of romiplostim therapy was 25 weeks (range, 4 to 118). The median dose of romiplostim used during SR was 3.9 microg/kg/wk. A durable and sustained platelet response (≥50x10^9/L) wasachieved in 24/27 patients (89%) after four weeks of treatment. The platelet response is seen to be between 50 and 470x10^9/L. Symptoms of bleeding were eliminated in all the patients (in whom a SR and even less than SR was achieved). Prednisolone was successfully tampered in 4/6 patients in 4 – 17 weeks from initiation of romiplostim. The most common adverse events were not severe: ecchymosis at the injection sites (30%), headache (20%), arthralgia (10%), and skin rash (5%). Serious adverse events were not registered.
Summary / Conclusion: Romiplostim administration has been associated with a durable platelet response in patients with CRITP. Romiplostim has been found to be well tolerated. Overall SR achieved was high, long term, and with no risk of severe adverse events. In conclusion, romiplostim therapy is effective and safe in adult patients with CRITP. No other illnesses have been recorded in this group of patients during the ongoing follow-up period.

B1924
REFRACTORY CYCLIC THROMBOCYTOPENIA
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Background: Cyclic thrombocytopenia (CTP) is a rare disorder of regularly oscillating platelet count and variable bleeding tendencies. Possible aetiologies include immune platelet destruction, insufficient platelet production, hormonal disturbance and T cell clonal disease.
Aims: We report a case of CTP refractory to multiple modalities of treatment that include corticosteroids, rituximab, Intravenous immunoglobulins (IVIG), anti D, cyclophosphamide, cyclosporine, azathioprine and romiplostim. We reviewed 53 cases of CTP reported in the literature to highlight the characteristics of this under reported disease.
Methods: The patient was 21 -year- old Caucasian lady, presented with excessive bruising, heavy menstrual periods and isolated low platelet count. She was diagnosed with Idiopathic thrombocytopenic purpura (ITP). Over the following three years she failed multiple modalities of ITP treatments including corticosteroids, rituximab, Intravenous immunoglobulins (IVIG), anti D, cyclophosphamide, cyclosporine and azathioprine. Consequently she had a bone marrow aspiration and trephine biopsy that showed absence of megakaryocytes. It was noticed that the patient has marked oscillation of platelet count between 1700 x 10^9/L and 0 in 5 week's cycles. When the platelet count fall the patient develops severe bleeding that requires treatment with platelet transfusion and pulses of recombinant factor VIIa (NovoSeven). The diagnosis of CTP was made and the patient received treatment with danazol, eltrombopag and then romiplostim without response.
Results: Patients with CTP present with symptomatic thrombocytopenia and frequently misdiagnosed as ITP. Platelet count fluctuates in cycles ranged between 1 week and 10 weeks and the average cycle is 4 weeks. Platelets fluctuate between a nadir that can reach zero and a peak that can exceed the normal limit of platelet count and the maximum reported platelet count was 2300 X10^9/l. CTP is more common in females with a female: male ratio of 3:1 and more common in Japanese. The age at diagnosis varies from infants to patients at their 80s. The mean age at diagnosis is 40 years. Not like ITP, the bone marrow in CTP shows absent or low megakaryocytes during the nadir phase of the cycle and normal or increased megakaryocytes during the thrombocytosis phase. There were several reports of isolated associations including Sjögren syndrome, LGL Leukaemia, H.Pylori, polycythemia vera and Clonal T cell. Conclusion: Most patients with CTP including our case fail to respond to conventional ITP treatments; nevertheless few reported cases responded to cyclosporine, corticosteroids, IVIG, azathioprine, low dose hormonal contraception and romiplostim. There is a reported case of CTP improved after H.Pylori eradication and there are also reports of spontaneous recovery.
Summary / Conclusion: Cyclic thrombocytopenia is an underreported cause for thrombocytopenia and need to be considered in the differential diagnosis of ITP. We report a case of CTP refractory to multiple modalities of treatment which highlights the need for better understanding of the disease and more effective treatments.

B1926
THROMBOCYTOPENIA DURING LONG-TERM LOW-MOLECULAR-WEIGHT HEPARINS (LMWH) FOR CANCER-ASSOCIATED VENOUS THROMBOEMBOLISM (VTE): TROPIQUE, A PROSPECTIVE, MULTICENTER, OBSERVATIONAL STUDY
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Background: Long-term treatment with LMWH is the recommended standard for treatment of venous thromboembolism (VTE) in cancer patients [1]. LMWH are known to be associated with the occurrence of thrombocytopenia. However, malignancy by itself, chemotherapy and radiotherapy increase the risk of thrombocytopenia in patients with cancer. Few prospective data are available on thrombocytopenia during long-term prescription of curative doses of LMWH in patients with cancer in real clinical practice
Aims: The study aims at documenting the prescription and use of curative doses of LMWH in patients with cancer in routine clinical practice. Clinical outcomes such as VTE recurrence, bleeding and thrombocytopenia will be recorded. Also, the study will assess whether the occurrence of thrombocytopenia may result in treatment discontinuation.
Methods: This is an ongoing prospective, observational, multicenter study in cancer patients with symptomatic VTE and an indication for treatment with LMWH for at least 6 months. Adult patients, aged 18 years or more, willing to participate, with diagnosed malignancy and recent symptomatic VTE in whom a treatment with LMWH has been initiated within less than 7 days, are consecutively included in the study. Patients already treated with LMWH for more than 7 days or with a contra-indication to LMWH are not eligible to participate. Main study outcome measures include VTE recurrence, bleeding, thrombocytopenia and adherence to recommended standards [1]. Assuming a normal patient distribution and the unfavorable hypothesis that only 50% of patients are treated according to the national recommendations, 384 patients are needed to

obtain conclusive results with a precision of 5% and a risk error of 5%. Incidences of 7% of VTE recurrence and 6% of major bleeding are expected. A total of 400 patients are therefore planned to be included in the study.
As of end of February 2013, 70 sites were activated and 136 patients were enrolled. Completion of enrollment is expected by June 2013.
Results: The results obtained from this study will add significantly to the knowledge regarding the long-term treatment safety of LMWH to prevent recurrent cancer-associated VTE in real clinical practice and will document whether thrombocytopenia influences treatment management.
Summary / Conclusion: Important prospective safety data on the long-term management of cancer patients with symptomatic VTE will be generated, and analyses of clinical parameters will add important information that may help to further tailor therapy and improve compliance with established recommendations.
[1] Farge et al. International clinical practice guidelines for the treatment and prophylaxis of venous thromboembolism in patients with cancer. J Thromb Haemost. 2013 Jan;11(1):56-70.

B1927

SLOW "DELAYING AND TAPERING" ROMIPLOSTIM ADMINISTRATION COULD ALLOW ITS DEFINITIVE SUSPENSION AND STABLE COMPLETE REMISSION IN PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) PATIENTS

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Background: Various lines of evidence indicate that Primary Immune Thrombocytopenia (ITP) is due to impaired thrombopoiesis in addition to the traditionally recognized autoantibody-mediated mechanism of platelet destruction. Thrombopoietin-receptor (TPO-r) agonist have demonstrated to be effective in ITP patients, given the high rate of sustained response while on long-term treatment.
Aims: We report 8 cases of patients treated with the TPO-r agonist romiplostim (R) for ITP unresponsive to different lines of therapy, that achieved a complete response while on-treatment, that have subsequently undergone a "delaying & tapering" (D&T) phase of R administration to ascertain if definitive suspension of the drug could be possible without relapse.
Methods: A retrospective observation of a single institution case series of ITP patients treated with R between Jan 2010 and Jan 2013 was performed.

Romiplostim remission induction in eight patients: main characteristics

Patien data, plt baseline	ITP duration before R	Prior treatment	R duration/ n tot doses admin	Current Plt count	R Off therapy (weeks)
M, 20y, 1x10^9/L	2 months	S, IVIG	15 wks/12	227x10^9/L	116
M, 67y, 1x10^9/L	2 months	S, IVIG	21 wks/17	194x10^9/L	84
M, 66y, 12 x10^9/L	2 years	S, IVIG, CSA, spl	22wks/13	235x10^9/L	74
F, 69y, 3x10^9/L	3 months	S, IVIG, rtx	37wks/32	132x10^9/L	12
F, 51y, 2x10^9/L	2 months	S, IVIG	64wks/60	156x10^9/L	23
F, 55y, 11x10^9/L	4 months	S, IVIG, azathiop	122wks/ 92	267x10^9/L	4
F, 41y, 5x10^9/L	2 months	S, IVIG	58 wks/55	202x10^9/L	4
M, 42y, 1x10^9/L	2 months	S, IVIG	14 wks/11	257x10^9/L	5

R: Romiplostim, S: steroid, IVIG: intravenous immunoglobulin, CSA: Cyclosporin, Azathiop: azathioprine, spl: splenectomy; wks: weeks

Results: Ten women and six men have been treated with first line steroid and IVIG and second or third line treatment (4 with azathioprine, 4 cyclosporin, 4 splenectomy, 2 rituximab) and considered non responders, so they started R administration. Eight patients (4 women and 4 men) received R for a mean of 42,6 weeks (11 to 101) and they all experienced a complete response (plt >100 x 10^9/L). Instead of continuing the scheduled weekly administration as indicated by the manufacturer, under strict weekly lab check, we started to delay of 7 days the expected dose. Additional weeks of delay were given if the platelet count remained > 50 x 10^9/l. In four out of eight patients we observed a drop of the platelet count under 50 x 10^9/lt without any bleeding and we resumed R treatment once at the same last given dose, then we tapered the dose of 1 mcg/kg the week after and so on until we stopped the treatment, and we observed a spontaneous rising of the platelet count to a normal value in all four patients. The other 4 patients showed a good stability during the "delaying period" of R treatment, and they did not need to resume the drug administration because their platelet remained normal. Eight patients were not considered for D&T procedure because of the following reasons: One woman was treated with R until platelet count rose and than splenectomy was performed with complete response achieved. One patient developed antibody against R: she lost the response after 48 weeks of ongoing treatment with sudden platelet drop and she was treated with rituximab that allowed a new complete response until now. One patient while on R treatment (14 months) experienced an acute myocardial infarction when platelet count was 89 x 10^9/L. A percutaneous transluminal coronary angioplasty was performed, and a bare metal stent was placed. R was suspended and we started rituximab when platelet count dropped, until we reached a safe platelet count for aspirin treatment. One patient is on chronic TPO-r ongoing treatment and her platelet count shows a complete dependence to R. One patient refused to continue R as chronic therapy because of a fluctuating response. Three other patients are currently under weekly R administration since few weeks now, with unstable response.
Summary / Conclusion: TPO-R Romiplostim is a handy treatment for ITP patients in different phases of the disease. A cautious attemp of "delay and tapering" of R administration in well stabilized responders could help to recognize patients potentially cured by the drug, that can be safely weaned from it and left without any therapy and a normal platelet count. Nonetheless attention has to be paid for possible adverse events.

B1928

SUCCESSFUL TREATMENT WITH THE TPO AGONIST ROMIPLOSTIM OF A YOUNG PATIENT WITH MYH9 RELATED THROMBOCYTOPENIA

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Background: MYH9 related platelet disorders are rare autosomal dominant syndromes characterized by macrothrombocytopenia with leukocyte inclusion bodies associated with mutations in MYH9, the gene for the nonmuscle myosin heavy chain IIA. Platelet transfusion is currently the primary medical treatment to improve hemostasis in symptomatic patients.
Aims: To evaluate the increase of platelet count in a patient with a severe thrombocytopenia associated with *MYH9* mutations (*MYH9*-related disease) treated with Romiplostim, a parenteral thrombopoietin mimetics.
Methods: a 39 years old female with symptomatic MYH9 related thrombocytopenia diagnosed at the age of 37 with indirect immunofluorescence and then molecular analysis of MYH9 gene. Platelet count before starting was 11x10^9/L (range 7- 23) with WHO grading of bleeding severity 2. She was treated with romiplostim once weekly by subcutaneous injection.
Results: romiplostim treatment was commenced at a starting dose of 1 mcg/kg, as recommended, by weekly subcutaneous injection performed in our outpatient department. The weekly dose was stepwise increased by 1 mcg/kg during the second and third week to improve platelets. At the beginning of the fourth week platelet count exceeded 50x10^9/L and Romiplostim treatment were continued at the dose of 3 µg/kg. Persistent platelet increase during the following weeks with disappearance of the bleeding manifestations allowed the progressive reduction of steroids. Romiplostim was well tolerated. The patient started home self injection during the fourth week. On sixty week the platelet count was 96x10^9/L, WHO grading of bleeding severity was 0.fl. Balduini et al. (Blood 2010) reported their experience on oral thrombopoietin mimetics of twelve adult patients with MYH9-RD with significative response on most, but not all patients (major responses were obtained in 8 patients, minor responses in 3. One patient did not respond).
Summary / Conclusion: At our knowledge, this could be the first case of MYH9 related platelet disorders treated with parenteral thrombopoietin mimetics (Romiplostim). Although performed on a single case, our experience suggest potential efficacy of such a treatment in this rare patients, thus offering new openings in the treatment of inherited thrombocytopenias both in chronic treatment and as preparation to surgical operations.

B1929

A COMPARATIVE STUDY OF NEW PLATELET INDICES IN PATIENTS WITH IRON DEFICIENCY AND HOMOZYGOUS OR HETEROZYGOUS SICKLE CELL DISEASE

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Background: The determination of platelet parameters, such as mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT) and immature platelet fraction (IMF), is provided easily by modern automated hematologic analyzers as a component of complete blood count, facilitating their further evaluation in patients with hematopoietic disorders.
Aims: The aim of the study to examine these platelet indices in patients with sickle cell disease and in those with iron deficiency anemia.
Methods: A retrospective study of 15 heterozygous (8 females, 7 males), 88 homozygous (52 females, 36 males) for sickle cell disease subjects and 47 iron deficient patients (29 females, 18 males) was conducted. Data were recorded and analyzed using Student's t test and Pearson's correlation test. P values <0.05 were considered as statistically significant.
Results: The participants were matched for age and gender. The mean PCT

value was 0,2611±0,121% in heterozygous, 0,4153±0,1198% in homozygous individuals and 0,2950±0,12203% in iron-deficient ones. PCT value was found to be significantly higher in homozygous patients when compared to the heterozygous (P<0,001) and sideropenic participants (P<0,001). IPF values were positively correlated with MPV (r=0,682, P<0,001) and PDW (r=0,759, P<0,001) in homozygous patients. A similar correlation was observed in subjects with iron deficiency [(r=0,694, P<0,001) and (r=0,739, P<0,001) respectively].
Summary / Conclusion: The study indicates that PCT levels were significantly higher in patients homozygous for sickle cell anemia than in heterozygous and iron-deficient ones. Regarding the relationships between the various platelet parameters, IPF was found to be positively correlated with MPV and PDV indices not only in homozygous sickle cell disease but in iron deficiency anemia as well.

B1930
COMPARISON OF MANUAL AND AUTOMATED PLATELET COUNTS IN THROMBOCYTOPENIA
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Background: Reliable platelet count is of prime importance in clinical management of thrombocytopenia where platelet transfusion, splenectomy, therapeutic or any appropriate intervention is concerned. Still most of the methods which accurately estimate the number of platelets remain at research or highly specialized laboratory set up. Automated analyzer count is based on impedance and optical technology whereas the manual chamber count method is based on phase contrast and naked eye observation. Results of the automated counts especially in severe thrombocytopenia seem to be uncertain and less reliable.
Aims: Determine how reliable the platelet count is in automated analyzer reports in thrombocytopenia, and how it compares with manual assessment of the platelet count.
Methods: In this research, a descriptive cross sectional study was carried out at the study setting of haematology clinic at Colombo South Teaching Hospital on 83 samples from patients with thrombocytopenia to determine the differences between the automated analyzer method and manual chamber count method especially in severe thrombocytopenia. Data record sheets were analyzed and stratified into two strata as moderate thrombocytopenia (<=150,000–50,000/mm^3) and severe thrombocytopenia (<=50,000/mm^3). Pearson Correlation and Levels of Agreement were carried out at each stratum according to the Bland and Altman comparison method.
Results: Pearson Correlations(R) was 0.897 as a whole and 0.78 at moderate thrombocytopenia where as it was low as 0.60 at severe thrombocytopenia. There was a tendency of around 20,000/mm^3 deviation of automated counts from the true count at moderate thrombocytopenia and that was 13,000/mm^3 at severe thrombocytopenia.
Summary / Conclusion: Pearson correlation was satisfactory and the levels of agreement were clinically acceptable at moderate thrombocytopenia. Both correlation and levels of agreement at severe thrombocytopenia were significantly beyond the clinically acceptable levels.

B1931
SAME DIAGNOSIS, DIFFERENT PROBLEMS, VARIABLE CLINICAL PROGRESS: IMPRESSIONS RELATING TO ELTROMBOPAG TREATMENT WITH 4 CASES
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Background: Eltrombopag treatment is indicated in chronic immune trombocytopenia (ITP) which is resistance or unresponsible to steroids or immunosuppressive treatments. In spite of the illness and the drug name is same, clinical progress and response to treatment is variable in every patient. In this abstract we present 4 chronic ITP cases who were unresponsible to steroid and immunosuppressive treatments and also each of these cases has different clinical progress and also have different kinds of problems .
Aims: To introduce the variable clinical progress and different problems of eltrombopag treatment
Methods: Case 1: Fifty-six year-old female was diagnosed as ITP at 2006. She was unresponsible to steroid, immunosupressive and azathiopurine treatments. Splenectomy was not performed because of reluctancy of patient and conflict of surgeon due to 300-1000 µL thrombocyte levels. The patients thrombocyte level was under 1000/µL when her nose bleeding was started. After these bleedings 50 mg/day eltrombopag treatment was started at September 2011. After two weeks of follow up process, patients thrombocyte levels was still low (1000-2000/ µL) and we increased the eltrombopag dose to 75 mg/day. Patients bleedings was stopped when her thrombocyte level increased to 10-15.000/ µL. The patients is still being followed with the same thrombocyte levels and 75 mg/day eltrombopag treatment without bleedings. Case 2: Thirty-three year-old female patient was diagnosed as ITP at 1987. Splenectomy was performed after five months from the diagnosis because of unresponsibility to steroid treatment. Accesory splenectomy was also performed 7 months later. Steroid, cyclophosphamide and azothiopurine treatment was given to the patient who was also unresponsible to splenectomy. Immunosupressive treatments was stopped at 2010 because of low thrombocyte levels (10.000/ µL). Rituximab treatment was started with the approval of the ministry of health. A partial response was achieved and the patient was followed without treatment. 50 mg/day eltrombopag treatment was started when the patient's thrombocyte level was 12.000/ µL at August 2012. Thrombocyte level was increased to 360.000/ µL in first week. The patient is still being followed in our clinic with the same treatment. Case:3 Forty-nine year-old female patient was diagnose as ITP at 2006. Splenectomy was performed at 2007 because of unresponsibility to steroid treatment. Azothiopurine treatment was given to the patient who was also unresponsible to splenectomy. Accesory spleen was detected at 2011 but the patient did not accept the surgery. 50 mg/day eltrombopag treatment was started at January 2012 when the thrombocyte level was 15.200/ µL. One month later thrombocyte level was 117.000/ µL but it was reduced to 15.000/µL in the second month. Because of that patient's diet and additional drug treatments was questioned. It was noticed that the patient was using oral calcium because of osteopenia. Her diet was changed and eltrombopag treatment was continued. Thrombocyte level was increased to 120.000/µL. The patient is still being followed in our clinic with the same treatment. Case 4: Forty-four years old male patient was diagnosed as ITP at 2011. Splenectomy was performed because of fail of response to steroid treatment. IVIG and vincristine treatments was given but thrombocyte levels was still low. 50 mg/day eltrombopag was started when the thrombocyte was 14.500/µL. Thrombocytes was still low and the dose of eltrombopag was increased to 75 mg/day. Follow of process cervical lymphadenopathy was detected and excisional biopsy was performed. Diffuse large B cell lymphoma was reported. Eltrombopag treatment was stopped and R-CHOP treatment was given. Thrombocyte level was > 100.000 /µL in the tenth day of the R-CHOP treatment.
Results: We presented 4 cases with different clinical progress and different problems.
Summary / Conclusion: In international guidelines, the response criteria for cronic ITP treatment of thrombocyte level criteria is defined under 5000/µL. But the resistant cases like our firs case, occurance of bleeding is more important than the guideline numbers. furthermore, 2500% at thrombocyte level was still under 5000/µL, should poiint that patient is unresponsible to eltrombopag treatment according to guidelines. But the ending of the bleedings directly means that the patient is well responded to the treatment. So when we are looking for references from guidelines, we should remember the word 'there is no illness, there is patient' and the response criteria to the treatment should be individualized. If response fails to the eltrombopag treatment occurs, diet and additional drug usage of the patient should be questioned. As in our third case, calcium usage and excessive calcium diet seems to cause response fail to eltrombopag. Unexpected decreases of thrombocyte levels from formely well responded patients, should be questioned about diet and additional drug usage again. Although not initially identified in chronic ITP,it should be noted that malignant disease may occur in the follow-up process like our fourth case.
The impression which we get from our four cases is that different problems and different clinical progress can be seen with the same diagnoses and same medicine usage.

B1932
PSEUDOTHROMBOCYTOPENIA ASSOCIATED WITH GRAVES' DISEASE
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Background: The association between hyperthyroidism and thrombocytopenia was described firstly in 1931. The shortened platelet life span, increased reticuloendothelial phagocytic activity, immune thrombocytopenia related to thyroid autoantibodies, genetic predisposition to the concurrence of immune thrombocytopenia and hyperthyroidism are most commonly reported mechanisms. Pseudothrombocytopenia (PTP) is a condition caused by anti-platelet antibodies in which agglutination of platelets in the complete blood count tube leads to incorrectly low platelet counts. It should be considered first in patients with low platelet counts without a bleeding diathesis. This phenomenon could be observed in healthy subjects, but it could also accompany certain diseases. The incidence is 0.09–0.21%. Misdiagnosis of PTP as true thrombocytopenia leads to unnecessary laboratory tests and unwarranted exposure to transfusion and related complications. Graves' disease, an autoimmune disease characterized by antibodies against the thyroid stimulating hormone (TSH) receptor, is the most common cause of hyperthyroidism. Graves' disease may be associated with autoimmune hematological disorders such as immune thrombocytopenic purpura. Association of PTP with Graves' disease was reported in only one case and the course of PTP was not defined in this paper.
Aims: A case with PTP associated with Graves' disease was presented in this report. After clinical healing and normalization of thyroid stimulating immunoglobulin, PTP was resolved completely.
Methods: A 27 year old woman admitted with palpitation, sweating and weight loss complaints. These complaints have started since six months. She had weight loss of 3 kg in spite of good appetite in this period. She had no fever or

diarrhea. Body temperature was 37.2 °C, arterial blood pressure was 120/60 mmHg and pulse was 104/min and rhythmic. The skin was moist. She had the nervous appearance. The thyroid gland was diffusely enlarged and painless. White blood cell count was 6.8 x10^9/L, red blood cell count 4.55 x10^{12}/L, hemoglobin 11.9 g/dL, hematocrit 35.2%, mean corpuscular volume 77.4 fL, mean corpuscular hemoglobin 26.3 pg and platelet count 33 x10^9/L in complete blood count with ethylenediaminetetraacetic acid (EDTA). Biochemical analysis, erythrocyte sedimentation rate and urine analyses were normal. Tests for thyroid disease were free T_3 16.63 pg/mL (2.2-4.2), free T_4 5.23 ng/dL (0.65-1.7), TSH 0.004 μIU/mL (0.4-4.2), and thyroid stimulating immunoglobulin (TSI) 69.42 U/L (0-14), anti-thyroglobulin antibody (anti-Tg ab) 5 IU/mL (5-100) and anti-thyroid peroxidase antibody (anti-TPO ab) 1.49 IU/mL (1-16). Thyroid ultrasonography revealed diffuse enlargement in the thyroid gland. Anti-nuclear antibody and anti-double strand DNA antibody were negative. Platelet clumps were seen sufficiently in peripheral smear examination. Platelets were counted mean 16.2 numbers in microscopic evaluation with x1000 augmentation. Complete blood count was repeated with heparin and platelet count was found to be 180 x10^9/L. PTP associated with Graves' disease was diagnosed and propylthiouracil 600 mg/day and propranolol 40 mg/day were started.

Results: Drugs' doses were adjusted with regular controls. White blood cell count was 5.8 x10^9/L, red blood cell count 4.61 x10^{12}/L, hemoglobin 11.5 g/dL, hematocrit 35.8%, mean corpuscular volume 77.6 fL, mean corpuscular hemoglobin 25.0 pg and platelet count 167 x10^9/L in complete blood count with EDTA after three months from diagnosis. Platelet count was found as 168 x10^9/L with heparin. Free T_3 3.21 pg/mL (2.2-4.2), free T_4 0.825 ng/dL (0.65-1.7), TSH 1.37 μIU/mL (0.4-4.2), TSI 15.37 U/L (0-14), anti-Tg ab 5 IU/mL (5-100) and anti-TPO ab 3.98 IU/mL (1-16) were found. Platelet clumps were observed in peripheral smear.

	Platelet count (with EDTA)	Platelet count (with heparin)	Free T_3 (2.2-4.2)	Free T_4 (0.65-1.7)	TSH (0.4-4.2)	TSI (0-14)	Anti-Tg ab (5-100)	Anti-TPO ab (1-16)
At diagnosis	33 x10^9/L	180 x10^9/L	16.63 pg/mL	5.23 ng/dL	0.004 μIU/mL	69.42 U/L	5 IU/mL	1.49 IU/mL
After three months	167 x10^9/L	168 x10^9/L	3.21 pg/mL	0.825 ng/dL	1.37 μIU/mL	15.37 U/L	5 IU/mL	3.98 IU/mL

Summary / Conclusion: PTP is frequently misdiagnosed which lead to inappropriate treatments. Therefore, this situation should be kept in mind primarily in the evaluation of thrombocytopenic patients.

B1933

CORRELATION BETWEEN PLATELET ACTIVATION INDICES AND METABOLIC PARAMETERS.

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Background: As we know diabetes mellitus and hyperlipidemia often present thromboembolic events. The calculation of the platelet activation indices, such as mean platelet volume (MPV) and platelet distribution width (PDW), is very easy with nowadays hematologic analyzers.

Aims: The aim of this study is to determine the possible correlation between platelet activation indices, such as mean platelet volume (MPV) and platelet distribution width (PDW), with different metabolic parameters,such as glycosylated hemoglobin level (HbA1c) and total cholesterol (CHOL).

Methods: In all, the results of the complete blood count (MPV and PDW) in 92 patients' cases were examined. These patients presented with diagnosed Metabolic Syndrome (MS) and were treated at the Outpatient Obesity, Hypertension and Dyslipidemia Clinic of the Department of Internal Medicine of the Psychiatric Hospital of Thessaloniki (group I). At the same time 95 random samples of the general population (group II) coming from various areas were examined. In all the samples the levels of glycosylated hemoglobin and total cholesterol were determined, while the results' statistical analysiswas performed with the use of the SPSS.

Results: In group II ofthe general population mean values and standard deviations of the examined parameters were: MPV: 9,51±1,18 fl, PDW: 16,22±2,17, HbA1c:6,49±1,88% and CHOL: 214±86 mgr/dl. Respectively, in group I the following values arose: MPV: 9,74±1,182 fl, PDW: 17,09±2,82, HbA1c:8,18±2,93 % and CHOL: 239±115 mgr/dl. Further statistical analysis revealed that for group I the correlation coefficient among HbA1c and MPV and PDW values was 0,33 and 0,39 respectively, while for group II the equivalent values of the correlation coefficient were 0,25 and 0,29.

Summary / Conclusion: It takes its toll that even though MPV and PDW measurements are useful, its sole determination is not enough for the estimation of the thromboembolic risk in relation to the HbA1c and CHOL values, since in no case (not even in group I of the patient suffering of MS), a statistically significant correlation between HbA1c and CHOL and the MPV and PDW valueswas observed. It is proved that thromboembolic incidents are multifactorialprocessesin which apart from platelets and other factors are involved, such as the endothelium and the coagulation factors and consequently there is no direct correlation between the platelet indices and biochemical findings, such as glycosylated hemoglobin and total cholesterol.

B1934

EFFICACY AND SAFETY OF TPO-R AGONIST IN NEUROSURGICAL SETTING: SUCCESSFULLY PERIOPERATIVE ROMIPLOSTIM TREATMENT OF A THROMBOCYTOPENIC PATIENT UNRESPONSIVE TO FIRST LINE THERAPY

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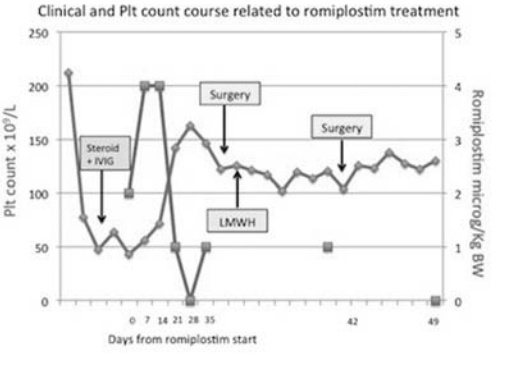
Background: Neurosurgery interventions should be performed with a normal or near normal platelet count to avoid serious bleeding complications, also in the postoperative period. Therefore thrombocytopenic patients requiring neurosurgery for expansive lesions are a difficult challenge because the need of a safe intervention is not always well balanced by the response to available therapies. Thrombopoietin-receptor (TPO-R) agonists have been used in different surgical/invasive procedure settings, mostly in patient with Primary Immune Thrombocytopenia (ITP) before splenectomy or in liver disease-related thrombocytopenia

Aims: We describe the case of a man with multiple brain lesions requiring surgical removal that developed a thrombocytopenia unresponsive to steroid and intravenous immunoglobulin (IVIG), safely and successfully treated with romiplostim in order to achieve and maintain a safe platelet count before, during and after the neurosurgical intervention

Methods: Clinical description of a neurosurgical thrombocytopenic patient treated with romiplostim in the perioperative period.

Results: A 63 years old man was admitted to our Neurosurgical Dept. with three newly diagnosed brain lesions. A total body CT scan excluded a solid metastatic cancer as explanation of the cerebral lesions and their surgical removal was scheduled, but patient started to develop a mild thrombocytopenia (54 x 10^9/L). We performed a bone marrow aspiration and biopsy to exclude myelophthisis. Megacaryocites are well represented and no pathological features were described in the bone marrow specimens. We excluded other common causes of thrombocytopenia (virus-related, autoimmune diseases-related) so we could conclude for an Immune Thrombocytopenia likely tumor-related. We started high dose dexamethasone (40 mg/day for 4 days, than 8 mg t.a.d) and IVIG in order to reach the required surgical platelet count (at least 100 x 10^9/L) without platelet transfusion. We didn't observe any response, and platelet count dropped to 43 x 10^9/L, so we decided to start romiplostim administration (2 microgr/kg as first dose), in order to avoid any immunosuppressant therapy. After 4 weeks of treatment the surgical removal of two lesions was performed when platelet count was 143 x 10^9/L, without complications. Low molecular weight heparin (4000 IU enoxaparin) was introduced as thromboprophylaxis and platelet count remained normal. A new surgical session, for the last brain lesion, was scheduled after 7 days, so romiplostim was administered once again the day before the procedure. Histological examination confirmed that a glioma was the reason of the expansive lesions, so chemotherapy was scheduled. Platelet count has remained normal after the last romiplostim dose so far, thus confirming the possible relationship between the cancer lesions and the thrombocytopenia.



Summary / Conclusion: Selected thrombocytopenic patient in surgical setting could be successfully treated with romiplostim, if first line therapy shows no effect, avoiding platelet transfusion thus allowing even neurosurgery to be performed without bleeding complications. Wide clinical studies are needed to provide more information about safety and efficacy of TPO_R agonist in this field.

B1935

RETROSPECTIVE ANALYSIS OF THERAPEUTICAL RESULTS IN IMMUNE THROMBOCYTOPENIC PURPURA

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Background: The investigation and management of patients with Chronic immune thrombocytopenic purpura (ITP) varies widely.
Aims: To evaluate the treatment of ITP patients in Departament of Hematology, County Hospital, Timişoara.
Methods: A retrospective study for 380 ITP patients was performed. Patients demographics, medical history, current treatments and side effects, were abstracted from the patient's medical charts for the 12 months prior to their most recent visit.
Results: The mean age was 47,1 years with 59% women and 41% men. Median time from the diagnosis of ITP to the start of the observational period was 25 months. Prior to the observational period, 39% of patients had been splenectomized and the most reported treatment was corticosteroids. During the observational period, 72% of all patients were treated. The most frequent reasons given for treatment were platelet count (60%), followed by bleeding symptoms (49%). Corticosteroids represented 61% of treatments, followed by IVIg (17%), azathioprine (12%) and rituximab. Splenectomies (7% of patients) and platelet transfusions (25% of patients) were performed during the observational period. For monitoring the platelet levels, 76% of patients visited their hematologist 1 to 8 times during the observation. Main reasons for a visit were a low platelet count (40% of visits) and bleeding (31% of visits). Overall, 40% of patients required hospitalization. Mean duration of hospitalization was 13,5 days.
Summary / Conclusion: The retrospective study of 380 patients provides the results of treatment practices in our country. It showed that bleeding symptoms remained quite frequent among patients with chronic ITP. Corticosteroids were the most widely used treatment.

B1936

THE EFFICACY OF REVOLADE(ELTROMBOPAG) IN IDIOPATHIC AUTOIMMUNE THROMBOCYTOPENIC PURPURA(ITP)-SINGLE CENTER EXPERIENCE:

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Background: ITP occurs when platelets undergo premature distruction as a result of autoantibodies or immune complex deposition on their membranes and there are no known etiologic factors. It is a diagnosis of exclusion and it is characterized by peripheral thrombocytopenia,normal or increased number of megakaryocytes in the bone marrow and absence of splenomegaly.
Aims: To demonstrate the efficacy of Revolade (Eltrombopag), a Tpo receptor agonist in treatment of ITP.
Methods: Thirty patients, ages between 35-56 years old, followed between 2010-2013,22 women(73,3%) and 8 men(26,6%);all received pulse-therapy with corticosteroids more then once and 17 (56,6%) had splenectomy. We started the treatment with 50mg ,od,when platelets count <30000/mm^3 and,then, lowered the dose to 25mg,od, when platelets >150000/mm^3. Five (16,6%) patients reached the level of platelets >250000/mm^3 with 50 mg od and needed to stop the medication for one week, than restarted with 25 mg od.
Results: All patients had platelets>50000/mm3 after the first week, no thromboembolic events; 7(23,3%)reported mild/moderate headache and 5(16,6%) had minor elevation of liver enzymes. No need to increase the dose to 75mg,od.
Summary / Conclusion: Revolade is a very strong option both for patients with ITP and splenectomy and for those who have contraindication for splenectomy. The medication is very well tolerated, despite the food restrictions(no mineral or diary products four hours before and four hours after the treatment with Revolade).

B1937

THE ANTIPHOSPHOLIPID ANTIBODIES, A RISK FACTOR TO DEVELOPED ANTIPHOSPHOLIPIDE SYNDROME OR SYSTEMIC LUPUS ERYTHEMATOUS IN THE PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA (ITP)

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Background: *Background*: The presence of antiphospholipid antibodies has been reported in immune thrombocytopenic purpura (ITP), but their role in the pathophysiology of disease is still unclear.
Aims: *Aim of study*: to evaluate the presence of antiphospholipid antibodies in ITP and the risk of developing an antiphospholipide syndrome or systemic lupus erythematous in patients with ITP.
Methods: *Methods*: we studied 49 patients with ITP hospitalized in Clinic of Hematolohy of Craiova (Romania) between 2007-2012. The diagnosis of ITP were established based on history, physical examination, complete blood count and reticulocyte count, bone marrow examination; 29 patients were tested for helycobacter pylori, HCV, antiphospholipid antibodies (APA- CLIA method), antinuclear antibodies (ANA-FEIA method).
Results: *Results:* the median age of patients was of 34 years, 80% were female and 20% were male. At diagnosis, 60% of them had a platelet count < 50 x10^9/L and 40% had a platelet count ≥ 50 x 10^9/L. The presence of APA was detected in four patients and ANA in seven patients (three of them had both APA and ANA). During the follow-up period (5 years), one patient with APA developed antiphospholipide syndrome and four patients with ANA (three of them both APA and ANA) developed systemic lupus erythematous, one of them with central nervous system manifestations.
Summary / Conclusion: *Conclusion*: the estimation of APA in patients with chronic immune thrombocytopenic purpura is indicated for the identification of patients who risk to develop an antiphospholipide syndrome or an autoimmune systemic disease.

B1938

MANAGEMENT OF PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) THERAPY - A RESTROSPECTIVE STUDY ON 49 PATIENTS

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Background: *Background*: Primary immnune thrombocytopenia (ITP) is characterized by a low platelet count which is the result of both increased platelet destruction and deficient platelet production. The development of autoantibodies in the pathophysiology of ITP still remains a problem.
Aims: *Aim of study*: to evaluate the response at therapy of 49 patients with ITP.
Methods: *Methods*: we studied 49 patients with chronic immune thrombocytopenia hospitalized in the Clinic of Hematology of Craiova (Romania) between 2007-2012. The diagnosis of chronic ITP was established based on history, physical examination, complete blood count and examination of the peripheral blood and bone marrow smear. All patients were initially treated with corticosteroids and the patients who did not respond were treated with Danazol, vinca alkaloid regimens, splenectomy or thrombopoietin receptor agonist as second line therapy.
Results: *Results*: 49 patients were initially treated with corticosterids (prednisone 0,5-2 mg/kg/day for 2-4 weeks or Dexamethasone 40 mg daily for 4 days every three weeks) and more than a half of them responded to this therapy; the patients who did not respond were treated, as second line therapy, with Danazol (two), Vinca alkaloid regimens (three), splenectomy (nine) or thrombopoietin receptor agonist (nine patients with refractory or relapsed chronic ITP and platelet count < 30.000/µl).
Summary / Conclusion: *Conclusion:* corticosterids remain the first line therapy in patients with chronic ITP but when they do not respond, a second line treatment option may be considered: Danazol, Vinca alkaloid regimens, splenectomy or thrombopoietin receptor agonist.

Bleeding disorders

B1939

THE RESULTS OF THE SAUDI NATIONAL SCREENING PROGRAM FOR FACTOR VIII AND IX HEMOPHILA INHIBITORS

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Background: Hemophilia A is an X-linked disease that affects males at prevalence of 1:5000-10000 while the prevalence of hemophilia B is 1:34,500 male. Although these disorders are rarely observed; it can be very serious (life threatening) and costly for families and countries. The development of factor inhibitor is a consequence of administration of blood products or manufactured factor concentrate. The reported prevalence of inhibitors for Hemophilia A ranges from 3.6-27% whereas for Hemophilia B very low 3-5%. There are few reports about the prevalence of these inhibitors in Arabs
Aims: we are reporting the final data analysis of a saudi screening survey for factor VIII and factor IX inhibitors.
Methods: A screening program for Hemophilia A and B inhibitor prevalence in Saudi Arabia had been launched in 2008 as a cross-sectional study. Seven centers in the country had participated in this survey. After approval of the proposal from research and ethical committee of each center, a representative from that center was included in a steering committee. Patients were requited from each center in addition to several Hemophilia awareness days were done to identify cases. All patients had been interviewed for clinical data collection and then subjected to confirmation of the disease in a central laboratory by measuring factor levels using standard chromogenic coagulation tests. Patients with confirmed Hemophilia A and Hemophilia B were included in the study and tested for presence of Factor inhibitors by Quantitative assay to measure Bethesda units (BU).
Results: Over 5 years 267 patients with diagnosis of hemophilia A or B were included in the study. Only 214 and been included in analysis, 53 patients were excluded mainly for incorrect diagnosis (most of these patients were von willebrand disease misdiagnosed as Hemophilia A). There were 158 hemophilia A (15 mild, 9 moderate and 134 severe) and 56 with hemophilia B (13 mild, 5 moderate and 38 severe). There were 208 males and only 6 female with hemophilia with mean age at diagnosis 1.6 years (range 0-20 years). Most of these patients 152 (71%) received treatment based on episodic bleeding and only 45 (21%) were on prophylaxis, while more than 100 (47%) received plasma derived clotting factors and 44(21%) recombinant concentrate still around 27 (13%) received Fresh frozen plasma. Chronic joint disability was encountered in 106 (50%) of patients with most commonly involved joints were Knees followed by elbows and ankles. The mean level of PTT was 86 sec. Factor VIII inhibitors were detected in 43 (28%) patients, of these 38 (88%) were detected in severe cases. Out of these patients 8 (19 %) had FVIII inhibitor level (1-5 BU), 23 (53%) titer (>5 BU) and in 12 (28%) titer was (< 1 BU). The total prevalence of inhibitors (> 1 BU) was 21%. Only one patient (1.8%) with severe hemophilia B had inhibitor at level of 135 BU.
Summary / Conclusion: we are reporting for the first time the prevalence of hemophilia A and B inhibitors in a cohort of Saudi patients. We found that the inhibitor level is similar with what had been reported in other ethnicity. We had detected 28 % with very low level of inhibitors which most likely representing transient inhibitor. We will conduct a follow up study to determine the frequency of development of high-level inhibitor in this group of patients.

B1940

LARGE INTER-INDIVIDUAL VARIABILITY OF THE RESPONSE TO NEW ORAL ANTICOAGULANTS

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Background: New, direct inhibitors of thrombin and factor Xa (FXa) show more stable pharmacokinetics than vitamin K antagonists do. Before the conclusion can be drawn that this warrants standard dose treatment it should be ascertained that the pharmacodynamic characteristics of the agents are predictable as well.
Aims: To investigate the response of the plasma from healthy subjects and patients with atrial fibrillation (AF) to a fixed dose of conventional and new anticoagulants.
Methods: Thrombin generation (TG) was determined by calibrated automated thrombinography (CAT). Platelet poor plasma (PPP) of 44 healthy, consenting subjects was spiked with otamixaban (direct FXa inhibitor), melagatran (direct thrombin inhibitor), unfractionated heparin, dermatan sulfate and pentasaccharide at concentrations around IC50. Thrombin generation was measured at 5 pM tissue factor and 4 µM phospholipids. The effect on TG of rivaroxaban (direct FXa inhibitor) in five consenting patients with AF was assessed in whole blood, platelet rich plasma (PRP) and PPP before therapy and in the 1st and 2nd week of therapy, three hours after ingestion of the drug.
Results: In the PPP of 44 healthy subjects the coefficients of variation (CV's) were 18% for the uninhibited endogenous thrombin potential (ETP) and 16% for the uninhibited peak height. A dose-dependent inhibition of all anticoagulants was observed in both ETP and peak height. The concentration of anticoagulant that inhibited ETP and peak around 50% was added to the individual plasmas. After inhibition the variation increased to 20-24% (ETP) and 24-43% (peak) for conventional and modern anticoagulants alike.
In the patients, both after 1 and 2 weeks of therapy, the ETP in PPP, PRP and whole blood decreased to a highly variable extent. The least variation was seen in PPP. The individual values of the response to rivaroxaban of the ETP were (1st week (%)/2nd week (%)): 21.9/29.3; 33.4/38.9; 9.1/11.3; 50.4/40.8 and 34.6/0.5. The values are to disparate and the number of patients too small to draw any quantitative conclusion, but it is obvious that the inter-individual variation is enormous. The differences in inhibition between the first and the second week in the same individual appear more similar than the inter-individual differences, which suggests that there may exist high and low responders.
Summary / Conclusion: This study indicates that the new anticoagulants cause quantitative changes in thrombin generation, that are as variable as those brought about by administration of vitamin K antagonists or by heparin treatment. The first results in patients suggest that, before relying on the effect of a fixed dose, the dose response in a patient should be determined.

B1941

POLYMORPHISM OF THROMBIN-ACTIVATABLE FIBRINOLYSIS INHIBITOR AND RISK OF INTRACRANIAL HEMORRHAGE IN FACTOR XIII DEFICIENCY

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Background: Factor XIII deficiency is an extremly rere coagulation disorder with estimated prevalence of 1/2000000 worldwide. The clinical manifestations of the disease are delayed wound healing, recurrent spontaneous miscarriage, severe bleeding and spontaneous intracranial hemorrhage as the main cause of death in these patients.
Aims: This study aimed to evaluates effect of a common polymorphism of thrombin activatable fibrinolysis inhibitor (TAFI) as a *antifibrinolytic factor in occurrence of* spontaneous intracranial *hemorrhage in patients with severe factor XIII deficiency.*
Methods: This case control study conducted on 34 factor XIII deficient patients with ICH and 36 patinents with factor XIII deficeincy but without history of ICH as control group. Initially all patients were moleculary analyzed for factor XIII deficiency and after confirmation of disorder both groups were assess for common TAFI Thr 325Ile polymorphism. And finally obtained data was analyzed by SPSS software.
Results: Molecular analysis for *TAFI* Thr 325Ile polymorphism revealed that almost all patients with ICH (89%) had this mutation that in 67% of patients was homozygot while in control group 18.7% was homozygot for Thr 325Ile polymorphism. There is a significant relationship between Thr 325Ile polymorphism in homozygote manner with incidence of ICH in severe factorXIII deficienty.
Summary / Conclusion: Co-existance of 325Ile polymorphism in homozygote manner with severe factor XIII deficiency increase risk of ICH about 20 fold.

B1942

THROMBIN GENERATION TEST FOR IDENTIFICATION OF PATIENTS AT HIGH THROMBOTIC OR BLEEDING RISK

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Background: Thrombin generation test parameters, particularly endogenous thrombin potential (ETP), obtained in plasma by the use of a fluorogenic substrate seems to reflect overall haemostasis better than traditional coagulation tests. Prognosis of patients with myeloproliferative neoplasmas is strongly affected by disease-related hemostatic complications and it is important for them to choose proper thrombosis prophylaxis without increasing risk of hemorrhages.
Aims: Aim of our study was to compare ETP in patients on low and optimal intensity vitamin K antagonists (VKA) treatment and to evaluate correlation

between ETP and international normalized ratio (INR) - standardized method of wide clinical utility.
Methods: The study involved 28 controls and 54 patients (M/F 30/24, mean age 58,2±14,8 yrs), treated with warfarin at list for 6 months and INR range of 0,9 to3,3. ETP in platelet poor plasma was measured using the Calibrated Automated Thrombogram (CAT), according to Hemker et al. both in the presence and in the absence of thrombomodulin (TM), which allow to assess influence of VKA treatment on the protein C system.STATISTICA 6.1 was used, data are given as mean±SD.
Results: Lag time, ETP, peak thrombin and time to peak obtained with and without TM demonstrated strong positive correlation (R between 0.93 and 0.98, P<0,05). Both in the absence and in the presence of TM strong inverse correlation with INR was found for ETP (R =-0,85 and R =-0,79, respectively, P<0,05) and peak height (R =-0,84 and R =-0,79, respectively, P<0,05). In patients with INR1,5-1,9 ETP (nMmin),obtained both with and without TM, was significantly (P<0,0001) higher (481,2±106,8 and 642,8±129,7 respectively) vs. those with INR2,0-3,3 (293,2±167,4 and 358,0±210,3, respectively), but significantly (P<0,0001) lower than corresponding parameters in controls (932,8±272,6 and 1731±253,6 respectively). None of the patients with INR1,5-1,9 had increased ETP, (i.e above 95th percentile measured in controls: >2114 nMmin in the absence of TM and >1433 nMmin in the presence of TM). In patients with INR 0,9-1,4 ETP was very close to controls (1487,4±210,3 without TM and 880,6±167,4 with TM). Two patients (both with INR =1) had increased ETP. In one case increased ETP in the absence of TM was accompanied by normal result in the presence of TM, and during the following18 months of observation patient did not demonstrate recurrent thrombosis. In the other patient with ETP 2343 nMmin in the absence of TM and 1603 nMmin in the presence of TM (i.e. increased) rethrombosis took place. In one patient with polycythemia vera, (INR 2.8), ETP and peak thrombin either in the absence or presence of TM were markedly decreased (73 and 71 nMmin ;4,6 and4,9 nM respectively), lag-time and time to peak were markedly prolonged (31,0 and 24,3 min; 41,6 and 34,3 min respectively), so the dose was corrected.
Summary / Conclusion: There is a strong correlation between ETP and INR both in the absence and in the presence of TM, but in some patients with INR within therapeutic range high risk of bleeding and/or rethrombosis can be better assessed by CAT and these patients could benefit from monitoring by this method. In some cases low intensity VKA is possible in aim of reduced risk of bleeding without increasing risk of recurrent thrombosis. Our data support the position that thrombin generation test may allow individualized approach when assessing the balance between bleeding and thrombotic risk and its modification by antithrombotic treatment.

B1943
NOVEL ORAL ANTICOAGULANTS - PROTOCOL TO MINIMISE BLEEDING AND EDUCATE DENTAL SURGEONS
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Background: As many patients attending dental surgery are taking medications which alter their bleeding tendency it has been a topic which receives much attention. In March 2004, the British National Patient Safety Agency (NPSA) issued a protocol for patients requiring invasive dental surgery while taking the coumarin anticoagulant warfarin. This has become an important aspect of patient safety and professional are aware of their responsibility for monitoring these patients' International Normalised Ratios (INR). Dental surgeons operate to these guidelines and are aware of a patient's potential increased bleeding risk and how to minimise the impact it will have following an invasive dental procedure, often by using simple local haemostatic measures. Eight years later, in March 2012, the British National Institute for Health and Clinical Effectiveness (NICE) approved the use of Dabigatran Etexilate for the prevention of stroke and systemic embolism for patients with nonvalvular atrial fibrillation and defined additional risk factors. Dabigatran Etexilate is one of the Novel Oral Anticoagulant group of drugs (NOAC) which have the potential to impact on dental surgeons providing invasive oral surgery both in primary care and in the hospital setting and currently there is little evidence and effective means of measuring patients' bleeding tendencies on these drugs.
Aims: To establish a working protocol for assessing and optimising patients receiving treatment with a NOAC drug who require invasive oral surgery procedures or conventional dental deep nerve blocks as part of their treatment in order to minimise their bleeding risk.
Methods: Current literature, national guidance and international experience was compared to the opinion of consultants in the region. A panel was established to review the literature develop expanded guidance given by the drug manufacturers. The functions of the panel were to review the evidence and to consider the implications in dental surgery practice in order to establish a safe working protocol for patients requiring dental and oral surgery while treated with NOAC drugs.
Results: The opinion of consultants from various specialties in the region varied greatly in both their experience of NOAC drugs and their advice on managing a patient requiring invasive dental treatment taking NOAC drugs. Through discussion with the haematology team a working protocol was established for patients requiring oral surgery within the Oral and Maxillofacial Surgery department.
Summary / Conclusion: Where new drugs are added to the formulary following approval by the National Institute for Health and Clinical Effectiveness the risk they pose to other healthcare professionals should be assessed. Within the hospital we have established a multidisciplinary team to examine the evidence and establish a protocol for the safe treatment of patients requiring oral and dental surgery while receiving treatment with drugs from the NOAC family. This information requires dissemination to colleagues providing care in both primary care and secondary care. The protocol will evolve with the further evidence, discussion and where more cases of increased bleeding and adverse events come to light.

B1944
MANAGEMENT OF DENTAL INVASIVE PROCEDURES IN HEMOPHILIA A/B AND VON WILLEBRAND DISEASE OUTPATIENTS
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Background: Dental procedures in hereditary bleeding disorders affected patients can be complicated by excessive bleeding. Replacement prophylactic therapy and local measures dramatically reduce the risk of complications in these surgical procedures. Moreover, self-infusion and home-treatment reduce therapy cost.
Aims: To describe the dental procedures performed in a group of hemophilia A/B and von Willebrand Disease outpatients utilizing systemic prophylactic therapy and local measures.
Methods: During the last six years, in our Dental Department, we performed one hundred and fifty four surgeries on forty nine patients (41 M, 8 F; median age 48 years, range 11-82) affected by hereditary bleeding disorders: 15 severe, 7 moderate, 10 mild hemophilia A; 2 severe, 2 mild hemophilia B; 6 type1, 1 type 2A, 3 type 2B, 3 type 3 von Willebrand Disease. The Hemophilia Center provided personalized therapeutic schemes, on the basis of the type/severity of the coagulopathy and type of surgery. Factor VIII concentrates were administered in severe, moderate and few cases of mild hemophilia A; FIX concentrates in severe and mild hemophilia B; desmopressin in mild hemophilia A, von Willebrand Disease type 1 and type 2A; FVIII/VWF concentrates in von Willebrand Disease type1, in case of low response or contraindication to Desmopressin, type 2B and type 3. One hundred and twenty one dental and roots extractions, ten third molar surgical extractions, one excisional biopsy, two cysts enucleation, eighteen scaling and roots planing, one gingival graft, one hypertrophic gingival tissue removal were performed under local and locoregional anesthesia. Local hemostasis was ensured by applying gelatine packing, fibrin glue, absorbable suture, fifteen-minute compression with tranexamic acid saturated gauzes. In the post-operative period, patients were treated with antibiotics and continued the self-infusion of concentrates/desmopressin for an average period of 5 days (3-7). Tranexamic acid mouthwashes (three times a day for 3 days) were prescribed; acetaminophen was used as the only pain-relief treatment.
Results: We observed no hemorrhagic or infectious complications. All patients completed the post-surgery management by home-based treatment.
Summary / Conclusion: A tailored prophylactic treatment ensures a good hemostasis in patients affected by hereditary bleeding disorders undergoing dental procedures. A strict collaboration between the hematologist and the dental surgeon is requested.A multidisciplinary approach allows to manage coagulopathic patients without bleeding complications; home-treatment reduces management costs.

B1945
PROPHYLAXIS WITH ACTIVATED PROTHROMBIN COMPLEX CONCENTRATE (FEIBA) IN CHILDREN WITH SEVERE HAEMOPHILIA A (HAEMA) AND HIGH RESPONDING FVIII INHIBITORS BEING ON IMMUNE TOLERANCE INDUCTION (ITI)
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Background: About one third of children with severe HaemA, mainly previously untreated patients, develop inhibitory antibodies towards infused factor VIII (FVIII) concentrates. The development of high–titer FVIII inhibitors [>5 Bethesda Units (BU)] renders treatment of patients problematic as they no longer respond to standard FVIII replacement. By passing agents are used to control or even prevent bleeding in these patients. Activated prothrombin complex concentrate (aPCC: FII, FIX, FX, activated FVII; FEIBA, Baxter, Vienna-Austria), licensed for use in our country in 2011;it has been suggested as preventive treatment in patients with severe HaemA and FVIII inhibitors in order to avoid serious and devastating haemorrhages
Aims: The safety and efficacy of FEIBA given as preventive treatment were evaluated in 4 children with severe HaemA and high responding FVIII inhibitors

(mean value: 342 BU, normal values < 0.6 BU), aged between 16 months and 5 years, being already on various ITI protocols (100 U/kg of FVIII concentrates for 3 or 4 times / week – 200 U/kg/24h) with different FVIII products (recombinant FVIII:3, plasma derived FVIII:1) for a median period of 15 months (1.5-37 months). Prophylactic treatment with FEIBA was administered for a period of 2-7.5 months in a dosage of 70 u/dl ±15 u/dl/day, and a frequency of 3 - 7 times/week, due to recurrent serious hemorrhagic episodes.
Methods:
Results: During the observational period, all patients showed improvement of their clinical status, as a threefold reduction of the mean number of haemorrhages under FEIBA prophylaxis was observed in comparison with those before FEIBA initiation (3, range 0-5 versus 9, range: 4-19 respectively). Neither new target–joint nor life-threatening haemorrhage was reported in all four children. It is worthy to note that one out of four boys had already suffered one serious episode of extensive haemothorax and two episodes of intracranial haemorrhage before initiating prophylaxis with FEIBA. No thromboembolic complications or any drug-related adverse reactions occurred during the period of prophylaxis while D-dimers were found negative in consecutive evaluations. All patients remained negative for HCV and HIV while they had maintained high titers of anti-HBs antibodies due to vaccination for hepatitis C. Elimination of FVIII inhibitors was reported in all four patients. Nevertheless, only additional administration of rituximab (MabThera, Rhoche) resulted in successful ITI and FVIII inhibitor eradication in one patient. Treatment with FEIBA was stopped when each patient's FVIII levels were measured >10 u/dl in a 30 minute post-infusion sample.
Summary / Conclusion: In our small series, FEIBA prophylaxis decreased the overall bleeding rate, and target-joint evolvement in children with severe HaemA and high responding FVIII inhibitors. In spite of the concomitant use of FVIII on any ITI protocol and infusions of FEIBA, a product containing activated FVII, no adverse events, mainly thromboembolic, were observed.

B1946
THE SIGNIFICANCE OF TEG AND TGA IN THE EVALUATION OF HEMOSTASIS IN CHILDREN WITH CYANOTIC AND ACYANOTIC CONGENITAL HEART DISEASES
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Background: Patients with cyanotic congenital heart disease (CHD) have hemostatic abnormalities, which result in an increased risk of bleeding. The cause is unknown, but recent studies have indicated that an elevated hematocrit, which is present in cyanotic patients, could be an important factor.
Aims: In this study, we aimed to evaluate the hemostatic state of children with cyanotic and acyanotic CHD by using thromboelastography (TEG) and thrombin generation assay (TGA).
Methods: Seventy four patients with CHD (39 acyanotic, 35 cyanotic; ages were between 0-18 years) were enrolled in this study. Twenty nine, age matched, healthy children were enrolled as a control group. Hemogram, PT, aPTT, TEG and TGA results were evaluated in all patients before angiography and cardiac surgery.
Results: In the cyanotic group, as expected, hematocrit levels were significantly higher than acyanotic and control groups (P=0.000). When compared to the acyanotic patients, PT anda PTT results were significantly higher in the cyanotic group (P=0.011 and P=0.037 respectively).
When TEG parameters were analyzed, TEG-R values were significantly longer in cyanotic patients than acyanotic and control groups (P=0.000 for both groups). Alpha-angle degree and maximum amplitude (MA) values were significantly lower in cyanotic group than acyanotic (P=0.001) and control (P=0,005) groups.
When TGA parameters were analyzed, peak thrombin levels were significantly lower in cyanotic group than acyanotic and control groups (P=0.000, P=0.000 respectively). When compared to control group, peak thrombin time results were significantly longer in cyanotic and acyanotic groups (P=0.014, P=0.028 respectively). Endogenous thrombin production was significantly lower in cyanotic group than acyanotic and control groups (P=0.000, P=0.000 respectively).
Summary / Conclusion: While it is well known that high hematocrit levels increase the tendency for thrombosis in cyanotic heart disease, TEG and TGA results in our study show that there are also coagulation disturbances in these patients.

B1947
INTRACRANIAL HEMORRHAGE IN HEREDITARY BLEEDING DISORDERS: THE EXPERIENCE OF ÇUKUROVA
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Background: Intracranial hemorrhage (ICH) is a life threatening complication of hereditary bleeding disorders in childhood resulting in high rates of mortality and disabling sequelae.
Aims: In this study, we evaluated our patients with intracranial hemorrhage and compared with literature.
Methods: From 1995 to 2012, 21 patients with intracranial hemorrhage were diagnosed and evaluated in Çukurova University Hemophilia Center. ICH episodes, the findings of physical examination, CT scan or MRI and treatment strategies including surgical interventions were reviewed retrospectively.
Results: We evaluated 22 episodes of ICH from 21 patients with hereditary congenital factor deficiencies (CFD). Age range was from 9 days to 12 years. There were 15 patients with hemophilia A, 2 patients with hemophilia B, 2 patients with factor VII deficiency, 1 patient with factor X deficiency, 1 patient with von Willebrand disease. Two patients with factor VII deficiency, 1 patient with factor X deficiency and 1 patient with von Willebrand disease were female. Except one patient with factor X deficiency, all patients had one bleeding episode. Two patients had a high titer inhibitor against factor VIII. The most important factor was trauma. A history of recent trauma was documented in 10 patients. Intracerebral and subdural hematoma were more frequently seen. The most frequent symptoms were seizure and headache. The diagnosis of hemophilia was established in 5 patients after intracranial hemorrhage who referred to our center with ICH . In 9 patients, hematoma was evacuated. Three hemophilia patients died due to ICH and 4 patients presented late sequelae.
Summary / Conclusion: Intracranial hemorrhage is the most serious complication in childhood, especially for hereditary bleeding disorders. Urgent establishing diagnosis and treatment with prompt doses of factors to initially maintain a normal concentration of circulating factor is mandatory. Despite the prompt treatment, death and late sequelae can be seen in this patients group.

B1948
HEMATURIA IN CONGENITAL COAGULATION FACTOR DEFICIENCIES
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Background: Spontaneous gross hematuria or subclinic microscopic hematuria is not an uncommon manifestation in severe hemophiliacs and has been thought to reflect tubular damage.
Aims: In this retrospective study, we evaluated the prevalence of hematuria in hemophiliacs and other congenital factor deficiencies in a comprehensive care center.
Methods: All medical records of hemophiliacs who attended this comprehensive hemophilia care centre between 1990 and 2012 were evaluated. 629 patients with congenital hemorrhagic diathesis (383 hemophilia A (HA), 77 hemophilia B (HB), 99 type-1, 16 type-2 and 14 type-3 vonWillebrand's Disease (VWD), 16 FVII deficiency, 8 FV deficiency, 5 FX deficiency and 11 rare factor deficiencies) were evaluated retrospectively in this study.
Results: Hematuria was determined in 39 of 383 HA patients, 12 of 77 HB patients, 5 of 129 VWD patients and 3 of 40 patients with the other factor deficiencies. The first attack of hematuria occurred at the age of 3-34 years (mean 15,5±6,9 years). Hematuria was seen in 59 of all hemophilia patients. Thirty four of these hemophiliacs had only one hematuria episode, 8 had two, 4 had three, 3 had four episodes and 10 had five and more episodes. Hematuria was seen in 29 severe, 20 moderate and 2 mild FVIII and FIX deficiencies. Inhibitors were positive in 7 of these patients.
While there were no significant findings in ultrasonography in 41 of 59 patients (70%) who had hematuria, nephrolithiasis was observed in seven patients (12%), pyelocaliectasis in one patient (1.7%) and renal cyst in one patient (1.7%). Ultrasonography was not performed in 9 patients.
While 8 patients (13.6%) received only hydration therapy, 43 patients (72.9%) received both hydration and factor replacement therapy. Five patients (8.5%) received hydration and factor replacement therapy. Two patients (3.4%) received fresh frozen plasma and 1 patient (1.7%) received fresh frozen plasma with combined prednisone therapy.
No cause for hematuria was identified in 46 of these patients (78%). Nine patients (15.3%) had nephrolithiasis, 1 (1.7%) had poststreptococcal glomerulonephritis, 2 (3.4%) had urinary tract infection, 1 (1.7%) had renal cyst.
Summary / Conclusion: Our results are consistent with the other investigations which rarely demonstrate urinary system abnormalities in these patients. The long-term outcome of hematuria is largely unknown.

B1949
MAJOR SURGICAL INTERVENTIONS IN CHILDHOOD RARE FACTOR DEFICIENCIES:A SINGLE-CENTER EXPERIENCE FROM TURKEY
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Background: Rare factor deficiencies(RFD) are autosomal recessively inherited coagulation factor deficiencies with an incidence ranging from one in 500 thousand to 2 million.
Aims: Data regarding to major surgical interventions of RFD are based on case reports and records of guidelines.There are not well-documented and separately prepared directories related to presurgical and prophylactic

approaches of surgical interventions of these deficiencies.
Methods: This study retrospectively reviewed 20 individuals with RFD who received 24 major surgical intervention selected among those who were monitored and treated by our clinic between 1990-2012. We retrieved information from patient records and the records contained in the dataprocessing environment introduced in 2005.
Results: Our retrospective study had consisted of 171 rare factor deficiencies that were followed up in our clinic and of whom 20 had 24 major surgical interventions between 1990-2012. Age range was 5-19 years and male/female ratio was 17/7 (71/29%).Seventeen (71%) of the major surgeries were performed for patients with FVII deficiency, 4 (17%) for FV deficiency, 2 (8%) for FXI deficiency and one (4%) for afibrinogenemia. Of the patients who underwent major surgery, 13 (54%) were asymptomatic and 11 (46%) symptomatic.In factor VII deficient patients, ventriculoperitoneal shunt insertion(n=1), ventriculo peritoneal shunt valve replacement(n=1), undescended testicle(n=1), adenotonsillectomy (n=3), tonsillectomy(n=2), hernioplasty(n=5), splenectomy(n=1), subdural haematoma discharge (n=1), fracture repositioning(n=1), adenoidectomy(n=1) operations were performed. In FV-deficient patients hernioplasty(n=2), subdural haematoma discharge(n=1), bone repositioning(n=1) operations were performed. In FXI-deficient patients, cleft lip (n=1) and hernioplasty operations were performed. In the afibrinogenemia patient, cochlear implant insertion was performed.In factor VII deficient patients, rFVIIa was used for 7(41.2%) of the major surgical interventions, FFP for 2 (11.8%), and FFP and rFVIIa was coadministered in one case (5.8%). No replacement therapy was administered in 7 interventions(41.2%). Six (60%) of the patients who received replacement for Factor VII deficiencies were symptomatic and 4(40%) were asymptomatic. rFVIIa was used at 15-35mcg/kg for 3-83 times (4±31) and in 4-12 hour intervals. Cases involving FFP treatments were before 2002, after which rFVIIa and rFVIIa stable at room temperature were used. The number of doses used in ventriculoperitoneal shunt operations was 83, and 60 doses of rFVIIa were used for replacement of the VP shunt valve of the same patient. For the two hernioplasty patients, it was not possible to control the bleeding with 9 doses of FFP in one of them with FVII:C 42% and haemostasis could be achieved with 4 additional doses of rFVIIa. During the other hernioplasty procedure, 4 doses of rFVIIa were sufficient for haemostasis. In one case of adenotonsillectomy and one case of tonsillectomy, haemostasis was achieved with 3 and 4 doses of rFVIIa, respectively. In undescended testicle operation, replacement with 3 doses of rFVIIa was required.In four FV-deficient patients, FFP at 20ml/kg was used every 24-36 hours. Number of doses ranged between 1 and 10.In two FXI-deficient patients, single dose (20ml/kg) of FFP were sufficient.In the afibrinogenemia patient, fibrinogen was used at 50 mg/kg/ in 2-4 day intervals for a total of 3 administrations.
Summary / Conclusion: Surgical interventions to RFD patients, which represent a population with varying characteristics, should be performed in hospitals with experienced haematology centers. Patients as well as the surgery and anaesthesia teams should be prepared and encouraged prior to the intervention.

B1950
RECURRENT NEONATAL INTRACRANIAL HEMORRHAGE AS THE SOLE INITIAL PRESENTING SYMPTOM OF ACQUIRED FACTOR II DEFICIENCY: A CASE REPORT IN AN EGYPTIAN CHILD WITH TYROSINEMIA TYPE1
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Background: Hypoprothrombinemia can be congenital or acquired due to another disorder as liver disease. Severe bleeding including intracranial hemorrhage typically occurs when prothrombin level (FII) is below 5%. Type 1 tyrosinemia is a severe metabolic disorder often presenting with liver disease or liver failure with a subsequent coagulopathy.
Aims: To report that acquired hypoprothrombinemia secondary to hepatic affection can present initially with the severe bleeding symptoms without any clinical or laboratory evidence of hepatic involvement.
Methods: A one year old boy of consanguineous marriage presented with recurrent intracranial hemorrhage and recieved replacement . He was investigated for an underlying coagulopathy, diagnosed and started on weekly prophylaxis.He was doing very well till he developed very mild jaundice several months later when he was invetigated for an underlying hepatic affection.
Results: Patient's hematological assessment showed mild FII deficiency whilst hepatological evaluation revealed mild hepatosplenomeagly and prominent pelicalyceal system on abdominal ultrasound.This in association with the transient hepatic dysfunction and coagulopathy raised the possibility of being a case of tyrosinemia. Investigations revealed high plasma lactate dehydrogenase, alkaline phosphatase, succinylacetone, tyrosine, methionine and alphafetoprotein. So investigations were consistent with the diagnosis of tyrosinemia type 1 but patient died while being prepared for hepatic transplantation.
Summary / Conclusion: Liver cell failure with a secondary coagulopathy is one of the known presentations of tyrosinemia but initial clinical presentation with a severe coagulopathy in the absence of clinical manifestations of liver disease is not recognized as the initial presenting symptom of this condition.

B1951
A CASE STUDY OF POSTPARTUM HEMOPHILIA: HETEROGENICITY IN CLINICAL PRESENTATION, THERAPY REQUIREMENTS AND THERAPY ADVERSE EFFECTS
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Background: Acquired hemophilia A is an extremely rare hemorrhagic disorder. Postpartum hemophilia A is considered to be relatively benign. The haemorrhage episodes are rarely fatal and mortality is usually associated with the immunosuppression therapy side effects. First and second line therapy recommendations include corticosteroids, with or without cyclophosphamide, and Rituximab respectively.
Aims: To exhibit the heterogeneity of postpartum haemophilia A regarding clinical presentation, therapeutic requirements and therapy related complications.
Methods: We present three women with postpartum haemophilia A. In each case we describe the clinical presentation, the diagnostic findings, the therapeutic choices and final outcomes.
Results: Patient 1. A 27-year-old primigravida presented with extensive subcutaneous haemorrhage, low blood pressure and melena two months after an uncomplicated delivery. A week ago the initial spontaneous subcutaneous haematomas were misdiagnosed and therapy with salicylic acid 100 mg was given by an orthopedician. Further investigation revealed severe anaemia and intra-abdominal haemorrhage. Patient 2. A 29-year-old primigravida presented with an abrupt onset of subcutaneous haematomas at the left upper extremity and finger numbness six months after an uncomplicated delivery. Clinical evaluation revealed appartement syndrome at the left forearm. Abdominal CT scan revealed a splenic haematoma. Patient 3. A 34-year-old admitted to our department because of the appearance of spontaneous ecchymoses of the upper extremities and of the right calf, eleven months after a third delivery by caesarian section. She had also mentioned the appearance of similar but spontaneously resolved superficial ecchymoses and episodes of haematuria three months ago. Laboratory tests showed in all three patients isolated prolongation of PTT, low factor VIII levels and the presence of a FVIII inhibitor by mixing studies. There were no findings of malignancy, autoimmune disease or antiphospholipid syndrome. Treatment with the by-passing agent FVIIa was administered only in patients 1 and 2. All three patients initially received immunosuppressive treatment with prednisone 1mg/Kg. Patient 1 did not respond well to prednisone and received additional immunosuppressive therapy with cyclophosphamide 1000mg. A normal FVIII activity and inhibitor eradication was achieved on day 90. The same patient developed Listeria Monocytogenes meningitis and later on bilateral femoral bone necrosis. Patient 2 responded to prednisone therapy without any complications and exhibited a normal FVIII activity on day 45. Patient 3 is in the first week of prednisone therapy and exhibits a gradual increase in FVIII activity.
Summary / Conclusion: Diagnosis of postpartum hemophilia A is based on spontaneous haemorrhagic manifestations, which develop during the first twelve months post labour, and is confirmed by low FVIII levels and the presence of a FVIII inhibitor by mixing studies. Although, the incidence is higher in primigravidas diagnosis cannot be ruled out in subsequent pregnancies. Interestingly, in postpartum haemophilia A the inhibitor may spontaneously disappear, so less immunosuppression therapy may be needed and this may indeed explain the lower mortality rate. Nevertheless, patient 1 required intensive immunosuppressive treatment and exhibited serious therapy related complications. We conclude that early diagnosis and management of postpartum haemophilia A is crucial and careful patient monitoring should be recommended during the immunosuppression period.

B1952
ILIOPSOAS HEMORRHAGE IN CONGENITAL FACTOR DEFICIENCIES:THE EXPERIENCE OF ÇUKUROVA UNIVERSITY, ADANA , TURKEY
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Background: Iliopsoas hemorrhage is a serious complication of bleeding disorders that occurs most commonly in patients with severe hemophilia and less commonly von Willebrand disease and Factor V deficiency and is considered as potentially life threatening condition and significantly associated with morbidity. Despite its clinical importance, there are few reports on the mode of presentation, treatment and sequela. In this study, we presented our experience about iliopsoas hemorrhage in patients with severe bleeding disorders.
Aims: In this study, we evaluated our patients with iliopsoas hemorrhage and compared with literature.
Methods: From 1995 to 2012, 19 patients with iliopsoas hemorrhage were evaluated in Çukurova University, Hemophilia center. The findings of physical examination, pain locations, complications were assessed. Iliopsoas hematomas were confirmed by ultrasonography, CT or MR scan. The treatment strategies were noted.
Results: We evaluated 29 episodes of iliopsoas bleeding from 19 patients with

congenital factor deficiencies (CFD). There were 13 with hemophilia A, 4 with hemophilia B, 1 with factor V deficiency, and 1 with von Willebrand disease. Age range was from 45 days to 19 years. Fourteen patients had one episode, three had two episodes, one had three episodes, one patient with severe hemophilia A with inhibitor had 6 episodes. Three patients had a high titer inhibitor against factor VIII. Iliopsoas hematomas were confirmed by ultrasonography in all patients. Five patients needed erythrocytes transfusion. The mean duration of therapy was 11,55±3,54 days, and the duration of hospitalization was 10,50 ±3,54 days. Patients with inhibitors were treated with by-passing agents. In 6 bleeding episodes of 1 severe hemophilia A patient with inhibitor treated with recombinant factor VIIa (rFVIIa). Activated prothombin complex concentrates (aPCC) was given in two episode in two severe hemophilia A patients with inhibitor. Long term complications included paresthesia in 5 patients in the distribution of femoral nerve and quadriceps atrophy in 4 patients.
Summary / Conclusion: Iliopsoas hematoma is a serious bleeding event in patients with hereditary clotting disorders. In this report, we present our experience and treatment modality in 28 episodes of serious iliopsoas hematoma in hereditary congenital factor deficiencies.

B1953
OUR CASES DIAGNOSED AS HEMORRHAGIC DISEASE OF THE NEWBORN
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Background: The nomenculature used for the entity called hemorrhagic disease of the newborn secondary to vitamin K deficiency which occurs in 1 % of the infants has been replaced by vitamin K deficiency bleeding of the newborn (VKDB), because this disease is not only caused by vitamin K (VK) deficiency**.** In 0.1-2 % of the healthy term newborns not receiving VK prophylaxis, classical VKDB occurs within the first 2 weeks of their lives.
Aims: We wanted to analyze our cases diagnosed as VKDB, and share our results in comparison with incidence rates of this disease cited in the literature.
Methods: Demographic data, information on medical history, clinical, and physical examination findings, bleeding sites, laboratory test results, treatment modalities, and their outcomes were analyzed in 21 cases with VKDB whom we followed up in the Division of Pediatric Hematology between 07.25. 2003, and 09.24.2012,
Results: These cases demonstrated clinical manifestations of early stage (n=2; 9.52 %), classical (n=3; 14.28 %), and late- type (n=16; 76.19 %) VKDB. The oldest infant with late-type VKDB was 2 years old. The infants with VKDB were born in the hospital (n=14; 66.66 %66.66) or at home (n=7; 33.33 %). They had (n=12; 57.14 %) or had not (n=7; 33.33 %) received vitamin K therapy, while in 2 cases (9.52 %) uncertainty existed. These infants were term (mature) (n=17; 80.95 %) , premature (n=3; 14.28 %), and postmature (n=1; 4.76 %) babies. They were receiving breast milk (n=16; 76.19 %), formula (n=2; 9.52 %), formula, and breast milk (n=2; 9.52 %). Neurological sequelae developed in 5 (23.8 %) cases, and 2 (9.52 %) infants died. Both of the deceased infants were in their early stage of VKDB..
Summary / Conclusion: Even though vitamin K therapy is mandatory during the neonatal period, currently in Turkey 33.33 % of the babies are still delivered at home, 33.33 of them don't receive vitamin K therapy, and 9.52 % of the infants die because of VKDB.

B1954
PERCUTANEOUS TRANSLUMINAL AORTIC VALVE IMPLANTATION (TAVI) FOR SEVERE AORTIC VALVE STENOSIS IN PATIENT WITH HEMOPHILIA A
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Background: Hemophilia is caused by the deficiency of coagulation factor VIII (A) or IX (B).The development of FVIII or IX inhibitors is the most severe and costly complication in the treatment of hemophilia.
The management of cardiovascular events in elderly hemophiliacs necessarily differs from that of non-hemophilic patients.
Aortic stenosis (SA) is the most common degenerative valve disease in Western countries, and its incidence increases with age. Nowadays, the surgical aortic valve replacement is the gold standard. However, approximately 30% of patients with severe symptomatic SA do not undergo surgical treatment because they are considered at high surgical risk because of advanced age and co-morbidity often coexist.
Aims: The TAVI has rapidly become an important treatment option for patients who are not candidates for surgical valve replacement or with high surgical risk.
Methods: A 56-years-old man with a severe hemophilia A with HCV-related chronic liver disease was admitted to hospital due to dyspnea on moderate exertion .A history of arterial hypertension, hyper-thyroidism and diagnosis of severe aortic valve stenosis was present in anamnesis. Electrocardiogram showed sinus rhythm, heart rate of 81 beats/min. An echo-colorDoppler excluded the presence of stenosing plaque in the right and left iliac and femoral arteries. During the hospital stay patient underwent coronarography which showed coronary arteries without any hemodynamically significant lesions, but dominant right coronary showed an abnormality of origin, deriving from ascending aorta. Aortic valve replacement was indicated, and because of the contraindication of surgery and his comorbidity, patient was subjected to TAVI that was performed in the cardiac catheterization laboratory under mild sedation and local anesthesia with fluoroscopic guidance. Echocardiographic analysis was repeated 24 hours after the TAVI procedure to assess prosthetic function and positioning and to exclude other complications. The outcome of the procedure was satisfactory. The patient did no experience any adverse events during and after the procedure. During hospitalization, under the supervision of haematologists, patient was treated with rFVIII 80 UI/Kg (Kogenate) bolus before surgery and then at a dose of 40 UI/Kg every 12 hours after surgery. During the procedure, heparin bolus 5000 UI. has been administered. Patient has continued home therapy as prophylaxis with recombinant FVIII infusion every 12 hours for 15 days and prophylaxis with EBPM 100 U/Kg/day.
Results: One year after TAVI procedure, patient maintains a satisfactory response in terms of cardiac outcomes and symptoms. The patient did not report any thrombotic or hemorrhagic events. Indeed, long-term follow-up has not revealed any complication.
Summary / Conclusion: The TAVI has rapidly become an important treatment option for patients who are not candidates for surgical valve replacement or with high surgical risk. The elderly patient is therefore an ideal candidate for percutaneous implantation especially in haemophiliac patients with high bleeding risk.

B1955
TRANEXAMIC ACID AS PROPHYLAXIS IN A YOUNG HAEMOPHILIA PATIENT WITH INHIBITOR PRESENCE
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Background: Inhibitor management remains a challenging issue in haemophilia patients. Tranexamic acid, an antifibrinolytic agent, is widely used both in inhibitor and non-inhibitor haemophilia patients as adjunctive therapy to factor replacement.
Aims: To report the clinical course of a 4 year old hemophiliac with persistent and exceptionally high inhibitor titers on a non-conventional prophylactic monotherapy with tranexamic acid.
Results: This is a report of a young haemophiliac diagnosed with severe FVIII deficiency (1%) soon after birth. During the first months of life he presented with repeated bleeding episodes requiring substitution therapy and by the age of 8 months inhibitor presence was established. Bleeding episodes were initially managed with recombinant activated factor VII, which subsequently became unavailable due to hospital economic reasons. ITI was planned, but due to poor venous access and difficulties in maintaining a central venous line (the child pulled off his Hickman catheter twice) was not started until the age of 2. During 12 months of ITI, the inhibitor titer rose from 128 B.U./ml to 2000 B.U./ml and ITI was discontinued as unsuccessful. The child continued to bleed frequently, with minimal access to factor VII as a result of hospital budget cuts. At the age of 3 a non-conventional non-established regimen of daily tranexamic acid prophylaxis was decided. During the months that followed the child demonstrated a dramatic change in clinical course, with great reduction in number of bleeds, almost none of which were of spontaneous nature. The child has now been on prophylaxis with tranexamic acid for 15 months, maintaining an unbelievably good joint status and an almost normal lifestyle.
Summary / Conclusion: Although not evidence-based or properly established, prophylactic treatment with tranexamic acid has proven extremely efficacious for this young haemophiliac patient who, as a result of the economic situation in Greece, cannot have access to appropriate treatment.

B1956

PERCUTANEUS TRANSLUMINAL ANGIOPLASTY (PTA) FOR AORTIC COARCTATION (COA) IN PATIENT WITH HEMOPHILIA A AND HIGH INHIBITORS TITRE USING FACTOR VIII INHIBITOR BYPASS ACTIVITY (FEIBA®)

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Background: Hemophilia is caused by the deficiency of coagulation factor VIII (A) or IX (B).The development of FVIII or IX inhibitors is the most severe and costly complication in the treatment of hemophilia. Controlling bleeding during and after surgery in haemophilia patients with inhibitors remains a challengefor physicians. Published studies demonstrate the efficacy, safety and tolerability of FEIBA in a variety of major and minor surgeries. CoA can be defined as a variable degree of narrowing of the aorta, resulting in pathologic obstruction of blood flow from the systemic ventricle to the systemic circulation. Treatment options depend on age and how aorta is narrowed and include surgical and transcatheter interventions. Recent developments in endovascular stent technology have extended the treatment options available and seem cost effective. The complications were represented by aortic aneursysm, aortic wall dissection/rupture, stent migration, embolic event, injury to access vessels, hematoma, bleeding, fistula formation.
Aims: We present a case report of a severe hemophilia A patient with inhibitors treated with FEIBA for the management of bleeding during transcatheter intervention (primary stenting) for post-isthmic CoA.
Methods: A 54-year-old man with severe hemophilia A and a high inhibitor titre (12 BU) and HCV-HBV related chronic liver disease referred to us because of acute pulmonary edema. A history of arterial ipertension and diagnosis of CoA were present in past anamnesis. Electrocardiogram showed atrial fibrillation, heart rate of 90 beats/min and complete left bundle branch block.
Because of the contraindication of surgery (high bleeding risk), PTA with stent implantation was performed in the cardiac catheterization laboratory, under deep sedation and local anesthesia with fluoroscopic and transesophageal echocardiographic guidance. Repeated echocardiographic examinations were performed 24 hours after the procedure to evaluate stent localization and the presence of residual gradients. The acute outcome of procedure was satisfactory. The patient had no adverse events during both the procedure and hospital stay. Before PTA 70 UI/Kg of FEIBA have been given to patientas a bolus and the same dose was repeated every 12 h for 7 days. As the coagulation factors was stable over time, and no bleeding was recorded during or after surgery, it was decided of increase the time intervals of administration from 12 h at 24 h for another 7 days, then move to prophylaxis regime for further 7 days.
Results: At 6 months after procedure, the patient has maintained a satisfactory response in cardiological profile and no thrombotic adverse events.
Summary / Conclusion: Endovascular stenting for the treatment of native and recurrent CoA appears to be a feasible, safe and efficient method in patients with hemophilia. Stent placement is a procedure that has a lower complication rate than balloon angioplasty and surgery. In patients with inhibitors, the administration of FEIBA at standard doses could be the gold standard for the management of bleeding.

B1957

FOLLOW-UP AND PROGRESSION OF HEMOPERITONEUM DEVELOPED IN A TYPE 3 VON WILLEBRAND PATIENT SECONDARY TO THE HEMORRHAGIC RUPTURE OF CORPUS LUTEUM

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Background: Haemoperitoneum can occur as a result of the rupture of a bleeding follicle and a few cases have been reported in patients with type 3 von Willebrand's disease. Adequate information is not available related to the treatment, and follow-up period of this condition.
Aims: We wanted to present treatment modality, ultrasonographic evaluation (US), and the process of complete cure of our case.
Methods: A 15.5-year-old female adolescent who had been diagnosed as type 3 von Willebrand disease started to suffer from abdominal pain, and dysuria 10 days before her due date of menstruation. Her physical examination of the patient with complaints of constipation and fever revealed an abdomen with its normal convexity, tendernes at suprapubic region, and both lower quadrants. Abdominal defence was detected at the left lower quadrant. Somewhat attenuated bowel sounds were auscultated. Urgent US could not demonstrate the left ovary clearly. A hemorrhagic cyst measuring 39 x 37 mm, and surrounded by a free fluid zone in the left adnexial region was detected. Doppler US demonstrated normal blood supply of the cyst. Dense free fluid collection was observed in the Douglas recess, and pelvic region. Contrast-enhanced CT disclosed hemoperitoneum, and a hemorrhagic-ruptured corpus luteum cyst. Hemoglobin (Hb) value was 12.1 g/dl. She was monitored for bleeding episodes. A concentrated solution which contained FVIII, and von Willebrand factor was administered at a dose of 20 IU/kg at 8 hour-intervals for a period of 24 hours, and then for 3 days at every 24 hours. Besides prednisolone (2 mg/kg/d), and desmopressine spray were given for 3 days. In addition, the patient received IV tranexamic acid (15 mg/kg) at 8-hour intervals for 3 days, then its oral doses were maintained for 7 days. During follow-up period, her Hb regressed to 10.6 g/dl. After recovery of normal menstrual cycles, oral contraceptive therapy was initiated.
Results: Daily US controls were performed, and on the 10. day, a hemorrhagic follicle in the left ovary measuring 16 x 25 mm , and minimal fluid collection in the Douglas recess, and around the left ovary were observed. Then she was discharged, and sent home with the recommendation of oral contraceptive use for at least 6 months. Her monthly US controls were performed, and at the end of the 6. month, her hormonal test results were assessed to be within normal limits. Another problem was not observed.
Summary / Conclusion: Conservative management in a type 3 von Willebrand's disease has been succesfull in avoiding surgery. If recognized early, this could be a way to avoid surgery.

B1958

EXPERIENCE WITH PROTHROMBIN COMPLEX CONCENTRATES FOR PROPHLAXIS AND TREATMENT OF BLEEDING

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Background: PCC is currently indicated for the treatment of bleedings and prevention (during surgery) caused by the congenital or acquired lack of vitamin K-dependent coagulation factors II, VII, IX and X in the blood.
Aims: In this presentation we discussed PCC and fresh frozen plasma (FFP) efficiency and side effects for prophylaxis and bleeding.
Methods:
From May-2012 to October 2012 total 50 patients evaluated retrospectively in our hospital. Twenty five patients used PCC and 25 patients used FFP for prophylaxis and bleeding.
Results: Patient characteristics and results are summarized in table 1.The PCC group was older than FFP group and this was statistically significant (P=0,021). No statistically significant for gender and indications both of the groups (P=0,773 and P=0,115). In PCC group before and after treatment prothrombin time (PT) was higher than FFP group (P=0,001 and P=0,001). Decreasing PT was higher in PCC group (P=0,001). In PCC group before and after treatment INR was higher than FFP group (P=0,001 and P=0,001). Decreasing INR was higher in PCC group (P=0,002). In PCC group before and after treatment active partial thrombin time (aPTT) was higher than FFP group (P=0,012 and P=0,048). No statistically significant for before and after treatment aPTT both of the groups (P=0,077). Efficiency time was lower in PCC group and statistically significant differences (P=0,011). But length of hospital stay wasn't different (P=0,762). There wasn't any side effect in both groups.

Table 1. Patient characteristics and results

		FFP	PCC	P value
Age		57,7 ± 19,4	70,1 ± 17,5	[a]0,021*
Gender	Women n(%)	16 (%64)	14 (%56)	[c]0,773
	Men n(%)	9 (%36)	11 (%44)	
Indications	Bleeding n(%)	15(%60)	21(%84)	[c]0,115
	Prophylaxis n(%)	10(%40)	4(%16)	
Before-PT		29,6 ± 17,0	61,1 ± 29,7	[b]0,001**
After -PT		19,8 ± 4,5	27,9 ± 6,8	[b]0,001**
PT difference		10,4 ± 13,4	33,2 ± 27,8	[b]0,001**
Before-INR		3,2 ± 2,2	7,2 ± 3,7	[b]0,001**
After-INR		1,5 ± 0,4	2,7 ± 0,8	[b]0,001**
INR Difference		1,7± 2,0	4,5 ± 3,4	[b]0,002**
Before-aPTT		67,0 ± 46,6	80,8 ± 37,5	[b]0,012*
After-aPTT		41,2 ± 13,0	46,8 ± 13,2	[b]0,048*
aPTT difference		27,7 ± 39,1	33,9 ± 39,4	[b] 0,077
Hospitalisation(day)		7,5 ± 11,6	7,4 ± 10,5	[b] 0,762
Efficiency time(hour)		32,6 ± 15,3	25,5 ± 17,0	[b]0,011*

[a] Student t Test [b] Mann Whitney U Test [c] Yates Continuity correction
*p<0,05 **p<0,01

Summary / Conclusion: We used PCC for the patients who were older and have higher coagulation parameters. It showed effect in a shorter time and corrected coagulation parameters more than FFP. PCC is an effective treatment for prophylaxis and bleeding. To achieve a precise results we need further studies with more patients.

B1959
SPONTANEOUS DUODENAL HEMATOMA IN A PATIENT WITH GLANZMANN'S THROMBASTHENIA
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Background: Glanzmann's thrombashtenia (GT) is a rare congenital disorder of platelet function associated with a prolonged bleeding time, a normal platelet count, abnormal clot retraction and defective platelet aggregation. Mucocutaneous bleedings including gastrointestinal bleeding are common in GT patients but spontaneous intramural duodenal hematoma is extremely rare.
Aims: We report a child who had GT presented with signs and symptoms of duodenal obstruction. We aimed to pay attention a rare presentation of GT patients.
Methods: A five-year-old boy with a known GT was brought to the pediatric emergency room by his parents. The patient had been complainting of the acute onset of periumblical and right upper quadrant pain and vomiting. There was no melena and hematemesis in admission. No history of trauma was present. The abdomen was flat with tenderness in the midepigastrium and right upper quadrant without evidence of peritoneal irritation. Rectal examination reveled black stool.
Results: Abdominal ultrasonography revealed appearent intramural duodenal hematoma and ileoileal invagination. Invagination was resolved via rectal barium application. Intramural hematoma was resolve recurrent administration of thrombocyte suspension. Erythrocyte suspension was given because of deep anemia. The patient did not need further surgical intervention. Intramural hematoma completely regressed on day 10.
Summary / Conclusion: Intramural duodenal hematoma is generally seen after abdominal trauma. It has been reported spontaneous intramural duodenal hematoma in coagulation disorders such as Von Willebrand disease, immune thrombocytopenic purpura, polyartheritis nodosa, hemophilia A (1-3). Only one report about GT and intramural duodenal hematoma was found in the literature and GT (1). The diagnosis of duodenal hematoma should be considered in any patient who has abdominal pain and a coagulation disorder. İntramural hematoma may be abundant even requiring blood transfusion. Nonoperative management of an obstructing duodenal hematoma was successful and potentially life-threatening surgery was avoided.

Thrombosis and vascular biology

B1960
PROTEIN C SERUM LEVELS ARE DECREASED IN HEART FAILURE: A GLIMPSE AT THE PULMONARY SIDE OF THE EQUATION?
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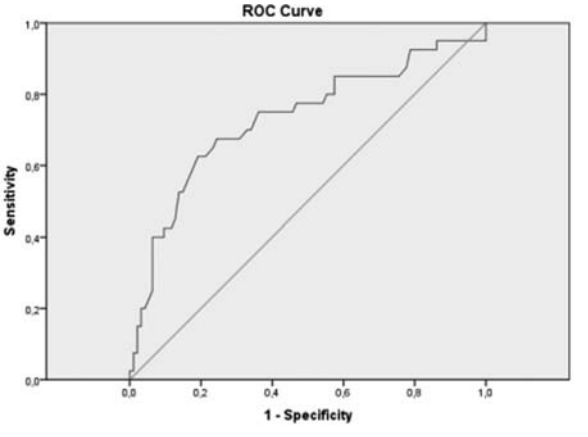
Background: Protein C (PC) is a fundamental piece of the endogenous anticoagulant system. It also has a role in inflammatory response, in endothelial permeability and in vessel remodeling through its receptor EPCR. The author has hypothesized that PC may be depleted in cases of pulmonary stasis and, thus, lower the threshold to pulmonary edema by increasing capillary permeability.
Aims: Assess the relation between levels of PC and the severity of heart failure.
Methods: Retrospective analysis of a series of consecutive patients who attended the emergency department of a Portuguese community hospital from September 2009 to September 2011.
Results: Patients with hematological diseases, coagulation disorders or hepatic dysfunction were excluded. A total of 149 patients were studied; median age 50 years (range 1-90), 46.5% females. The most prevalent primary diagnoses, as listed by the attending physician, were myocardial infarction (64 patients, 44.7%), stroke (16 patients, 11.2%) and heart failure (8 patients, 5.6%). Levels of PC (expressed as percentage of activity) are significantly lower in patients with levels of NT-proBNP superior to 2000 pg/ml (compatible with decompensated heart failure): average level 82.2 vs. 103.6 ng/ml in other patients, P<0.001, 95%CI of the difference: 11.9 to 30.9. Results of transthoracic echocardiography are available for 58 patients. Patients with PC levels in the lowest quartile have a significantly lower left ventricle systolic shortening fraction (P=0.011, 95%CI of the difference: -14.2 to -1.2). Receiver-operator characteristic curve for PC and heart failure has an area under the curve of 0.73.



Summary / Conclusion: PC levels are decreased in cases of severe systolic dysfunction. Although limited by sample size and missing data, these results support that PC may have a role in the pathogenesis of heart failure, concerning specifically episodes of exacerbation. Further studies are needed to confirm this novel hypothesis, as well as the possible role of PC as a biomarker.

B1961
THROMBOEMBOLISM PROPHYLAXIS AFTER CESAREAN SECTION (PRO-CS) TRIAL
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Background: Background: Pregnancy and the post partum are well-established risk factors for venous thromboembolism, with higher risk if delivered via cesarean section. However, current evidence cannot justify deep vein thrombosis thromboprophylaxis on the basis of caesarian section alone in all women with different risk factors especially low risk, thus cannot be recommended for the time being. This study was conducted to determine the efficacy and safety of DVT prophylaxis in low risk women who delivered via cesarean section, and to identify the risk factors. The study regesisted in clinical trial, NCT01321788.
Aims: This study was conducted to determine the efficacy and safety of DVT prophylaxis in low risk women who delivered via cesarean section, and to identify the risk factors

Methods: Methods: Prospective, randomized study of adult female patients aged 18 – 35 years old presenting to OB-Gyn Department of Security Forces hospital, Riyadh Saudi Arabia who delivered via cesarean section were included in the study. Subjects were randomized into two groups; a) Drug group – received Tinzaparin 4500 IU subcutaneously once daily 12 - 24 hours after cesarean operation and, b) placebo group – received once daily 12 – 24 hours after cesarean operation for tow weeks. Both groups received mechanical prophylaxis using graduated compression stockings. Patient's demographics, physical data, obstetrical history, medical histories for thrombosis, presence or absence of clinical signs and symptoms of deep vein thrombosis and co-morbidities were all noted. Laboratory tests were done to all patients. Patients were observed within one week of hospital stay for signs and symptoms of DVT, minor and major bleeding events. After discharge patients followed-up after two weeks, at 6th week, at 3rd month and 6 months interval thereafter for one year.
Results: Results: A total of 200 patients (mean age: 28.6 years) consented for the study. The demographic data was similar to both groups. There were only one incidences of deep vein thrombosis in the 200 placebo group in comparison to 100 tinzaparin study group . There has been no record of death after 1 year of follow-up.
Summary / Conclusion: Conclusion: Our study showed the low risk caesarian section are not at risk of developing DVT as will no benefits of LMWH confirm no safety and efficacy of thromboprophylaxis using LMWH.

B1962

THROMBELASTOMETRY IN DUAL ANTIPLATELET TREATMENT IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Background: Dual antiplatelet therapy (acetylsalicylic acid (ASA) and thienopyridins) has an important role in the management of patients with acute coronary syndrome (ACS) undergoing coronary intervention. However, there can be a high individual variability in response to antiplatelet therapy.
Aims: We initiated this study to investigate the utility of thrombelastometry in assessing the response to dual antiplatelet treatment in patients presenting with acute coronary syndromes (ACS) and to compare these results with established monitoring techniques.
Methods: Our study included 20 patients with ACS (13 men, 7 women, mean age 67.6 years) who received a loading dose of ASA (400 mg, n=20) and clopidogrel (600 mg, n = 15) or prasugrel (60 mg, n = 5). The control group consisted of 21 healthy blood donors (17 men, 4 women, mean age 50.1 years). The first sampling was conducted to monitor the loading dose of antiplatelet drugs prior to the implementation of selective coronarography. The second sampling served to monitor the efficacy of antiplatelet therapy in maintenance doses. As laboratory methods served optical agregometry and rotation thromboelastometry (ROTEM).
Results: Optical aggregometry showed a significant decrease in platelet aggregation between the first and second sampling after stimulation by arachidonic acid (33.2% vs. 21.1%, controls 74.6%) as well as by adenosine diphosphate (51.4% vs. 37, 1%, controls 72.7%). There was found a significant decrease in aggregation for the antiplatelet combination clopidogrel-ASA (57.3% vs. 45.1%, P=0.05) and even more significant decrease was seen for combination prasugrel-ASA (35.0% vs. 17.5% p <0.001). The standard parameters of ROTEM (CT, MCF) didn´t record significant results.
Summary / Conclusion: In our study the treatment with prasugrel was more effective than clopidogrel in patients with ACS as measured by optical aggregometry. Conversely, standard parameters of ROTEM do not seem to be sufficiently sensitive for monitoring the antiplatelet therapy. The role of ROTEM in this field requires further studies.

B1963

CONTRIBUTORY RISK FACTORS FOR DEVELOPMENT OF THROMBOSIS IN CHILDREN WITH NEPHROTIC SYNDROME

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Background: Diagnosis and treatment of thrombosis is important in the management of children with nephrotic syndrome (NS).
Aims: The aim of this study was to evaluate the prevalence of thrombosis and contributory risk factors for development of thrombosis in children with NS.
Methods: Nephrotic syndrome registry data was retrospectively evaluated since July 2008 in Hacettepe University. Among 187 children with the diagnosis of NS (80 girls and 107 boys; mean age 12.4±5.5 years) followed up in Hacettepe University Faculty of Medicine, Pediatric Nephrology Unit; 17 children (16 boys and 1 girl) with the mean age of 11.6±6.7 years (1.3-24.0) identified as having thromboembolic complications. All 17 children with NS and thrombosis screened for laboratory risk factors for thrombosis (protein C, S, and antithrombin III, homocystein, lipoprotein, triglyceride and cholesterol levels, antiphospholipid antibodies (APA), factor V Leiden, prothrombin, MTHFR 677 and 1298 mutations, plasminogen activator inhibitor (PAI) polymorphisms, factors II, V, VII, VIII, IX, XI, and XII levels). The diagnosis was confirmed by cranial magnetic resonance imaging (MRI), doppler ultrasonography (USG), and echocardiography.
Results: The mean age of the 17 children was 4.5±3.2 years (0.08-11.0) at the time of NS diagnosis and that was 7.1±4.9 years (0.25-14.0) at the time of thrombosis. Among 17 children with thrombosis; 4 had catheter related thrombosis in jugular veins, 2 had cerebral infarct, 2 had portal venous thrombosis, 2 had intracardiac (right ventricle and right atrium; respectively) thrombosis, 3 had sagittal sinus thrombosis, 1 had cerebral infarct and intracardiac thrombosis at tricuspid valve and patent ductus arteriosus, 1 had left sigmoid and transverse sinuses thrombosis, 1 had superficial right femoral vein thrombosis, and 1 had cephalic vein thrombosis. Inherited risk factors for thrombosis were identified in 16 (94.1%) children. High factor VIII levels was detected in 11/17, high factor V levels in 4/17, decreased protein C level in 3/17, decreased protein S level in 2/17, antithrombin III deficiency in 5/17, high homocystein level in 4/17, high lipoprotein a level in 4/17, antiphospholipid antibodies in 1/17, anticardiolipin antibodies in 2/17, factor V Leiden heterozygote mutation in 2/16, MTHFR 677 heterozygote mutation in 6/16, MTHFR 677 homozygote mutation in 1/16, MTHFR 1298 heterozygote mutation in 3/13, MTHFR 1298 homozygote mutation in 1/13, PAI (4G/5G) polymorphism in 2/13, and PAI (4G/4G) polymorphism in 1/13 child. Two children had1, seven children had2, three children had3, and four children had 4 inherited risk factors for thrombosis. Furthermore; 4 children had central venous catheters, 2 had infection, and 1 had rejection episode of transplanted kidney as clinical risk factors for thrombosis. Most of the children (n:13, 76.4%) treated with only low molecular weight heparin (LMWH). Thrombectomy was performed for 2 children. The duration of anticoagulation therapy was 6 months for most of the children (n:13, 76.4%). Only 1 child received 12 months therapy and other 3 children received 3 months therapy. Twelve children (70.5%) had complete recovery from thrombosis, whereas 3 had partial recovery with sequela and 2 had died due to sepsis and multiple organ dysfunction syndrome and other systemic diseases except for thrombosis.
Summary / Conclusion: It should be kept in mind that children with NS and additional inherited risk factors should be followed up carefully for development of thrombosis. The early diagnosis and appropriate intervention can improve outcomes, reduce mortality and morbidity significantly.

B1964

FIBRINOGEN GENETIC VARIABILITY AND PROTHROMBOTIC PROFILE IN PATIENTS WITH HYPERTENSION

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Background: The G455A fibrinogen polymorphism is associated with the risk of coronary artery disease. However, it remains unknown whether it affects the prothrombotic profile of patients with hypertension (HT). We aimed to examine the impact of this polymorphism on fibrinogen, D-dimers, factor V (fV) and factor X (fX) levels in hypertensive patients.
Aims: Fibrinogen Genetic Variability And Prothrombotic Profile In Patients With Hypertension
Methods: The study population consisted of 457 HT and 224 non HT. The G455A polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, while circulating levels of fibrinogen were measured by the von Clauss method. D-dimers levels, fV and fX levels were measured by standard coagulometry techniques.
Results: Genotype distribution for non HT and HT was GG: 50.9%, GA: 41.8%, AA: 9.7% and GG: 51.5%, GA: 37.6%, AA: 10.9% respectively. There was no significant difference in fibrinogen levels (mg/dl) between 455AA homozygotes and 455G allele carriers in non HT patients (448.3±34.6 vs 395.4±9.9, P=NS). Importantly, 455AA genotype presented with much more elevated levels of fibrinogen compared to the GG+GA in HT patients (535.4±25.3 vs 414.2±8.0, P<0.001). Moreover, HT 455AA homozygotes had significantly increased D-dimers levels (μg/l) compared to 455G allele carriers (640.3±83.6 vs 485.5±27.2, P<0.05). No difference was observed for non HT regarding D-dimers between the 455AA genotype and GG+GA (477.6±74. vs 450.8±40.7, P=NS). Interestingly, 455AA genotype presented with higher fV(%) and fX(%) levels compared to GG+GA in HT patients (133.6±5.8 vs 117.8±3.3, P<0.05, for fV) and (101.9±4.6 vs 92.2±2.4, P<0.05, for fX). However, no difference was observed in fV and fX levels between 455AA and GG+GA in non HT (105.8±11.6 vs 118.7±4.4, P=NS for fV) and (95.8±8.0 vs 119.4±29.1, P=NS for fX).
Summary / Conclusion: The G455A fibrinogen genetic polymorphism has a remarkable impact on prothrombotic profile of patients with hypertension, by affecting fibrinogen, D-dimers, factor V and factor X levels. These findings provide evidence that this polymorphism modifies further the atherosclerotic effects of hypertension via alterations in the coagulation cascade.

B1965

IDIOPATHIC PURPURA FULMINANS, DIFFERENTIAL DIAGNOSIS OF CUTANEOUS THROMBOSIS IN CHILDHOOD, ANTITHROMBOTIC THERAPY AND MONITORING

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Background: Purpura fulminans (PF) is a thrombotic skin disorder, usually caused by a deficiency of Protein C or S-mediated autoimmune mechanism, characterized histologically by extensive venous thrombosis of the dermis with hemorrhagic infarction of adjacent tissues. There are three clinical situations described: neonatal PF, infectious PF, and idiopathic PF. Differential diagnosis must be done with hypo/dysfibrinogenemia and disseminated intravascular coagulation (DIC).
Aims: We describe a case of a patient who had idiopathic PF; diagnosis, treatment and monitoring.
Methods: Case report
Results: 2 year old Moroccan male patient with no medical history, admitted by fever 48 hours of evolution associated with rejection of the motion and two lower extremity purpura. After admission rapid progression of lesions coalesce in 6 hours, with a central area and very painful ischemic, prothrombin activity noticeable decrease to 16.3%, establishing initial treatment with vitamin K and antibiotics. Because clinical suspicion of coagulopathy, coagulation test were extended revealing normal antithrombin (85%), fibrinogen (Claus von: 0.2 g/L), and protein C (121%) levels. The ethanol test was negative, prothrombotic polymorphisms (FV Leiden and prothrombin G20210A) were absent, and a weak positive lupus anticoagulant was detected. As expected, D-dimer levels were high (32,550 ug/dL). Relevantly, severely reduced protein S (4%) determined by *functional assay Protein S HemosIL TM*. A protein S inhibitor is suspected because after mixing with normal plasma this parameter was not corrected completly. Parents' protein S levels were normal. Cultures pharyngeal, blood, urine and skin negative and seronegative (HAV, HBV, HCV, mycoplasma, Varicella - zoster IgG and IgM, CMV, EBV and parvovirus B19). Skin biopsy: thrombotic vasculopathy compatible with PF, fibrinoid thrombosis in practically all and venular capillaries of the dermis and adipose tissue, hematic extravasation between dermal collagen bundles.
After diagnostic confirmation of PF anticoagulation with enoxaparin 1.5 mg/kg/12 hours was started performing titration by determining levels of anti-Factor Xa (therapeutic levels from 0.5 to 1.0 IU/mL) and fresh frozen plasma therapy, thus obtained a progressive increase in serum levels of protein S and clinical improvement.
The patient fully recovered without sequelae, and after 3 months anticoagulant therapy was discontinued. We highlight a relevant decrease of protein S levels during a new infectious event, but because the absence of clinical symptoms, no treatment was required.
Surface Plasmon Resonance on protein S-CM5 chips, revealed no anti-protein S antibodies in patient's plasma at any infectious event. Moreover, western blot of plasma protein S revealed normal total protein S levels of similar size than in controls. Finally, molecular analysis of *PROS1* gene revealed no significant mutation.
Summary / Conclusion: 1) Despite its low incidence, the PF is an entity to be considered in the differential diagnosis of cutaneous thrombosis in childhood, 2) In this framework, it is necessary to carry out early histological analysis to find evidences sustaining a definitive diagnosis. 3) Anticoagulant therapy should be initiated at an early stage to prevent damage; 4) In addition to anti-protein S antibodies; other factors triggered by infections may interfere with the anticoagulant activity of this protein C cofactor. Further studies are required to identify this factor in this patient.

B1966

MONITORING OF LOW-MOLECULAR-WEIGHT HEPARIN IN HIGH RISK PREGNANCY

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Background: The molecular-weight Heparin (LMWH) is the preferred anticoagulant for the prevention and treatment of pregnant women with high risk of venous thromboembolism (VTE) and adverse pregnancy outcomes**.** Dosing of LMWH based on weight alone is standard for most non-pregnant patients, the concept of using a target antifactor Xa level for VTE prophylaxis during pregnancy remains controversial, with currently alternatives including: previously defined prophylactic dose, intermediate-dose or periodic monitoring of antifactor Xa activity.
Aims: To describe demographic and clinical characteristics, and to evaluate the dosing requirements and monitoring patterns when LMWH is used during pregnancy in our institution.
Methods: A retrospective, observational, cohort study of patients who were given prophylactic or therapeutic LMWH during pregnancy and who were monitored with antifactor Xa between January 2011 to February 2013. We analized demographic parameters, history of congenital or acquired thrombophilia, obstetric and/or VTE history, treatment monitoring and dose adjustment, complications and obstetric outcomes.
Results: Data were obtained on 46 pregnancies in 46 women. Median age 34 years (18-45). Table below shows observed LMWH parameters.
Trimester LMWH was started N (%)
First 33 (71.7%)
Second 12 (26.1%)
Third 1 (2.2%)

Starting dosis

Enoxaparin 40 mg/24 h 40 (86.9%)
Enoxaparin 60 mg/24 h 5 (18.9%)
Enoxaparin 60/12h 1 (2.2%)
Indications for LMWH prophylaxis/treatment
Adverse pregnancy outcomes 5 (10.9%)
Antiphospholipid syndrome 12 (26.1%)
Thrombophilia, no VTE 19 (41.3%)
History of VTE and thrombophilia 3 (6.5%)
History of VTE 5 (10.9%)
Current VTE 1 (2.2%)
A mean of 2.3 (ranges 1-16; median: 6) antifactor Xa levels were obtained for each patient. The mean antifactor Xa level was 0.343 UI/mL (range 0.07-0.87). 18 patients (39.1%) required dose changes (increasing dose) , 6 of them requires more than one dose change (both increase and decrease). Complications (embolic events and/or hemorraghe) were not observed. 1 patient (2.2%) under target antifactor Xa levels presented fetal death at 37 week due to eclampsia.
Summary / Conclusion: LMWH dose cheges throughout pregnancy according to antifactor Xa levels were common in our patients, similar to data refered in literature. Increase in the LMWH dosage requirements suggest that more frequent monitoring may be appropiate to achieve a predetermined target or antifactor Xa levels. Although dosing recommendations for LMWH in pregnancy are fairly well described, monitoring guidelines to attain acceptable anticoagulant safety and efficacy are still needed.

B1967

THROMBIN-LIKE AMIDASE ACTIVITY IN PLASMA REMAINS HIGH AFTER LUNG DISEASES EXACERBATIONS

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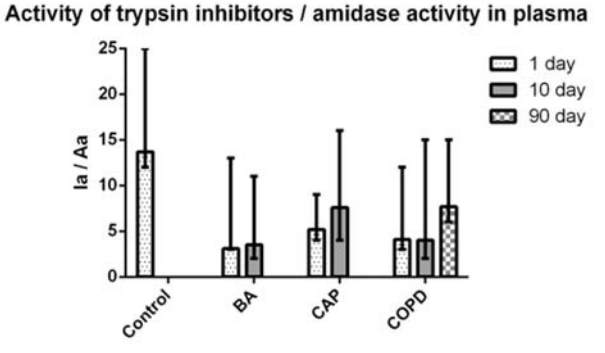
Background: It is known, patients with chronic obstructive pulmonary disease (COPD) are at an increased risk of venous thromboembolism. Courses of hypercoagulable state in this case are not clean. Protease imbalance plays key role in COPD pathogenesis and coagulation cascade is enzymatic cascade consist mainly of serine proteinases with trypsin-like activity. Besides thrombin, such proteinases, as kallikrein or trypsin can carry out factor XII activation. Serine and other proteases modify the properties of angiogenic growth factors and cytokines able to degrade of the endothelial and interstitial matrix. Theese proteases can cleavage of amino acid bond in BApNA (Na-benzoyl-L-arginine p-nitroanilide) and it is possible chromogenic assay of amidase activity.
Change of plasma thrombin-like amidase activity (Aa) can reflect system reaction on hypoxia or relate to systemic inflammation.
To analize whether any airflow limitation is a course of the imbalance between the procoagulant and anticoagulant pathways we analysed plasma samples of COPD, bronchial asthma (BA) and community-acquired pneumonia (CAP) patients.
Aims: To measure amidase activity and activity of serine protease inhibitors in plasma of patients with COPD, BA, CAP to estimate risk of thrombotic complications.
Methods: Informed agreement was received from all patients. There were two times to blood assay – the 1-st day (exacerbation) and 10-th day at a hospital. COPD patients visit us at 90-th day too. They received standart therapy in City clinical hospital No. 70 of Department of Healthcare of Moscow. A total of 15 patients (mean age 43±10) were on study. There were 7 COPD, 5 BA and 3 CAP patients. Control group include 10 subjects, age 40±11. Blood samples were collected in 4 ml tubes, 60 USP Units of Lithium Heparin, after processing plasma samples stored frozen. The ability of the plasma samples to catalyse the hydrolysis of BApNA is referred to as Aa. Simultaneously in microwell plate the activity of trypsin inhibitors in plasma (Ia) was measured using BApNA hydrolysis. In microwell plate in duplicate we added 50 µl of samples at a dilution of 1:25, 200 µl of a 1 mg/ml BApNA solution in 0,1 M tris-HCl buffer at pH 7.65, 50 µl and 50 µl buffer (Aa analysis) or 50 µl trypsin solution with activity 225 U/ml (Ia analysis). 1 U corresponds to the amount of enzyme which increases the absorbance at 410 nm by 0.001 per minute at pH 7.65. We read

absorbance of each microwell on a spectro-photometer using 410 nm as the primary wave length (620 nm as the reference wave length). The plate was incubated at 25°for 30 minutes on a microplate shaker set at 100 rpm. After that was the second absorbance reading using 410 (ref. 620) nm and the difference of absorbances (ΔOD) was found. We created a standard curve by plotting ΔOD for each standard on the ordinate against the standard trypsin activity (from 899 U/ml to 14 U/ml and 0 U/ml) on the abscissa. Calculated Aa was multiplied by the dilution factor. Ia was calculated using abscissa $\Delta OD_Z = (\Delta OD_{225} + \Delta OD_{Aa}) - \Delta OD_{Ia}$ to consider proteinase activity of the sample. We didn't use soybean trypsin inhibitor to this analysis because ratio Ia/Aa was evaluated to estimate proteinase imbalance.

Results: We examinated proteases and serpins activity in the presence of heparin that inhibition of thrombin by antithrombin was accelerated.

All COPD, BA, CAP samples showed a markedly increased Aa in comparison with conrol group. So lung inflammation and airflow limitation are the sufficient reasons to the changing plasma Aa activity within 10 days after exacerbation of lung disease. For example, it can be a consequence of kallikrein activation. We assumed that increasing of Ia testifies to reaction to Aa increasing. But Ia showed tendency to increase only in COPD group (Mann-Whitney's criterion, P=0,065). We next examined Ia/Aa ratio. Results of long-term follow-up study are presented in Figure. This ratio in 10 day BA and CAP groups and even in 90 day in COPD group didn't reach control value. Smoking status and other COPD risk factors were not analyzed and it is limitation of the study.



Summary / Conclusion: The current study demonstrates continuous proteinase imbalance in plasma of patients with lung diseases. It can be cause to pay attention to the procoagulant activity after CAP or exacerbations of chronic lung diseases.

B1968

STUDY OF CONSTITUTIONAL THROMBOTIC RISK FACTORS AND ACQUIRED RETINAL VEIN THROMBOSIS. EXPERIENCE A CENTER.

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Background: Retinal vein thrombosis (RVT) has been associated with vascular disease and the main risk factors associated are hypertension (HT), diabetes mellitus (DM) and atherosclerosis. Thrombophilia caused by hemostasis disorders is a well known cause of the increase of risk for suffering thrombotic events in your people as a consequence its role in the RVT is being investigated.

Aims: We conducted a retrospective study ofthrombophilia abnormalities in patients with RVT in order to see the association of molecular pathology or plasma associated thrombotic risk in this group of patients.

Methods: We analyzed a database of our Service with 818 patients during 8 years. Adquired risk factors were identified as: HT, obesity, DM, smoking, dyslipidemia, family history of thrombosis (FT), oral contraceptives, and immobility; and presence of possible alterations that cause thrombophilia as: mutation for Factor V Leiden (FVL), G20210A prothrombin gene (P20210A), and plasma deficits such as protein S deficiency, protein deficiency C, resistance to activated protein C (APCR) and lupus anticoagulant.

Results: We identified 26 patients with TVR, 16 women and 10 men, with a mean age of 43.46 years (range 13-73). 34% patients did not collect any risk factors in the medical record. Of the remaining patients, 53% had one risk factor: 4 smoking, 2 FT, 2 HT, 1 obesity; and 35.3% had two or more (FT with smoking, obesity and DM). Regarding thrombophilia studies findings were : - Heterozygous for FVL mutation in 7.7% (2 patients), one patient had a family history of thrombosis. - Heterozygous mutation for P20210A by 3.8% (1 patient), and obesity asociated. - In the analysis of the deficits of plasma factors, the 88.46% of the results were normal, in other cases, one patient had protein S deficiency and one cases a lupus anticoagulant, not associated with other risk factors.

Summary / Conclusion: Our results reveal an incidence in population diagnosed of RVT similar to the normal population and confirm the association of RVT with age ≤ 45 years, family history of thrombosis or cardiovascular risk factors. However, it seems necessary to conduct prospectives studies in patients with RVT to determine the role of hereditary and acquired thrombophilia in these patients.

B1969

EVALUATION OF HEALTH LITERACY AND ADHERENCE TO THROMBOPROPHYLAXIS IN PREGNANCY

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Background: A report carried out in 2002 by the National Adult Literacy Agency stated there is a critical need for additional Irish research into health literacy. There has remained a paucity of work in this area. Thromboembolic disease remains a leading cause of morbidity and mortality in pregnancy and puerperium as evidenced by statistics gathered such as in the Centre for Maternal and Child Enquiries (CMACE) report 2010.

Aims: The primary goal of this research was to analyse the relationship between health literacy and adherence rates to LMWH (low molecular weight heparin) thromboprophylaxis in pregnancy.

Methods: The Rapid Estimate of Adult Literacy in Medicine (REALM) was used to measure health literacy in pregnant patients on LMWH thromboprophyalxis. A second questionnaire entitled the Beliefs in Medicines Questionnaire (BMQ) was also administered to assess patient beliefs, concerns and knowledge surrounding their medication. Both of these are standardised tools widely used in research. With patient consent, their pharmacies were contacted and information gathered regarding their prescription collection. This information was used as a surrogate indicator of adherence.

Results: Of the 24 patients studied, 25% were found to have inadequate health literacy. Of the thirteen patients for whom information was available, eight were found to have incomplete adherence. The BMQ showed that in general participants believed that their medication was necessary, and their concerns did not outweigh theses beliefs. Patient knowledge surrounding their medication was relatively good. Analysis was carried out to look for possible associations between inadequate health literacy and adherence, along with patient beliefs, concerns and knowledge.

Summary / Conclusion: Results suggest that the level of inadequate health literacy may even be higher than expected. Adherence to LMWH regime is suboptimal in the majority of cases. We postulate that there is a significant relationship between health literacy levels and adherence. This is a pilot study in one centre with a small sample size. Indications are that further studies would be useful and that implementation of services such as focused information leaflets and a text messaging reminder service may result in higher rates of adherence to LMWH in these patients.

B1970

LOCAL EXPERIENCE IN THE DIAGNOSIS AND TREATMENT OF DEEP PELVIC SEPTIC THROMBOPHLEBITIS

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Background: There are two types of septic pelvic thrombophlebitis (SPT): ovarian vein thrombophlebitis (OVT) and deep septic pelvic thrombophlebitis (DSPT). Patients with DSPT usually present within a few days after delivery or surgery with unlocalized fever that persists despite antibiotics, in the absence of radiographic evidence of thrombosis.

A variety of factors (Cesarean section, Pregnancy, Pelvic infection eg, postpartum endometritis, pelvic inflammatory disease, Induced abortion, Pelvic surgery, Uterine fibroids, Underlying malignancy. Hormonal stimulation) confer increased risk for SPT.

The diagnosis of SPT can be challenging and is often a diagnosis of exclusion. In the absence of definitive diagnostic findings, many clinicians presume SPT in patients with appropriate risk factors and persistent fever despite antibiotic therapy who defervesce within 48 hours of empiric systemic anticoagulation.

There have been no studies to determine the optimal duration of anticoagulation therapy in SPT. In the absence of documented thromboses or underlying hypercoagulable state, most clinicians favor discontinuing anticoagulation following resolution of fever for at least 48 hours.

Aims: To review the experience in our center regard concerning patients with suspected DSPT.

Methods: Medical records from patients with suspected DSPT in the last year were reviewed to obtain relevant data.

Results: We present three cases in which DSPT was suspected. In all them fever was unresponsive to antibiotics and appeared in the immediate postpartum period associated with increased levels of acute phase reactants. Blood

cultures showed no bacterial growth in all three cases and abdominal CT scans were normal except where noted. Patient #1: a 29 year old woman complained of fever 24h after a vaginal delivery complicated with fetal distress. Once DSPT was suspected sodium heparin was started at therapeutic doses. Fever disappeared soon after heparin therapy. Patient #2: a 34 year old woman complained of abdominal pain and febricula 9 days after an uncomplicated vaginal delivery. On clinical suspicion of DSPT, LMWH was started with good clinical response. A study of thrombophilia performed afterwards detected a lupus anticoagulant as well moderately increased IgM anticardiolipin antibodies. Low dose AAS was administered onwards. A subsequent pregnancy went uncomplicated under antithrombotic prophylaxis. Patient #3: a 39 year old had undergone cesarean section due to premature rupture of membranes; fever started seven days after the procedure. In abdominal CT scan only a previously known leiomyoma was noticed. LMWH was started due to suspicion of DSPT but in this instance anticoagulation did not solve the clinical problem. CT scan was repeated showing red degeneration of myoma and thus this patient was not considered to have DSPT.

Summary / Conclusion: Although SPT is usually diagnosed by obstetricians, this entity must be known by hematologists, since antithrombotic therapy leads to diagnosis as well as clinical resolution.

B1971

THROMBOPHILIC STATUS IN PATIENTS WITH EXTRA-HEPATIC PORTAL HYPERTENSION

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Background: The extra-hepatic portal hypertension due to portal thrombosis was belived to be a rare condition (about 10-20% of all cases of portal hypertension). Chronic myeloproliferative disorders (MPD) were considered to be the main cause of thrombotic complications in adult patients (pts), and thrombophilia - to be a predisposing factor.

Aims: To compare the rate of antiphospholipid syndrome and mutations causing predisposition to thrombophilia in patients with portal vein thrombosis (PVT) associated with chronic myeloproliferative disorders (MPD) and without MPD.

Methods: 183 patients (77 males, 106 females, Median age – 41 years) with portal thrombosis confirmed by Doppler sonography were included into this study. The period from the first manifestation of portal hypertension (splenomegaly, gastro-esophageal varices, variceal bleedings) to examination in our Center varied from 6 to 480 months (Median 60 months). Only 73 (40%) patients had bone marrow morphology of chronic myeloproliferative disorders(MPD+ group) The other patients had normal pattern of bone marrow, normal blood picture or cytopenias (MPD- group). The both groups were comparable by age, sex and duration of disease.

All patients were screened for heritable thrombophilic gene mutations (G1691A Factor V Leiden, G20210A prothrombin, C677T MTHFR mutations, polymorphism of PAI-1gene (4G/5G, 4G/4G) and lupus anticoagulant with antiphospholipid antibodies (APL-Abs).

Results: Homozygous C677T MTHFR mutation was found in 1% of pts in MPD+group and in 9%>in MPD-group, heterozygous factor V Leiden mutation – in 13,5% and 13% pts, respectively, heterozygous G20210A prothrombin gene mutation – in 3% pts in MPD+group and 8% in MPD-group. 4G/4G PAI-1 polymorphism was revealed – in 11% and 13%, 4G/5G – in 30% in MPD+group, in 19% in MPD-group. The combination of 3 and more thrombophilic gene mutations was found in 35% patients. Lupus Anticoagulant was found in 62% MPD+ pts, and in 55% pts without MPD. The antiphospholipid antibodies (APL-Abs) to membranes phospholipids and β2-glycoprotein I were studied in 37 pts. APL-Abs were found in 22 pts LA+ and in 15 pts LA-, most frequently in pts with cytopenias. Statistically significant difference in MPD+ group and patients without MPD was not revealed.

Summary / Conclusion: The use of molecular diagnostic methods reveals the high frequency of mutations causing predisposition to thrombophilia in patients with portal vein thrombosis (PVT) associated with chronic myeloproliferative disorders and without MPD. Antiphospholipid syndrome was diagnosed in 58% patients with MPD and 65%> without MPD. Patients with portal thrombosis due to hereditary and acquired thrombophilia have a high risk of recurrence, and so prescribing anticoagulant/antiaggregant therapy in patients without MPD should be necessary.

B1972

THROMBOPHILIC RISK FACTORS AND THE EFFICIENCY OF PROPHYLACTIC ANTICOAGULATION THERAPY IN CHILDREN WHO UNDERWENT RENAL TRANSPLANTATION

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Background: In pediatric renal transplantation, graft loss due to thrombosis is a major problem. Although thrombosis has been identified as the most important risk factor for early graft loss, there is limited data about the effect of prophylactic anticoagulation therapy on graft survival, rejection episodes, and graft function in childhood.

Aims: The aim of this study is to evaluate inherited and acquired risk factors for thrombosis and the efficiency of anticoagulation treatment protocol used in our center for renal transplant recipients.

Methods: Twenty-two children (10 boys and 12 girls) with the mean age of 14.2±3.7 years (6.8-18.9) were underwent renal transplantation in Hacettepe University Faculty of Medicine between 2009 and 2012. All 22 children screened for inherited and acquired risk factors for thrombosis (protein C, S, and antithrombin III, homocystein, lipoprotein, triglyceride and cholesterol levels, antiphospholipid antibodies (APA), factor V Leiden, prothrombin, MTHFR 677 and 1298 mutations, plasminogen activator inhibitor (PAI) polymorphisms, factors II, V, VII, VIII, IX, XI, and XII levels) to extend prophylactic anticoagulation therapy in those with an increased risk for thrombosis. Prophylactic anticoagulation therapy protocol using in our center include intravenous heparin administration at a dosage of 10 U/kg/h for approximately 2 weeks start after the procedure and continue during hospitalization and switch to low-molecular-weight heparin (LMWH) at a dosage of 1 mg/kg after discharged from the hospital for at least 3 months. LMWH prophylaxis has extended to 6 months for children with thrombophilic risk factors. The effect of this protocol on incidence of acute rejection episodes has also been analyzed.

Results: No thrombosis occurred in early post-transplant period in these 22 children who underwent renal transplantation. Thrombophilic risk factors were identified in 17/22 (77.2%) of these children. High factor VIII levels was detected in 9/16, decreased protein C level in 2/14, decreased antithrombin III level in 1/14, high homocystein levels in 4/14, high lipoprotein a level in 4/14, high triglyceride levels in 6/22, high cholesterol levels in 2/22, factor V Leiden heterozygote mutation in 1/18, MTHFR 677 homozygote mutation in 3/18, MTHFR 1298 homozygote mutation in 2/7, PAI 4G/5G polymorphism in 2/7, and PAI 4G/4G polymorphism in 2/7 children. The mean follow up duration for these children who underwent renal transplantation was 24.6±12.5 months (3-48). Acute rejection episode and graft loss was not observed in any children during this follow up period. Macroscopic hematuria was observed in one patient after 10 days of transplantation and heparin was decreased to the dosage of 5 unit/kg/h until discharge from the hospital. Other two patients had thrombosis in their arteriovenous fistula 2 months and 1 year after transplantation. These patients received LMWH treatment for an additional 6-month for thrombosis.

Summary / Conclusion: Children with thrombophilic risk factors should be identified before renal transplantation. Prophylactic heparin at a dosage of 10 U/kg/h approximately 2 weeks during hospitalization and LMWH therapy at a dosage of 1 mg/kg at least 3 months should be administered for all renal transplant recipients, whereas LMWH treatment should be extended for children with identified thrombophilic risk factors. This prophylactic anticoagulation therapy will decrease the risk of thrombosis in renal transplant recipients.

B1973

ANTITHROMBOTIC PREVENTION IN NEUROSURGERY

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Background: Venous thromboembolism (VTE) is a serious but preventable disease with possible acute and chronic complications. VTE is one of the leading causes of deaths in Europe. VTE leads to hospitalizations or prolongation of hospitalizations. Quality of life of the VTE patients is worth mainly due to chronic complications such as postthrombophlebitic syndrome and pulmonary hypertension.

Aims: To describe the necessity and benefits of risk stratification and perioperative antithrombotic prophylaxis in neurosurgery patients.

Methods: All 54 patients operated at Neurosurgery department in month January 2013 are evalulated ex post. The group of patients consisted of 17 male and 37 female, aged 19-81 years, average age 53 years. The operations performed were: intracranial tumor extirpation (2 cases), hemilaminectomia or laminectomia (7), dorsal lumbal somatic fusion (23), anterior lumbal somatic fusion (2), anterior cervical somatic fusion (5), baloon kyphoplastic (5), nerve decompression (9). Patients´ risk status was evaluated for intermediate or high risk. No low risk patient was identified because of recent neurosurgery intervention. Into the high risk group were considered patients with oncologic diagnose, long-term immobilization and previous VTE episode. No inherited trombophylia markers were tested. Prophylactic low-molecular-weight heparin was considered for all patients. In those at intermediate risk lower dose fo the time of hospitalization (maximum up to 14 days) and in those at high risk high dose for the hospitalization periode followed by lower dose for up to 6 weeks.

Results: No VTE event has been observed in the reported group of patients during the hospitalization periode and in the close follow up (4-8 weeks).

No bleeding or other complications were reported in the observed group of patients.

Summary / Conclusion: Authors describe their philosophy in VTE risk evaluation and prophylaxis, based on most recent national and international guide-

lines. The right risk stratification of patients, selection of the proper method, timing, dosage and duration of antithrombotic prophylaxis can minimize the occurence of VTE events in neurosurgery patients.

B1974

DEEP VENOUS THROMBOSIS SECONDARY TO MAY-THURNER SYNDROME: A CASE REPORT

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Background: We report a case of a 31 year-old female with pain and swelling of the left lower extremity as the initial presentation of acute deep venous thrombosis (DVT) secondary to May-Thurner Syndrome, a rare case with unknown overall prevalence, that is due to an anatomical anomaly where the left common iliac vein is compressed between the right common iliac artery and the lumbar vertebrae. She had a two-day history of pain and swelling of the left lower extremity, warmth and difficulty in ambulation. On examination, the left lower extremity had purplish discoloration, varicosities, grade three pitting edema, warmth, tenderness from the medial part of the thigh to the calf and limitation of movement. Its circumference was greater than the right lower extremity by 10 cm. Pulses, reflexes and sensory were intact with no inguinal lymphadenopathy.
Aims: May-Thurner Syndrome is often unrecognized due to the prevalence of other more easily recognized risk factors for DVT. A more invasive study, such as venography is necessary to correctly identify and manage this anomaly to prevent debilitating sequelae.
Methods: Duplex scan of lower extremities showed acute DVT partially occluding the left common iliac and distal external iliac veins, proximal to distal femoral vein, and popliteal vein. CBC, PT and PTT were unremarkable. She had elevated Factor VIII levels at 201%, and low protein S level at 19%, prompting investigation for thrombophilia which yielded negative findings. On further workup, venography revealed stenotic, anomalous vein originating from the left iliac vein, obstruction from the left common iliac vein to mid-proximal femoral vein and collaterals in the common femoral vein to the left common iliac vein. Workup for anti-phospholipid antibody syndrome, systemic lupus erythematosus, malignancy, lymphedema, and infection were negative.
Results: Management was aimed at clearing the thrombus present and correcting the underlying compression of the left iliac vein. Methods of clearing the thrombus include anticoagulation and thromboreductive strategies. Initial anticoagulation with Enoxaparin was overlapped with Warfarin for long-term anticoagulation. She also underwent catheter-directed thrombolysis with Alteplase, and ilio-femoral, popliteal, and posterior tibial thrombectomy after IVC filter insertion; however, treatment failed as evidenced by persistence of pain and swelling on the left lower extremity and occurrence of pulmonary embolism. Subsequently, femoro-femoral vein bypass using cross-over great saphenous vein and arteriovenous fistula creation to the femoral venous bypass graft were successfully performed, followed by chronic anticoagulation with Rivaroxaban. Clinical improvement was noted with completely resolved pain and swelling on the left lower extremity after 6 months. Follow-up duplex scan of lower extremities also showed marked improvement.
Summary / Conclusion: A comprehensive diagnostic approach is essential to accurately identify May-Thurner Syndrome as a cause of iliofemoral DVT, especially in a young female with unilateral pain and swelling of lower extremity. Long-term anticoagulation and thromboreductive strategies, while indicated, are not adequate to prevent long-term sequelae in this condition, and a more invasive therapeutic approach, surgery, is indicated.

B1975

WARFARIN-INDUCED BREAST NECROSIS ASSOCIATED WITH FV LEIDEN, PROTEIN S DEFICIENCY AND PROTHROMBIN G20210A MUTATION

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Background: Warfarin induced skin necrosis is a rare side effect of warfarin therapy. It occurs in only 0.01-0.1% of patients taking the drug and is frequently associated with low levels of protein C,S or FV Leiden mutation.
Aims: The investigation of a 28 year-old obese woman admitted for cholecystectomy. She had a history of deep vein thrombosis (DVT) during a pregnancy (4th month of gestation) and a subsequent still birth. Two days after the operation, oral anticoagulation was initiated without a preceding heparin administration. Six days after the onset of warfarin the patient complained for a strong pain in her right breast and on the next day in the left breast as well. Both breasts were painful, with a red color which quickly developed to a blue-purplish. A few days later a large necrosis was observed in the left breast and the patient underwent mastectomy.
Methods: Postoperative haematological investigation and thrombophilia profile waw ordered.
Results: INR: 3.9, D-dimers: 13.6 μg/ml, AT: 89%, protein C: 91%, protein S (total): 51%, pro C global FV. N.R.: 0.46, factor V Leiden: present (homozygous), prothrombin 20210A mutation: present (heterozygous).
Summary / Conclusion: The patient had multiple genetic factors predisposing to thrombotic events. It is worth noting that her mother who had low protein S levels, was heterozygous for FV Leiden and prothrombin variant 20210A, presented no thromboembolic events and had given birth to four children. Reinstitution of low dose warfarin therapy to our patient was successful, starting under simultaneous cover with LMWH.

B1976

THROMBOSIS PREDICTORS IN SAUDI PATIENTS WITH INFLAMMATORY BOWEL DISEASE: A PRELIMINARY STUDY

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Background: Recent studies report that inflammatory bowel disease (IBD) is associated with an increased risk of vascular complications. The most important of these complications are arterial and venous thromboembolism, which represent a significant cause of morbidity and mortality in IBD patients, yet there is no clear evidence who is at risk to develops thromboembolic event and what are the risk factors. The incidence of thromboembolism in IBD ranges between 1% and 7.7% in clinical studies. Many IBD patients with thromboembolic disorders either have active disease or have undergone recent major abdominal surgery. Most of the studies on thrombosis in the Saudi population were retrospective. The main purpose of this prospective study was to identify main thrombosis predictors in Saudi patients with IBD.
Aims: Recent studies report that inflammatory bowel disease (IBD) is associated with an increased risk of vascular complications. The most important of these complications are arterial and venous thromboembolism, which represent a significant cause of morbidity and mortality in IBD patients, yet there is no clear evidence who is at risk to develops thromboembolic event and what are the risk factors. The incidence of thromboembolism in IBD ranges between 1% and 7.7% in clinical studies. Many IBD patients with thromboembolic disorders either have active disease or have undergone recent major abdominal surgery. Most of the studies on thrombosis in the Saudi population were retrospective. The main purpose of this prospective study was to identify main thrombosis predictors in Saudi patients with IBD.
Methods: This was a prospective, cohort study conducted in tertiary care teaching hospital in Riyadh. Patients with IBD, were invited to participate in this study. Patient recruitment lasted over a year. Study patients had a definitive diagnosis of UC or CD that had been confirmed by radiological, endoscopic, and histological studies. Patients on any medication that might have caused platelet or coagulation abnormalities during the last eight (8) weeks before blood sampling, had Impaired renal or liver functions, had myeproliferative disorders, or diagnosed with cancer were excluded. None of the study participants had any previous thrombotic episode. Appropriate ethical approval was obtained. Baseline characteristics were summarized using descriptive statistics. Categorical variables were examined using the Pearson $\chi 2$ test or the Fisher's Exact test, as appropriate. The designated level of significance was .05.
Results: A total of 100 Saudi patients with IBD were followed up and included in the analysis, 51 were women. Average age was 32 years (min, max), 73 had Crohn's disease, and 27 had ulcerative colitis. No mortality was reported so far. Eight patients (8%) had at least one thrombotic event. Predictors of thrombosis in this sample were mainly family history of deep venous thrombosis (DVT), P=.072 and pulmonary embolism (PE), P=0.05.
Summary / Conclusion: The main predictor of thrombosis in this patient population was family history of Thromboembolic event.

B1977

LIPOPROTEIN(A) AND ISCHEMIC STROKE IN CHILDREN: A SINGLE CENTER EXPERIENCE IN SOUTH AMERICA

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Background: Ischemic stroke is a rare and heterogeneous disease in childhood, with an incidence of approximately 5/100.000 individuals per year and has a significant impact on morbidity and mortality. Clinical presentation and risk factors are not the same as in adults and the most frequently reported risk factors are congenital heart malformations, acquired heart diseases, hemoglobinopathies, collagen vascular diseases, some rare inborn metabolic disorders, trauma, infection and thrombophilia.
Aims: The aim of this study was a retrospective analysis of inherited prothrombotic risk factors in pediatric patients with first ischemic stroke.
Methods: Data from all patients admitted in our service from 2008 to 2012 were identified. Heart diseases, hemolytic anemia, vasculitis, vasculopathies, structural anomalies of the cerebrovascular system and trauma were excluded. Elevated homocysteine, lipoprotein (a) [Lp(a)] and factor VIII; antithrombin, protein C and protein S deficiency; factor V Leiden; factor II G20210A and antiphospholipid antibodies were evaluated.
Results: Ten patients (five girls and five boys), younger than 18 years were

studied. The median age was6,07 years, ranging from 0,6 to 13 years. In seven patients (Table 1) were detected increased levels of Lp(a) [cut-off 30mg/dL, in at least two samples and dyslipidemia was excluded]; one patient had sinusitis associated and three had a family history of ischemic stroke. The clinical presentation was hemiparesis (4 patients) and convulsion (3 patients). One patient was treated with low molecular weight heparin for 6 months and six patients received aspirin in the acute treatment. All patients are using aspirin for secondary stroke prevention and until now no one had stroke recurrence. Two patients present neurological sequelae (hemiparesis and epilepsy).

Tabe 1: Patients characteristics

Patient	Age	Gender	Clinical presentation	Treatment	Lp(a) mg/dL patient	Lp(a) mg/dL parents	Infection	Family history	Sequelae
1	12y11m	F	Hemiparesis and convultion	Aspirin	47 / 41	mother 56 father 33	no	ischemic stoke father's family	no
2	7m	F	Convultion	Aspirin	200 / 91	father 83	no	ischemic stroke mother's family	no
3	5y1m	M	Convultion	Aspirin	163 /269	unknowm	sinusitis bilateral	unknowm	lost following
4	1y7m	F	Convultion	Aspirin	30 / 70	mother 37	no	no	hemiparesis
5	3y6m	M	Hemiparesis	Enoxaparin	90/128	mother 203	no	ischemic stroke mother's family heart attack father's family	no
6	5y1m	M	Hemiparesis	Aspirin	269/137	father 145	no	no	epilepsy
7	13y	M	Hemiparesis	Aspirin	70/78	mother 73	no	no	lost following

Aspirin 1-5mg/kg/d
Enoxaparin 1mg/kg/BD

Summary / Conclusion: Although the association between circulating levels of Lp(a) and stroke is well established in childhood and the measurement of Lp(a) should be included in screening programs in children suffering from arterial ischemic stroke, no uniform approach exists for the treatment and the recommendations in children are extrapolated from adult guidelines.
Further studies are needed to understand the underlying mechanisms involved and thereby improve primary and secondary prevention of childhood stroke.
In our service, we use aspirin as secondary prophylaxis.

B1978

THE INCIDENCE OF VENOUS THROMBOEMBOLISM IN UNSELECTED MYELOMA PATIENTS ATTENDING A TERTIARY REFERRAL CENTRE

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Background: Multiple myeloma is a malignant disorder characterized by clonal proliferation of plasma cells. VTE is one of the leading causes of death in patients with cancer. Patients with haematological malignancies have the highest risk, adjusted for age and sex. Myeloma therapies such as thalidomide and lenalidomide (IMiDs) further increase the risk. There are few dedicated studies in the literature looking at the incidence of VTE in myeloma patients. Almost all published data is extrapolated from treatment studies where they look at the incidence of thrombotic side effects in highly selected populations. Myeloma is a disease of the elderly and in this population, co-morbidities are inherent. Therefore, looking at the rate of VTE in randomised controlled trials designed to evaluate treatment efficacy may not be generalisable.
Aims: To estimate the prevalence of VTE in a 'real world' myeloma population and to assess the risk factors associated with VTE.
Methods: The medical charts of patients with a diagnosis of myeloma attending a tertiary referral centre between Jan 2007 and Dec 2012 were retrospectively reviewed.
Results: 217 patients were identified. The average age was 65±12 years. 62% (n=134) were men. The median follow-up per patient was 35 months (Range 1-231 months).Myeloma subtypes included IgG 53%, IgA 24%, Light Chain 17%, Non secretory 4%, IgD 2% and IgM <1%. The ISS stage was known for 77% - 28% (49/168) were stage I, 49% (82/168) were stage II and 23% (39/168) were stage III. 66% (n=143) received 1-2 lines of therapy, 32% (n=69) received 3-4 and 2% (n=5) received >4. 69% (n=149) received IMiDs and 98%(n=146) of these were administered with either high dose steroids or chemotherapy. 7% had a second malignancy. 12% (n=27) had an episode of VTE (7% PE, 5% DVT). Non-parametric tests were performed to see if there was an association between the use of IMiDs, co-existing malignancy, number of treatment courses and the occurrence of VTE in this population but none were significant.
Summary / Conclusion: The prevalence of VTE in this population was similar to previously published reports. Myeloma patients have many risk factors for VTE but in this population, no one was predictive. As myeloma therapies and outcomes are rapidly changing, a dedicated prospective study is required to evaluate all risk factors to mediate prophylaxis of VTE.

B1979

EXCESS BLOOD CLOTTING FACTOR VIII AND THE RISK OF THE INFRINGEMENT OF CEREBRAL CIRCULATION

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Background: Currently, an important role in the development of disorders of the arterial and venous circulation belongs to thrombophilia. Definite interest thrombophilia is associated with excess content of blood clotting factors. The increasing level of factor VIII in the blood is one of the major risk factors for arterial and venous thrombosis. The increasing level of factor VIII over 150% reflects a 6-fold risk of venous thromboembolism, compared to those where the level of antihemophilic factor is less than 100%. Causes of these disorders have not been fully elucidated. These patients have the likelihood of recurrence of venous thrombosis.
Aims: Determine the clinical significance of increasing factor VIII in patients with cerebral stroke and possible association with other disorders of hemostasis.
Methods: A total of 56 patients, including 46 women and 10 men aged 22 to 50 years, mean age - 30.8 years. Most of women had miscarriage in early pregnancy, as well as the failure of in vitro fertilization, 4 women had a history of acute ischemic stroke, and 1 - arterial thrombosis fingers. In men, the treatment causes were: 1 patient had pulmonary embolism, thrombosis of the femoral artery - 1 patient, repeated acute ischemic stroke - 3 patients, thrombosis of the internal carotid artery of one patient, venous thrombosis of the lower limbs - 4 people. Research methods included the identification of indicators of hemostasis: activated partial thromboplastin time, international normalized ratio, fibrinogen, thrombin time, the level of protein C, protein S, antithrombin III, plasminogen, factor VIII, factor IX, von Willebrand factor, platelet count, determination platelet aggregation under the influence of inducers, such as ADP and collagen. RT-PCR method is determined by genetic mutations of blood coagulation: MTHFR C677T, MTRR Ile22Met, prothrombin G20210A, inhibitor of the plasminogen activator PAI-1, platelet receptor ITGB3 Leu33Pro, fibrinogen 455G/A, FV Leiden Arg506Gln.
Results: Group of patients with a factor VIII level more than 150% (normal value of 50 - 150%) consisted of 24 patients, including 18 women and 6 men. The level of factor VIII in the group was 224.7±4.2% (P <0.05). A group of patients with normal antihemophilic globulin were 32 patients, including 28 women and 4 men. The average content of factor VIII in the comparison group was 104.4%±2.2%. Of the patients examined all seven people, that is 29.2% with acute ischemic by ischemic type, enter a group with a high content of antihemophilic globulin. In the comparison group of violations of cerebral blood flow was observed. In these seven patients, the average level of factor VIII was 231.6%±2.4%. Among other hemostatic disorders in these patients revealed a polymorphism of PAI-1, 5 - homozygous variant, at 1 - heterozygous. 1 patient had a heterozygous polymorphism MTRR Ile22Met, at 1 - MTHFR C677T and heterozygous fibrinogen 455G/A, 2 patients had homozygous polymorphism MTHFR C677T, 1 patient - heterozygous polymorphism ITGB3 Leu33Pro and elevated von Willebrand factor (190%), one patient - a heterozygous mutation in the prothrombin G20210A.
Summary / Conclusion: Thus, the increase in factor VIII in the blood is an additional risk factor for vascular events in the vessels of the brain. Increase in factor VIII is more than 200% to 29.2% increased risk of acute ischemic even in young patients. A combination of excess factor VIII procoagulant with other defects of hemostasis is unfavorable for such patients, in particular, with the polymorphism of PAI-1. Thus, a comprehensive study of the blood coagulation system, including the determination of factor VIII in the blood, is advisable at patients, especially with cerebral accident.

Quality of life, palliative care and ethics

B1980

TO ADMIT OR NOT TO ADMIT HEMATOLOGIC PATIENTS IN THE ICU? THAT'S THE QUESTION!

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Background: The admission of patients with hematological diseases to the Intensive Care Unit (ICU) has been an issue of controversy for many decades. In the 80s the mortality rates of these patients were 80%, but newer studies have shown an improved outcome (mortality of 50-60%). Nevertheless, due to the serious complications that these patients commonly develop and their difficult management, ICU specialists are still reluctant to admit them.
Nowadays, the therapeutic advances improved the long-term prognosis of these patients and it is now urgent to evaluate their predictor outcomes in the ICU, in order to facilitate the challenging decision to admit an hematological patient.
Aims: Characterize the hematological patients (Hp) admitted to a polyvalent ICU, evaluate their mortality and prognostic factors and compare their evolution and predictor outcomes with a paired sample of non-hematologic patients (nHp).
Methods: Observational, retrospective study in Hp admitted in a polyvalent ICU from March 2007 to March 2012. Statistical workup was done using SPSS® v19.0. The group of non-hematological patients (nHp) was selected during the same period and matched to the Hp group by sex, age (±1), Acute Physiology and Chronic Health Evaluation score II (APACHE II) (±5), Simplified Acute Physiology Score (SAPS) (±5) and cause of admission.
Results: During the analyzed period we have treated in our ICU 24 Hp (1,6% of all admissions), median age 65 years, 7 (29%) female. Hematological diagnosis: 9 Myeloproliferative/Myelodisplastic Syndromes, 8 NonHodgkin lymphomas/Multiple Myelomas, 2 Aplastic Anemias, 5 benign disorders. The main reason for admission was respiratory infection (67%). Median APACHE 17±8,9, SAPS 36,3±10,9. Median ICU length of stay 11,5±13,7 for Hp and 9,2±5,9 for nHp. The mortality rate in ICU of the Hp was 45,8% and the nHp 54,2%.
In order to evaluate mortality influencing factors, several predictors of outcome (PO) were tested between both groups (Hp and nHp) using Fischer Test: (APACHE, SAPS, reason for admission, neutrophil and platelet count, required ventilatory, renal and inotropic support, duration of mechanical ventilation, sepsis and transfusional needs), but we only found statistical significance for sepsis (P=0,03) as a predictor of mortality. When analyzing the relative risk (RR) of death, for the different PO, they were similar in both groups and the need for inotropic support had the highest RR (3,4).
Summary / Conclusion: When comparing the Hp to nHp admitted to ICU for the same reason, with similar age and scores (APACHE and SAPS), the Hp had a lower mortality than the matched group of nHp. Selection bias was excluded using T-student test. The hematological characteristics of the Hp (neutropenia, anemia and thrombocytopenia) *per se* did not influenced mortality in our group of patients. The Hp had similar RR of death as the nHp in all the tested PO. This study shows that despite historical bad outcomes, the Hp should have the same admission criteria as nHp.

B1981

METRONOMIC THERAPHY IN VERY OLD PATIENTS : WHEN TO TREAT AT HOME BED IT'S NOT SO BAD.

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Background: Patients 80 years old or older frequently are affected by aggressive B or T lymphomas. Which is the best treatment in these patients is still unclear, because standard treatment are complicated by an higher toxicity and mortality.
Aims: Aim of this study is to verify if metronomic therapy, already used in solid tumours, is not inferior and less toxic than standard chemotherapy in treatment of aggressive lymphomas of very old patients.
Methods: We considered 26 patients from 2009 to 2013. To calculate frailty of patients CHARLSON, CIRS-G, CRASH and GISL score were used.
In group A patients were treated at home with metronomic therapy with cyclophosfamide 50 mg days 1to5, etoposide 50 mg days1-3-5, prednisone 25 mg days 1to7, lenalidomide 10 mg days 1to21, all orally, every 28 days for 9-12 cycles (Large B Cell Lymphoma and Mantle Cell Lymphoma), or with cyclophosphamide 50 mg days 1to3, fludarabine 25 mg days 1to3, etoposide days 4to6, prednisone 25 mg days 1to15, all orally, methotrexate 15 mg im day15, every 28 days for 9-12 cycles (T cell Lymphoma). In group B patients received at hospital i.v. Rituximab 375 mg/sqm day1, Cyclophosphamide 750 mg/sqm day1, adryamicin 50 mg/sqm day1, prednisone 50 mg/sqm orally day 1to5 (Large B Cell Lymphoma, T cell lymphoma and Mantle Cell Lymphoma). In group A M/F:8/8, median age was 85.5 years (R85-94), TNHL/DLBCL/MCL:5/4/1, median IPI 4(R2-5),median follow-up was 6 months(R2-13), 9 patients showed 1 comorbidity (56%), 7 patients 2 or more (44%); CHARLSON>5:12pat(75%),CIRS-G=4:9pat.(56%),CRASH>9: 7pat(43%),GISL FRAIL:12pat.(75%). In group B M/F:4/6, median age was 85 years (R85-91), TNHL/DLBCL:2/8, median IPI 4(R2-5),median follow-up was 6 months(R1-24), 2 patients showed 1 comorbidity (20%), 2 patients 2 or more (20%), 6 patients no comorbidities (60%) CHARLSON>5:4pat(40%),CIRS-G=4:5pat.(50%),CRASH>9:3pat(30%),GISL FRAIL:5pat.(50%). SF8 questionnaire was used to evaluate quality of life of patients.
Results: In group A median hospitalization was 0 weeks (R0-12), complete remission 4 patients (25%), partial remission 8 patients (50%), progression of disease 4 patients (25%), G3/G4 toxicities (hematologic 25%, not hematologic 25%,infection 37%, transfusion 19%,death 37.5%), days of hospitalization/days of global survival 5%(R0-25), cost per month of survival € 5000 (R250-9100), SF8 60 (R40-100). In group B median hospitalization was 9 weeks (R3-17), complete remission 5 patients (50%), partial remission 2 patients (20%), progression of disease 3 patients (30%), G3/G4 toxicities (hematologic 70%, not hematologic 50%,infection 40%, transfusion 80%,death 60%), days of hospitalization/days of global survival 33%(R20-100), cost per month of survival € 21000 (R5000-37000), SF8 40 (R20-50). At Kaplan-Mayer analysis median survival was 18 months for both groups.
Summary / Conclusion: Metronomic therapy is cost-effective and warrants a good quality of life and survival in very old patients.

B1982

IMPROVEMENT OF QUALITY OF LIFE AND INCREASE HEMOGLOBIN CONCENTRATION IN ANEMIC PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS TREATED ERYTHROPOIESIS-STIMULATING AGENTS

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Background: Anemia is a frequent and under-appreciated complication lymphoproliferative disorders (LPD) and antitumor therapy decreasing survival rate and overall quality of life (QoL). Anemia's pathogenesis is based on suppression by proinflammatory cytokines, decreasing erythroid precursor's sensitivity to serum erythropoietin and the myelosuppressive effects of chemotherapy. Therefore erythropoiesis-stimulating agents (ESA) are used as effective pathogenetic treatment of anemia in LPD patients significantly increasing hemoglobin concentration, reducing a number of red blood cell (RBC) transfusions and improving QoL.
Aims: To study the efficacy of ESA, to compare increasing Hb concentration in LPD patients with anemia whom were administrated ESA treatment versus similar patients without ESA (control group), to find out improvement of QoL in group ESA patients.
Methods: We performed this interventional prospective study in LPD patients (n=135) with anemia (Hb 3.9-10 g/dl). Median age of patients was 66 years (range 26-88). ESA (n=88) group included patients with low-grade non-Hodgkin's lymphoma (NHL, n=11), chronic lymphocytic leukemia (CLL, n=21) and multiple myeloma (MM, n=56). A control group (n=47) included similar patients with low-grade NHL (n=7), CLL (n=10) and MM (n=30). ESA was administrated subcutaneously. Epoetin alfa (n=39) on 40.000 IU weekly, Epoetin beta (n=27) on 30.000 IU weekly and Darbepoetin alfa (n=22) 500 µg once per three weeks. All patients had received two or more cycles of antitumor treatment before start of ESA treatment. If Hb concentration was <8.0 g/dl, it was treated by RBC transfusion before ESA administration. The target Hb level was 11-12 g/dl. Positive response was estimated as increasing Hb concentrating ≤2.0 g/dl (without RBC transfusions) or achieving target Hb level during 8-16 weeks of ESA therapy. QoL was assessed using the FACT-Anemia subscale questionnaire.
Results: Mean baseline Hb concentration was similar in both groups ESA and control (8.89±1.20 g/dl and 8.54±1.51 g/dl, respectively, P>0.05). On the whole in the ESA group Hb concentration increased from baseline to 11.18±2.21 g/dl (P<0.001). We observed positive response at 60 patients (68.2%) in the ESA group, their Hb concentration increased from 9.03±1.14 g/dl to 12.24±1.15 g/dl (P<0.001). In non response grope Hb concentration was almost the same (from 8.68±1.26 g/dl to 8.44±1.16 g/dl). Having compared efficacy of different ESA we didn't find out significant statistical difference between of them. Epoetin alfa showed positive response in 26/39 patients (66.7%), Epoetin beta – in 19/27 (70.4%), Darbepoetin alfa – in 15/22 (68.2%). Positive response in the control group was observed at 14/47 patients (29.8%), it's significant less than in ESA group (P<0.05). However Hb concentration increased from 8.54±1.51 g/dl to 10.48±1.19 g/dl in the control group of patients. We compared QoL in ESA patients before and after ESA therapy and observed improvement in the most items but statistically significant improvement (P<0.05) was found out only in the next items: 1) I feel fatigued; 2) I feel weak all over; 3) I feel listless ("washed out"); 4) I feel tired; 5) I have trouble starting things because I am tired; 6) I have trouble finishing things because I am tired; 7) I need sleep during the day; 8) I have been short of breath; 9) I have pain in my chest.
Summary / Conclusion: In this prospective study was shown the efficacy of ESA therapy improving QoL and increasing Hb in LPD patients with anemia.

B1983

DISSOCIATIVE DISORDERS IN STRUCTURE OF PSYCHOGENIC REACTIONS AT THE BLOOD SYSTEM DISEASES PATIENTS

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Background: There are some types of psychogenic reactions at the blood system diseases patients necessary to study for development of the therapeutic techniques directed on correction of these disorders
Aims: Clinical studying of the dissociative disorders revealed at patients with blood system diseases patients, and development of the therapeutic techniques directed on correction of these disorders
Methods: 192 patients with various blood system diseases were studied by the psychopathological method. Dissociative psychogenic reactions were revealed at 86 (44,8%) patients.
Results: 2 types of psychogenic reactions with dissociative symptoms are revealed – anxious-dissociative (n=67 (77,9%) and schizophrenic dissociative (n=19, (22,1%) ones.
Anxious-dissociative reactions:
- Proceed with the phenomena of real blood system disease alienation and signs of the latent somatizing anxiety, being accompanied by abnormal behavior in illness (Barsky A.J. Klerman G.L., 1983). Into the forefront the consciousness fragmentariness (dissociative disorder of identity (DSM-IV-R) acts. Alienation of blood system disease signs is associated with significant depersonalization and derealization. The events occurring in a hospital connected with diagnostics and treatment of blood system disease are perceived as though from outside. Patients categorically deny any fears connected with illness outcome. Thus the dissociation covers basically cognitive aspect of reaction («cognitive dissociation»). There are doubts concerning existence of blood system disease, correctness of the diagnosis, statement of absolute recovery, ignoring of the somatic status deterioration symptoms, or their interpretation as manifestation of less serious illness. - persons with shizoid and anancastic personality traits prevail. Dissociative schizophrenic reactions: - the phenomenon of blood system disease alienation, a complete negation of the fact of a disease dominates, patients completely refuse further care, motivating it with that they are almost healthy and don't need the specialized help. As a rule, such patients repeatedly ask for the help only at emergence of the complications which are directly menacing to life. - persons with schizotypal disorder (DSM-IV) (n=15) or with the personality changes at juvenile one catadrome schizophrenia (n=4) prevail. The basic principles of the revealed mental disorders therapy were: - empirical selection of medications taking into account specific features - prescription of psychotropic drugs according to their main indications on the basis of the clinical picture analysis of psychopathological disorders - choice of psychotropic drugs on the basis of an assessment of risk/advantage proportion with taking into account the tolerance and safety profile, and also potential medicinal interactions. Efficiency of psychopharmacotherapy with of drugs standard dosages using depended on the type of psychogenic reaction. At the anxious-dissociative disorders the quantity of responders reached 76,1%, and at the schizophrenic dissociative reactions – 68,4%.
Summary / Conclusion: Dissociative reactions at patients with blood system diseases are rather frequent and accompanied considerable resistance to psychopharmacotherapy

B1984

HEALTH-RELATED QUALITY OF LIFE AND PRODUCTIVITY IMPACT OF MYELODYSPLASTIC SYNDROMES (MDS): THE PATIENT PERSPECTIVE

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Background: One of the most important clinical features of myelodysplastic syndromes (MDS) is the presence of chronic anemia which is directly related to fatigue. Patients' experiences of fatigue associated with chronic anemia are well-documented in the literature; however, consensus on measurement of fatigue is lacking.
Aims: To perform a qualitative interview study to understand the effect that fatigue has on the health-related quality of life (HRQL) of MDS patients, and the economic burden of the disease.
Methods: Following a targeted review of the existing literature, 60-minute face-to-face in-depth qualitative interviews were conducted with MDS patients (n=17) in the US with a clinically confirmed diagnosis according to the World Health Organization (WHO) 2008 classification Interviewer questions were open-ended and focused on patients' HRQL and indirect costs associated with the disease. The 12-item Short-Form Health Survey (SF-12v2®) was administered after the interview to gain further insight into the impact on patients' HRQL. Qualitative data were analyzed using a grounded theory approach that combined semi-quantitative and qualitative methods.
Results: The mean age of patients interviewed was 70.8 years (range: 42.2 to 85.5 years); 59% of patients were male. The median time from diagnosis was 3.8 years; (range: 0.2 to 8.7 years). The sample included patients of each WHO 2008 classification subtype; the most common diagnosis was refractory cytopenia with multilineage dysplasia (47%). Based on the International Prognostic Scoring System (IPSS), 59% and 41% of patients were diagnosed with low and high risk MDS, respectively, with 76% of patients currently taking treatment for their MDS. Current treatments included erythroid growth factor support (53%), hypomethylating agents (18%), and lenalidomide (6%). One patient reported transfusion-dependence (≥2 transfusions in the past eight weeks) – however the number of patients receiving occasional transfusions was not recorded. The most frequently reported comorbidities included diabetes (53%), high blood pressure (35%), and cancer (24%). This qualitative study found that MDS is associated with a considerable financial impact to patients. Based on the qualitative interviews with patients, the indirect financial costs reported (i.e., decreased work activities) were equally as burdensome to patients as their direct medical cost for treatment. Seven patients (41%) discussed the impact of MDS on their ability to work, either as a result of fatigue or their constant need for treatment. Patients reported being unable to complete work activities, taking time out of work for medical appointments, and reduced productivity during working hours. Patients also reported financial impacts associated with travel to medical appointments (n=11, 65%), and caregiver assistance with daily activities that patients had difficulty completing as a result of their disease (n=5, 29%). A total of 30 HRQL concepts within 11 domains (Table 1) were spontaneously reported by patients during the interviews, with the largest HRQL impairment being physical activity (n=16, 94%) directly related to patients' fatigue. These results are consistent with findings from the SF-12v2® questionnaire, on which patients scored a mean Physical Functioning domain score of 37.5 (SD 30.28) on a scale from 0-100 where a higher score indicates a better health status. The national norm for the US is a mean of 50.0 with a standard deviation of 10 for the Physical Functioning domain score. MDS patients also frequently reported the social (n=12, 71%), sleep (n=11, 65%), functional (n=10, 59%), and emotional (n=9, 53%) impacts of the disease.

Table 1. Number of patients who reported impacts in HRQL domains during qualitative interviews

HRQL domain	n (%) N=17
Physical	16 (94)
Social	12 (71)
Sleep	11 (65)
Functional	10 (59)
Emotional	9 (53)
Cognitive	7 (41)
Activities of daily living	7 (41)
Consumption of time	4 (24)
Ability to travel	4 (24)
Leisure activities	3 (18)
Self-care	2 (12)

Summary / Conclusion: These qualitative findings provide additional insight into the HRQL and financial impact of MDS which has been considered yet not fully articulated in the literature for these patients. Specifically, in addition to decline in HRQL, fatigue and the constant need for treatment in MDS appear to impact patients' work activities as well as their productivity within society.

B1985

AUDIT MORTALITY WITHIN 30 DAYS OF CHEMOTHERAPY IN A DISTRICT GENERAL HOSPITAL IN UK

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Background: Over the last three decades, there has been a large increase in both the number of chemotherapy. Over the last three decades, there has been a large increase in both the number of chemotherapy treatments available for patients with haematological malignancy and its usage. There is clear evidence of the beneficial effects of these treatments. There is also considerable experience and in grading and managing toxicities associated with these treatments. In UK there are no national benchmark figures for early mortality due to chemotherapy unlike for surgical interventions. NCEPOD (National Confidential Enquiry into Patient outcome and death) published a document in 2008 which has reviewed the care of patients who died within 30 days of receiving systemic anti-cancer therapy. The document recommended that all deaths within 30 days of chemotherapy should be considered at morbidity and mortality or clinical governance meetings.

Aims: The aim of the study was to monitor all deaths within 30 days of chemotherapy in patients with haematological malignancies at the Countess of Chester Hospital, a district general hospital in Chester, UK covering population of 250,000 residents.
Methods: Databases were searched for all deaths within 30 days of chemotherapy in those patients who received one or more cycles of chemotherapy between Jan & Dec 2012. Information regarding treatment intention-curative or palliative-, cause of death, number of previous treatment was collected from patients' case notes and Somerset cancer register (national database application which contain clinical cancer data).
Results: Between January2012 and December 212, 17 deaths occurred within 30 days of chemotherapy. Of these, 9 deaths (53%) were due to disease progression. Eight (47%) deaths were related to chemotherapy, of which 5 (29%) were due to neutropenic sepsis. 11 0f the 17 deaths occurred in patients who were receiving potentially curative chemotherapy. Performance status prior to chemotherapy was not documented anywhere in the notes in 8 (47%) of patients.
Summary / Conclusion: The authors believe regular collection of this data including accurate recording of performance status may add weight to the decision-making process. We feel this is an audit system that could be used for haemo-oncology centres nationally. We practice this form of audit yearly and feedback to the treating teams so that deaths are discussed and practice monitored.

B1986
QUALITY OF LIFE AND DISEASE UNDERSTANDING: IMPACT OF ATTENDING A PATIENT CENTERED CANCER SYMPOSIUM
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Background: Various instruments have been developed to measure quality of life (QOL) among cancer patients. Components of QOL assessment include symptoms (e.g., fatigue), physical function, social interactions and financial burden. To date no studies have evaluated the effect of a cancer symposium as an educational intervention to improve quality of life among cancer patients.
Aims: The aim of this study is to evaluate the impact of a patient centered cancer symposium on a broad population of cancer patients.
Methods: Surveys were distributed to attendees of the Mayo Clinic "Living with Cancer" patient symposium in January 2013. Surveys items included the EORTC QLQ-C30, questions regarding disease comprehension (response options of not at all, a little, quite a bit, and very much), and Distress Thermometer. Attendees also completed validated disease-specific questionnaires based on cancer type. These questionnaires included the MPN-SAF, EORTC disease-specific modules, and FACT disease-specific scales.
Results: 158 patients completed the pre-convention survey. There was equal gender representation among participants (51%F). Median age was 67.5 years (range 30-86). Disease type included 37% hematologic malignancies, 23% breast cancer, 23% prostate cancer, and 17% other. The large majority of respondents were greater than 1 year from cancer diagnosis (76%) with 40% greater than 3 years from diagnosis. Most respondents endorsed understanding their disease quite a bit (54%) or very much (29%). Most respondents reported disease comprehension of "quite a bit" or greater in regards to treatment options (84%), screening modalities (83%), disease symptoms (74%), and cancer-related side effects (71%). The lowest percentage of understanding ("quite a bit"/"very much") was reported for legal issues of disease and treatment (27%), managing disease related fatigue (41%), risk factors of disease (49%), and managing disease related stress (49%). Subjects with heme malignancies (N=58) had significantly less understanding of risk factors (31% vs 60% in nonheme, Jonckheere-Terpstra [JT] P<0.001) and treatment options (78% vs 89% in nonheme, JT P=0.03) with a trend for less understanding of treatment side effects (66% vs 75% in nonheme, JT P=0.07). 158 respondents completed the QLQ-C30 with mean scores of 84.4 (SD=16.6) for physical functioning, 83 (SD=22.3) for role functioning, 80.7 (SD=22.9) for social functioning, and 72.7 (SD=20.2) for global health status. The three symptoms with highest mean scores were insomnia (mean=35.5, SD=32.4), fatigue (mean=29.3, SD=22.4), and pain (mean=22.7, SD=24.7). Mean distress was 3.8 (SD=2.8) with respondents identifying a median of 5 problems. Subjects with heme malignancies had statistically significantly lower physical functioning (mean 80.6 vs 86.7 in nonheme, t-test P=0.02) but did not significantly differ on any other QLQ-C30 scale. Mean distress among subjects with heme malignancies was 3.5 (SD=2.6) with respondents identifying a median of 6 problems neither being statistically different from nonheme subjects.
Summary / Conclusion: Cancer patients had high baseline disease understanding and appeared motivated for an educational teaching program via a patient centered cancer symposium. However, subjects with heme malignancies reported less understanding of risk factors, treatment options, and treatment side effects. Heme respondents also reported worse physical functioning than nonheme respondents. Further results of cancer knowledge and QOL following the symposium are being assessed.

B1987
THE FACTORS OF KETAMINE THAT AFFECT SEDATION IN CHILDREN WITH HEMATOLOGY/ONCOLOGY PROCEDURES: PARENT SATISFACTION PERSPECTIVE
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Background: The pain and its complication during sedation with ketamine remains a significant problem for children with hematologic malignancy.
Aims: The purpose of this study is to further evaluate the parental satisfaction for procedural sedation and analgesia during pediatric hematology/oncology procedures performed by pediatric hematologist in the department of pediatrics, Phramongkutklao hospital.
Methods: A prospective audit of intravenous ketamine use in department of pediatrics requiring invasive procedures. Children received intravenous of ketamine 1 mg/kg. The informed consents and assents were obtained .The procedure was assessed by way of a physician completed form and by evaluation of questionnaires given to parents to estimate levels of pain by using a 0 to 10 mm Visual Analog Scale (VAS).
Results: A total of 46 children aged 6 months to 15 years were observed at pediatric unit for a total of 46 procedures. The indications for procedural sedation and analgesia included lumbar puncture and intrathecal chemotherapy (50%), bone marrow aspiration or biopsy (21.7%), and both procedures (28.3 %). The mean VAS scale during oncology procedures were 3.39±2.39 which was expressed by all the parents/guardians of the children treated. Adverse effects were observed in all children include hallucination (39.1%),nausea (30.4%),hypersalivation (26.1%), vomiting (21.7%)No child required admission to hospital and there were no serious complications.
Summary / Conclusion: Intravenous ketamine 1 mg/kg is effective for invasive procedures in children with malignancy. The use of intravenous ketamine may produce psychedelic effects in children. These adverse effects may alter the child's comfort and parental satisfaction especially in the age group which are younger than 10 years old**.**

B1988
IMPACT OF HYPOMETHYLATING AGENTS ON THE BLOOD PRESSURE OF THE PATIENT WITH HEMATOLOGIC MALIGNANCIES: SAFETY AND RECOMMENDATIONS FOR USE.
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Background: DNA hypomethylating agents are currently important drug in the therapeutic approach from myelodysplastic syndromeand myeloblastic leukemias. Alikhani-Koopaei (j. Clin. Invest. / 2004) and S. Friso(Atherosclerosis 2008) demonstrated the relationship of epigenetic in arterial hypertension (AH) and the effect of agents such as 5-azacytidine as regulatory hypomethylation of the gene of 11-beta-hydroxysteroid dehydrogenase, involved in AH epigenetic control.
Aims: Determine the relationship of blood pressure with hypomethylating agents and assess the security and management of hypotension in patients with hematologic malignancy treated with azacitidine. We consider hypotension a decrease of 20 mmHg with respect to your baseline blood pressure.
Results: We have reviewed 109 treatment cycles of azacitidine of 26 patients (14 men, 12 women) with a median age of 74.5 years (range 39-83) treated by hematological malignancies (16 AML, MDS-10).50% of the patients receiving treatment for chronic hypertension. In our Center 18 of the 26 patients (69.23%) presented an episode of hypotension, but stratifying by groups, in the 46 first cycles of azacitidine is have found an incidence of 73.9 %(N=34) of arterial hypotension. After the high rate of adverse effects, we customize our Protocol, by removing antihypertensive in the week receiving azacitidine, decreasing the total incidence of 73.9% to 30.3% (P<0.001) respectively.
Summary / Conclusion: Our data confirm the role of hypomethylation of DNA in the epigenetics of hypertension described by Alikhani-Koopaei (j. Clin. Invest. / 2004) and S. Friso (Atherosclerosis 2008). Azacitidine, as agent hipometilante, presents a synergistic effect with drugs used in the treatment of high blood pressure. After monitoring and surveillance at our Center, we suggest blood pressure control all patients treated with agents hipometilantes and avoid the concomitant use of antihypertensive /azacitidina in the week of treatment, improving security, tolerance and avoiding adverse effects potentially serious.

B1989

SECOND PRIMARY MALIGNANCY IN HEMATOLOGY PATIENTS IS A RARE ENTITY?

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Background: The reported incidence of multiple primary cancer (MPC) is rare, and it is even less common to observe hematological malignancy with solid tumor. The etiologic implicating factors are a first primary tumor itself, genetic predisposition, environment exposures, chemotherapy(CT), radiotherapy(RT), hormonotherapy(HRT), immunosuppresion and also a better overall and prolonged survival achieved due to improved treatment.
Aims: To evaluate occurrence of second primary malignancy (SPM) in hematology patients.
Methods: We retrospectively studied the files of 563 (307men, 256 women)with median age 62years (43-81) and a male to female ratio 1.7:1 who were consecutively admitted in our unit.Between January 2010 and January 2013, 8(1.42%)patients developed metachronous second primary malignancy(SPM), 5(1.62%)men and 3(1.17%)women .
Results: The median interval time to the diagnosis of the SPM was 94 months (14-216). Half of the SPM occurred between 14 and 33months while the others were detected after a 5-year interval. 5/8 cases were associated with a first hematologic malignancy, and 3/8 with solid tumor. These cases included myeloproliferativesyndrome/ polycythaemia vera with lung cancer, chronic lymphocytic leukemia with lung cancer,myelodysplastic syndrome/acute myeloid leukemia with lung cancer , chronic myelomonocytic leukemia with lung cancer, lung cancer with myelodysplastic syndrome, prostate cancer with myelodysplastic syndrome, melanoma with gastric MALT lymphoma, B- chronic lymphocytic leukaemia with breast cancer.5/8 cases had diagnosis lung cancer as primary or second malignancy. 5/8 pts had treatment for the first malignancy which included CT, only Hydreoxyurea (HU),RT, surgery, HRT and 3/8 did not receive any treatment. 3/5 pts with lung cancer were heavy smokers.
Summary / Conclusion: The risk of developing SPM among hematology pts surviving>5 years was low in our series, considering the prevalence of MPC varying between 0.73% and 11.7% Based on small numbers, an unexpected presence of solid tumors was reported.Lung cancer was the commonest site of metachronus tumour. Our findings support a role for a shared susceptibility(genetic, enviroment) that predisposes to certain solid tumors.We did not find prevalence of lymphoid or myeloid malignancies as second tumor in contrast to the references.The administration of chemotherapy and or irradiation did not seem to increase the risk.

B1990

ROMANIAN PEOPLES OPINION ABOUT THE UTILITY OF CHEMOTHERAPY IN ACUTE LEUKEMIC PATIENTS

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Background: It is useful to regularly test the opinion of Romanian people according to the various issues raised by medical practice. The problems about the management of leukemic patients are among them. Their responses can provide starting points for improving it.
Aims: We aimed to study the opinion of Romanian inhabitants about the issues related of acute leukemia chemotherapy.
Methods: A transversal study was performed on a sample of 221 Romanian inhabitants: 62 consecutive hospitalized patients in January 2013 and all those 159 subjects who responded to a questionnaire on the management of acute leukemic patients posted on Internet. The results were analyzed and they have allowed conclusions that we hope to have implications for clinical practice.
Results: The mean age of surveyed patients was 40.64+/-19.31 years. Distribution by gender: women 58.37%, men 41.63%. Most responders (78.13%) agreed with chemotherapy in acute leukemic patients. 88.69% of them agreed to idea that all chemotherapeutics for leukemic patients must be present in hospitals and their treatment must be free of charge. Unfortunately, essential drugs for their treatment are missing in Romania in the last months. If the responders were in their situation, almost half of them could not buy them and 33.94% of them would not know what to do. Only 47.06% felt that they could buy them from other countries and 32.58% had no idea how to buy them. More than half of them didn't agree to the idea that chemotherapeutics could be replaced by herbal medicine and 33.03% had no idea about this. The vast majority of those surveyed (80.54%) felt that chemotherapy delay may worsen leukemic patient outcome. Only 27.15% didn't agree to the idea that resveratrol could replace chemotherapeutics and 65.16% didn't know this drug. Most (77.38%) feel that the government is responsible for the absence of chemotherapeutics in hospitals. If they have a leukemic child 71.94% of them would go abroad to treat him. 85.07% of them, would agree to donate blood for these patients, even if they could not receive chemotherapy, as palliative treatment. To avoid the absence of chemotherapeutics in the future, 52.49% of them felt that these drugs should be made in Romania and only 17.19% agreed with their import. Most (71.04%) were optimistic: they felt that public opinion could influence the solving of chemotherapeutics absence, but only 34.84% of them agreed to the idea that the popularization of this problem on Internet could sensitize authorities to solve the vital drug absence; 35.29% weren't sure, 15.38% didn't know, and 14.48% didn't agree to this idea.
Summary / Conclusion: Most people are aware of the importance of chemotherapy made in time for effective treatment of leukemic patients, and agree to buy missing chemotherapy drugs or go abroad for their child treatment. They agree, as blood donors, to participate voluntarily in helping them. Most responders were optimistic regarding the influence of public opinion on solving the chemotherapeutics absence but only third of them felt that Internet is the right place to discuss and solve this problem.

B1991

PALLIATIVE CARE FOR CHILDHOOD CANCER IN COUNTRIES WITH LIMITED HEATH RESOURCES: CURRENT CHALLENGES

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Background: Cancer patients suffering from a terminal illness require special care and support. There is deficiencies in the current practice guidelines and lack of quality assurance in the provision of these services. The main target in that group focus on symptoms management, psychological rehabilitation.
Aims: The study aimed to plan a standard of care in our center aiming at assessment and management of all symptoms encountered in children with terminal childhood cancer, to provide maximum comfort to patients and families, to compare with the previous service provided over the preceding period and to highlight the deficiencies in the current provision of care.
Methods: The study was conducted prospectively (Group A=20 patients) on terminal cancer patients recruited from Oncology Unit - Pediatric Hospital- Ain-Shams University in the period from JAN2009 through JAN 2011.This group was compared to (Group B= 13 patients),studied retrospectively from patients hospital records over the previous 2 years prior to study . They were enrolled based on less than 10% predicted overall survival in international results and/or progressive, refractory disease or relapse with standard of care protocol and second line salvage protocol. Parental consents were obtained. Patients were subjected to proper assessment of type of malignancy, detailed review of all given therapies and their response thorough clinical examination and system review. Pediatric palliative care overview included anticancer therapy and symptoms management. Integral assessment for all symptoms (gastrointestinal, respiratory, neurological, pain, anorexia/cachexia), together with psychiatric evaluation of parents and patients were carried in three phases: initial screening, assessment and interventions and reassessment
Results: The frequency of various symptoms were pain in 80% of patients, GI in 65% , anorexia / cachexia (50%) , Cancer related fatigue (35%), neurological (15%) and respiratory (40%); the duration of patients on palliative care was 12.37±9.807 months in group A compared to group B(4.250 ±2.527 months)(P<0.001). Marked symptoms focus improvement was detected in group A after implementation of the program. In group A, 62.5% were given analgesia around-the-clock (ATC) and 37.5% were given analgesia PRN. All patients in group B were given analgesia PRN. Depression and anxiety was present in6, 10 (30, 50 %) of group A patients before treatment which dropped to2,2(10, 10%) respectively after treatment. Group B was not subjected to any psychological rehabilitation. Ten patients had weight loss, and 10 had more than 10% weight loss in group A , after rehabilitation all 20 patients had normalized weight.
Summary / Conclusion: Timely and planned management of pain and other symptoms is effective in relieving patients and parents distress and improving their quality of life.

Health economics

B1992

THE COST OF PRODUCING A UNIT OF BLOOD IN GREECE: AN ECONOMIC ANALYSIS FOR THE CASE OF "AGIOS SAVVAS" REGIONAL CANCER HOSPITAL

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Background: The economic burden of blood collection and processing is substantial and there are many studies which have provided useful insights and estimates in this field in several different healthcare settings across the world.
Aims: Given that healthcare resources are scare, especially in the presence of the current crisis in Greece, an economic analysis was undertaken to determine the mean cost of "producing" a unit of blood from a hospital and societal perspective. This abstract describes the preliminary findings of a project which attempts to estimate the cost of a blood unit across all the public hospitals nationwide.
Methods: In accordance with official guidelines, an activity based costing model with five steps was adopted, to produce more accurate results than conventional approaches. In this light, the cost of blood "production" from a hospital perspective accounted for a) the cost of collection, b) the cost of processing, c) the cost of laboratory testing, d) storage cost, and e) the cost for elimination of a blood unit if a component fails for any reason. In addition, from a societal perspective, indirect cost comprises the donor's productivity loss, assuming that each individual expends half of a working day per unit donated. The cost associated with donor recruitment, pre-transfusion preparation and administration, follow up due to adverse events or other relevant resources consumed in the long-term were not taken into consideration. It must be noted that in Greece there are not separate blood bank centers, which may operate as a supplier for local hospitals, and thus, the storage cost was estimated specifically for "Agios Savvas" hospital. As an allocation parameter for distribution of institutional overhead costs we used the square meters (m^2). The calculation of the medical equipment's depreciation was assumed to be fifteen years, with a discount rate at 3.5%. Main input parameters of the model include personnel cost, reagent costs, equipment related cost, percentage of wastage of blood units, institutional overheads, yearly blood production, the cost of Nuclear Acid Tests (NAT), other exams and relevant consumables. Data were collected through a structured questionnaire filled with data obtained by the blood centre of "Agios Savvas" Regional Cancer Hospital, the database of National Blood Centre of Greece, the National School of Public Health and the Ministry of Health. All data referred to the year 2012.
Results: The mean cost for "producing" a blood unit from a hospital perspective was estimated at €224.70. In particular, the cost of personnel was estimated at €99.18, the cost of institutional overheads at €8.52, the cost of consumables at €3.34, the cost of computerization at €0.80, the cost of blood bag (different types) at €28.30, the cost of examinations at €47.29, the cost of NAT at €37, the cost of blood distruction at €0.19 and the cost of equipment at €0.08. The indirect cost per blood unit was estimated at €31.67. Hence, the total societal cost per blood unit was estimated at €256.37.
Summary / Conclusion: The preliminary findings of this study represent an update of previous estimates, as well as an advance in the methodology used for the determination of the cost of blood unit in Greece. It must be mentioned that several cost elements which are associated with the cost of transfusion are missing from the present analysis and their consideration will certainly lead to higher estimates. A common issue in studies which use similar methodologies relates with the fact that the estimation of blood cost was based only at the Red Blood Cells production, without taking into consideration the rest of blood products, such as the Fresh Frozen Plasma or the platelets.

B1993

UNDERSTANDING PATTERNS OF ORDERING COMMON BLOOD TESTS IN PATIENTS ADMITTED TO HOSPITAL – AN AUDIT TO EVALUATE NUMBER AND FREQUENCY OF COMMON BLOOD TESTS IN ADMITTED PATIENTS IN A MEDIUM SIZED DGH

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Background: Blood tests play a very important part in the diagnosis and management of most diseases and pathology costs form significant proportion of the health service budget. The Independent Review of Pathology Services in the National Health Service UK by Lord Carter postulated that savings of £500 million annually are possible by reducing wastage.
In cost reduction strategies most savings are predicated on economies of scale and service redesign, creating larger pathology units with Hub and Spoke models to reduce waste and improve efficiency by up-scaling automation and down-scaling staffing levels etc.
However, inappropriate and unnecessary blood tests also contribute significantly to waste but there is a dearth of literature or guidance on this subject.
Aims: The aim was to evaluate number, frequency and appropriateness of common blood tests in adult patients admitted to the hospital in order to assess for possible wastage
Methods: In our study we conducted a retrospective audit on approximately four hundred patients admitted to a medium sized District General Hospital. Obstetric and paediatric patients were excluded from this study. The remaining 309 patients were evaluated using the electronic patient records. . The purpose was to study the number and frequency of common blood tests e.g. Full Blood Count, Renal and Liver blood tests. The data on Length of stay was also evaluated. The cohort was subdivided into following subgroups for data analysis: Medicine, Care of the Elderly, Orthopaedics & Surgery.
Results: Our results showed that over 50% of admitted patients were medical patients who also had a longer average length of stay than the surgical cohort. Consequently, average number of tests per patient was higher in medical patients but frequency of repeating blood tests appeared broadly similar in medical and surgical groups. The repeat ordering of blood tests did not appear to be influenced by abnormal results as similar frequency of repeat blood tests was noted in patients with normal and abnormal results.
The repeat ordering appeared to follow a recurring pattern rather than being reactive to the results of the previous test. There was significant wastage as blood tests were frequently repeated even when recent results were within normal range. On the contrary in some patients repeat tests were not ordered despite abnormal results. It was also interesting to note that there was no significant difference in repeat ordering of blood tests between cases showing severe abnormalities of Full Blood Count and those with milder abnormalities.
Summary / Conclusion: Although our audit had a number of limitations, it highlights the need for further studies to evaluate the pattern of ordering common blood tests in hospitalised patients with a view to identify wastage and minimise it.
There is also need for developing evidence-based consensus on how to reduce unnecessary testing without compromising patient safety. Published literature on this subject is limited and much innovative research is possible.

B1994

WORTHINESS OF BONE MARROW TRANSPLANTATION AS A TREATMENT OPTION FOR THALASSEMIA PATIENTS IN DEVELOPING COUNTRIES

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Background: Thalassemia is a serious prevalent chronic genetic disorder worldwide, with carrier rates reaching 9% in some countries as Albania and Egypt. The long life course of thalassemia causes continuous suffering for the patients, parents and community. The problem aggravates more in developing countries where there are limited financial and human resources capable to cope with increasing numbers of patients and their needs. Treatment of thalassemia is of two types. The first is long life course treatment in the form transfusion and chelator administration. The other is the conclusive only cure for the disease, which is Stem cell transplantation, from which the most recommended type is bone marrow transplantation.
Aims: We aimed to study thalassemia in Albania and Egypt, as an example of developing countries with different circumstances.
Methods: Data collection and calculations for treatment costs in Albania and Egypt.
Results: We found that long life treatments of patients can reach 25, 400 and 12,300 euros in each country respectively. In Albania, there are no enough treatment facilities for the long life demanding patients, most importantly, lack of adequate blood and treatment costs that approaches European standards. Similar situation is present in Egypt where there is no enough resources to overcome the annually increasing number of patients. Moreover, and due to high carrier rate with lack of preventive program, increase of disease is found to be uncontrollable as well. Accordingly, curing thalssemia in young age by bone marrow transplantation; as a once per life treatment, can be the solution for the long life health and financial suffering for the disease.
Summary / Conclusion: Curing thalassemia in early age with - once in life - bone marrow transplantation can be the solution to avoid progressing health and financial problems.

B1995

SURVIVAL AND TREATMENT RELATED DEATHS IN PEDIATRIC ONCOLOGY PATIENTS: A SINGLE CENTER RETROSPECTIVE COHORT STUDY

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Background: Improved survival for pediatric cancer patients is one of the great success stories of the late 20th century.
Aims: To estimate survival, event free survival (EFS) and causes of treatment related deaths in childhood malignancies.
Methods: A retrospective review of patients' data from Hematology Oncology

Clinc, Children's hospital, Ain Shams University in the period from 1999 to 2008.
Results: 506 patients were included. The 5-year survival rate of the studied childhood malignancy was 49%. Patients were divided into; leukemia group (307 patients): 71% ALL, 22.5% AML, 0.2% biphenotypic and 4.3% chronic leukemias. The 5-year survival rate of these groups was 55, 23, 33 and 14% respectively, while; the 5-year EFS was 48.1, 25.2, 31 and 18% respectively. Lymphoma group (85 patients) included; Hodgkin disease (41%) and non Hodgkin lymphoma (59%), their 5-year survival rate was 81 and 57% respectively, and the 5-year EFS was 78 and 90% respectively. The solid tumors group (114 patients), comprised 43% with neuroblastoma and 27.2% with wilms' tumor, their 5-year survival was 19 and 60% respectively, and the 5-year EFS was 66 and 71% respectively. Infection was the most common cause of treatment related death representing 25.3% in leukemia patients, 30.7% in lymphoma patients, and 20.4% in solid tumor patients. Deaths related to progressive disease ranged from 25 to 45%. The commonest complication of chemotherapy was infection requiring hospital admission in 95% of leukemia patients, 82% of lymphoma patients and 85% of solid tumor patients. The commonest infections were febrile neutropenia and bronchopneumonia.
Summary / Conclusion: The highest survival rate was in patients with Hodgkin disease followed by those with wilms' tumor, while the lowest survival rate was for chronic leukemia followed by neuroblastoma and AML. More attention should be given to supportive care and infection control for oncology patients.

B1996
MORBIDITY RATE OF MALIGNANT NEOPLASMS OF LYMPHOID AND HEMATOPOIETIC TISSUE IN SAINT PETERSBURG
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Background: The analysis if the indexes of morbidity rate of malignant neoplasms of lymphoid and hematopoietic tissue in certain regions allows detecting of disease risk factors, forecasting morbidity level, planning hematological aid, evaluating its quality and detecting organization gaps.
Aims: To determine morbidity of malignant neoplasms of lymphoid and hematopoietic tissue in St.Petersburg in 2000-2010 y.
Methods: The research of morbidity rate indexes was performed for the period of 2000 – 2010, based on the state statistic account form No. 7 "Data of the diseased with malignant neoplasms", with involvement of the data from population cancer registry of Saint Petersburg and of Federal State Statistics Service about number, basic demographic trends of the population and morbidity rate indexes in Russia and Saint Petersburg.
Results: In the period of 2000 – 2010 the number of the newly diagnosed diseased with malignant neoplasms of lymphoid and hematopoietic tissue increased on 39%, while the number of the newly diagnosed diseased with all malignant neoplasms increased in Saint Petersburg on6,6% (and 15,2% in Russia). It provides evidence of high rates of malignant neoplasms of lymphoid and hematopoietic tissue morbidity rate increase in Saint Petersburg. The morbidity level among men exceeded the morbidity level among women. In the morbidity rate structure the lymphomas head the list: the standardized ratio of non-Hodgkin's lymphomas in 2010 was equal to 4.64 per 100 000 of population, of Hodgkin's lymphomas – 2.31. Next to it chronic lymphocytic leukemia ranked (2.23 per 100 000), the next was multiple myeloma and immunoproliferative diseases (2.14 per 100 000 of population), acute lymphocytic leukemia (1.48 per 100 000 of population), chronic myeloid leukemia (1.35 per 100 000), acute myeloid leukemia (1.17 per 100 000). The morbidity rate increase in the last 10 years took place due to the morbidity rate increase of non-Hodgkin's lymphomas (from 3.65 to 4.64 per 100 000), multiple myeloma (from 1.48 to 2.14 per 100 000), acute myeloid leukemia (from 0.83 to 1.17 per 100 000) and chronic myeloid leukemia (from 1.26 to 1.35 per 100 000 of population).
Summary / Conclusion: High levels of malignant neoplasms of lymphoid and hematopoietic tissue morbidity rate in Saint Petersburg result from a variety of causes, the main of which are: maintaining of the trend of senior and old people in the city population structure, ecological factors typical for large industrial cities, climate conditions that exacerbate the effect of the ecological factors. The morbidity rate indexes level is influenced also by the quality of primary diagnostics and optimality of the registration and account of the detected diseases. The quality is provided in Saint Petersburg by operation of the population cancer registry.

B1997
GOOD SURVIVAL RATES IN ELDERLY PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA COME AT A PRICE
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Background: Cases of lymphoma are on the rise in view of our ageing population. Haemato-oncologists are often faced with the dilemma of how aggressive one should be when treating very elderly patients hereby defined as >70 years, since very often similar patients have multiple comorbidities and relatively poor performance status.
Aims: The aims of this retrospective review were to define the survival rate and patient characteristics including costs of care of an unselected group of patients who were over 70 years old when diagnosed with Diffuse Large B-Cell Lymphoma (DLBCL) from January 2010 to February 2013 in Malta.
Methods: This was a case note review of 19 consecutive patients over 70years old with DLBCL including those with transformed follicular NHL. We looked at patient characteristics such as stage, gender, treatment regimes, ECOG performance status, costs of care.
Results: Mean age was 78.4 years (range 72-89years). 68.4% were female with an average ECOG performance status of 2. The mean Stage of the DLBCL was 2 with elevated LDH at 392. The eGFR was as expected at similar ages with a mean of 75 and mean albumin normal at 40.
All but 2 patients were treated with standard infusional chemotherapy (mainly reduced doses of Rituximab, Cyclophosphamide, Adriamycin, Vincristine and prednisolone or dexamethasone (R-CHOP). In patients with pre-existing cardiac problems the anthracycline was substituted with Etoposide (R-CEOP). The other 2 patients received radiotherapy only since one had a very poor performance status and the other only had minimal disease in one groin (Stage 1 A) and multiple co-morbidities including cardiac arrhythmias and severe ischaemic heart disease. 5 patients died with a mean survival of 354.2 days (24-830). 3 patients died in remission of conditions unrelated to the DLBCL and 2 died of complications of chemotherapy. 73.7% are alive with a mean follow-up time of 377 days. Only 14% of bone marrow biopsies were positive for lymphoma. The mean number of days of hospitalisation was 43.3 days with many requiring antibiotic and GCSF support.
Summary / Conclusion: It is evident that very elderly patients still have an excellent survival against DLBCL using tailored therapy. However, a large proportion required prophylactic GCSF and in-patient therapy±longterm care due to their multiple co-morbidities and lack of appropriate support in the community. This should be taken into consideration when one is planning future haemato-oncology services.

B1998
A PROSPECTIVE STUDY ON THE COST AND UTILIZATION OF AZACITIDINE IN A CANADIAN PROVINCE: IMPLICATIONS FOR BUDGET IMPACT ANALYSIS (BIA)
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Background: Cost effectiveness analysis (CEA) and incremental cost effectiveness ratio (ICER), based on data generated by Phase III trials, are often used to fund expensive drugs. Innovative drugs with proven efficacy may not be approved if they exceed arbitrary thresholds. The UK agency NICE initially did not recommend use of azacitidine (AZA) despite clinical efficacy and survival advantage based on a phase III trial. (Fenaux et al. Lancet Oncol 2009; 10:223-32).
Aims: To study the use and cost of azacitidine in the province of Manitoba (MB) and implications for cost analysis.
Methods: In MB (population 1.2 million), AZA became available in 2009 on compassionate basis and was later funded through CancerCare Manitoba. All Pts who were started on AZA from March 2009 to 30 April 2012 were prospectively studied. Drug utilization included any administration until 30 June 2012. Responses were as per IWG 2006 criteria, until 31 July 2012. Use was considered an 'approved indication' (AI) if it met Health Canada criteria: Intermediate-2 and high risk MDS or AML with 20-30% blasts. All other use was classified as a non-approved indication (NAI). Those on clinical trial were excluded. The cost of AZA per 100 mg vial was taken as $628 (listed price in Canada).
Results: A total of 27 Pts (8 females), received one or more doses of AZA for 20 AIs and 9 NAIs. In AIs, the diagnoses were: RCMD in5, RAEB-1 in3, RAEB-2 in 7 and AML (RAEB-t) in 5. The overall response was 50%: CR in 3 (15%), marrow CR with hematologic improvement (HI) in 2 (10%), and stable disease with HI in 5 (25%). Diagnoses in NAIs were: (a) RCMD (Int risk-1) in 2 (b) AML (blasts >30%) in 5 (with relapse after CR, failure of standard chemotherapy, or as a bridge to BMT) and (c) Relapse after BMT in 2 (these Pts had earlier received AZA for AIs). Response in NAIs was 11% (Stable disease with HI in 1). Detailed utilization and cost analysis is shown in Table 1. In the AZA 001 tri-

al, a median of 9 cycles were given at a schedule of 75mg/m^2 per day for 7 days, every 4 weeks. For an adult with BSA ≥1.4 mg/m^2, 2 vials of 100mg/day are needed (including wastage). Hence the estimated cost per cycle is $8,792. The projected cost of treatment per Pt is $ 79,128. Our study showed that the actual cost per Pt for AIs was $ 51,464. The median number of cycles given was less than in the AZA 001 trial (5 vs. 9) and in 33% cases, the doses were reduced or cycles attenuated. Our results are similar to a larger study from France, where patient received a median of 6 cycles (Itzykson et al. Blood 2011. 117: 403-11) Use in NAIs represented 18.9% of the cost incurred in AIs.

Utilization of Azacitidine in Manitoba over 3 years

Indications for AZA use	Median cycles (range)	Total cycles	Reduced dose or duration	Total vials used	Total cost ($)	Cost per cycle ($)	Cost per Patient ($)
Approved (n=20)	5(1-25)	141	33%	1639	1,029,292	7300	51,465
Non-Approved (n=9)	3(1-5)	23	9%	311	195,308	8492	21,701
AZA,azacitidine; $, Canadian dollars							

Summary / Conclusion: The importance of opportunity costs and budget impact have not received emphasis in recent economic literature. For decision makers, budget impact may have more relevance than ICER alone. The critical factors in a budget impact analysis (BIA) are the number of patients utilizing the drug and the magnitude of use. Data from RCTs may overestimate the amount of drug used per patient, while any information on the anticipated numbers for an orphan drug is likely to be unreliable. Moreover, clinical trials cannot provide information on the NAIs after licensing. To generate reliable data for BIA, phase IV studies are essential. For drugs which have proven clinical efficacy, conditional approval may be granted to generate phase IV data, before a final pharmaco-economic evaluation.

B1999

OVARIAN FUNCTION IN FEMALE SURVIVORS OF CHILDHOOD MALIGNANCIES

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Background: Chemotherapy-induced infertility is a common side effect observed in women of fertile age after treatment for malignant disease
Aims: to study gonadal function and fertility in female survivors of childhood malignancies.
Methods: Study included 30 female cancer survivors and 30 age matched healthy females as a control group. Data collected regarding; type of malignancy, age at diagnosis, duration on and off treatment, treatment received (radiation or chemotherapeutic regimens), sexual, menstrual, pregnancy and fertility histories were also recorded. Laboratory investigations included; T4, TSH, LH, FSH and AMH. Pelviabdominal ultrasound was done to estimate the mean ovarian volume.
Results: Among patients; 80% had normal menarche and 6 (20%) had delayed menarche (P>0.05). There was higher LH and FSH levels and lower AMH levels in patients (P<0.05) with no significant difference in thyroid function tests (P>0.05). Lower mean ovarian volume was observed among female survivors (6.32±2.31 cm^3) (P=0.041). There was a higher FSH & LH levels among female survivors of solid tumors compared to those with hematological tumors (P=0.05 and 0.04 respectively). There was a significant positive correlation between FSH level and patients' age at start of malignancy (r=0.65, P=0014), age of menarche (r=0.74, P=0.036) and duration of treatment (r=0.54, P=0.025).There was a significant negative correlation between age of menarche and AMH level (r=-0.61, P=0.03).
Summary / Conclusion: Female survivors of childhood malignancies had reduced ovarian reserve and reduced mean ovarian volume, especially those with older age, older age of menarche and longer treatment duration.

B2000

CHEMOTHERAPY ORDERING, PREPARATION AND ADMINISTRATION USING COMPUTERIZED OREDR ENTRY SYSTEM – HOSPITAL AMPANG (MALAYSIA) EXPERIENCE

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Background: Chemotherapy errors are serious as cytotoxics have very narrow therapeutic window, are toxic even at therapeutic dosages and cancer patients are generally a vulnerable group. The complexity of regimen and drug schedule predisposes to medication error, one study showed hemato-oncology has the highest error rate.
Aims: We developed a stand-alone application using Microsoft Access to order, prepare and administer chemotherapy in Hospital Ampang. The application is built to suit the work process and automating calculation process. The final chemotherapy request and administration form complies with most of the standard recommendations by American Society of Oncology safety standards on chemotherapy.
Results: From Feb 2011 to end of 2012, a total of4,567 chemotherapy regimens, comprising 27,842 cytotoxic drug items were requested and processed via this application. 99% of chemotherapy order was made via electronic ordering. Regular meetings and surveillance are conducted to rectify and prevent error in programmed formula. Users' survey conducted after 24 months showed 100% satisfaction over the old manual prescription process.

Summary / Conclusion: With the introduction of computerized order entry system, we experience marked improvement in efficiency of ordering and processing chemotherapy requests. Since chemotherapy regimens and doses are computerized and autocalculated, improved safety via minimizing errors by manual ordering is also achieved. We strongly recommend centers handling chemotherapy treatment to develop a similar system that is customized to suit the work process in their practices. It is worthwhile to invest time and money to develop such an application as it reduces medication errors and potential litigation, which are both costly and potentially fatal.

Acute lymphoblastic leukemia – Clinical

B2001

EVALUATION OF SERUM LEVELS OF MULTIPLE CYTOKINES AND ADHESION MOLECULES IN PATIENTS TREATED FOR ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) shows a high degree of heterogeneity due to a variety of mutations and mechanisms involved in leukemogenesis. This heterogeneity is often not fully reflected in standard treatment approaches. Cytokines are soluble molecules that take part in intercellular communication, with a specific role in cell proliferation control. Postconsolidation immunotherapy with interleukin-2 and histamine dihydrochloride improved the leukemia-free survival of adult patients with acute myeloid leukemia in complete remission. Increased levels of soluble adhesion molecules have been shown to correlate with better outcome. Further knowledge gained from multiple cytokine and adhesion molecule analysis should allow better diagnosis and disease management.
Aims: The aim of our study was to evaluate serum levels of multiple cytokines and adhesion molecules and changes related to disease activity in patients treated for AML.
Methods: A total of 20 AML patients, mean age 53.5±11.8 years, median 55.4, 7 males and 13 females, 4 with better risk, 7 with intermediate risk, 9 with high risk according to cytogenetics and molecular genetics, treated with cyclic chemotherapy (3+7, 2+5, HiDAC) alone or in combination with high-dose chemotherapy (FIAG-Ida, preparative regimen Bu/Flu/ATG) followed by allogeneic hematopoietic stem cell transplantation in 5 cases were studied. We evaluated serum levels of the following 22 cytokines and adhesion molecules: interleukins (IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-23), vascular endothelial growth factor (VEGF), tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma), epidermal growth factor (EGF), monocyte chemotactic protein-1 (MCP-1), E-selectin, L-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1). All biomarkers were measured by biochip array technology on Evidence Investigator analyzer (Randox) at the diagnosis of AML (active leukemia) and in durable complete remission (CR) at circa 6 months after completion of chemotherapy.Probability values (p) < 0.01 were considered statistically significant.
Results: Comparing serum cytokine and adhesion molecule levels in active leukemia and in durable CR, we found significant increase in serum IL-7 (6.43±4.91 ng/L vs. 21.36±7.72 ng/L; P<0.00005), EGF (17.52±16.01 ng/L vs. 48.66±28.15 ng/L; P<0.0001) and VEGF (65.48±41.33 ng/L vs. 158.92±60.75 ng/L; P<0.0005). On the other hand, we found significant decrease in serum L-selectin (2430.93±1032.85 mcg/L vs. 1340.15±308.27 mcg/L; P<0.0005) and IL-13 (6.97±3.41 ng/L vs. 4.02±4.02 ng/L; P<0.01). Serum levels of other evaluated cytokines and adhesion molecules were without significant differences.
Summary / Conclusion: Our results indicate that serum levels of some cytokines and adhesion molecules (IL-7, EGF, VEGF, L-selectin, IL-13) are significantly altered in patients treated for AML, reflecting activity of the disease. Whether these alterations could serve as a prognostic marker for AML is not known. To assess their predictive value for patient outcome, further studies comparing cytokine and adhesion molecule levels with established prognostic markers (cytogenetics, molecular genetics) in a larger number of patients is necessary.
The work was supported by Specific research project "Analysis of defined prognostic factors in acute myeloid leukemia" (FMHS) and by a long-term organisation development plan 1011 (FMHS).

B2002

PREDICTION OF CLINICAL RESPONSE USING A PERSONALIZED MEDICINE *ex vivo* TEST IN A PATIENT DIAGNOSED WITH MIXED PHENOTYPE T/MYELOID ACUTE LEUKEMIA

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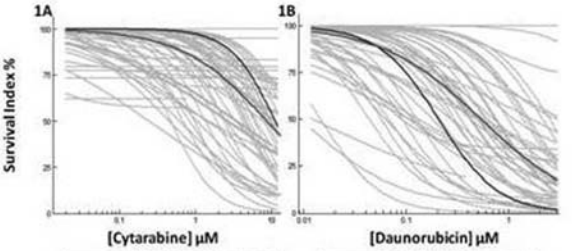
Background: Mixed phenotype acute leukemia, T/ myeloid, is a rare entity accounting for less than 1% of leukemias. Treatment of these entities is not well defined, since response to standard chemotherapy regimens is usually poor. A patient presenting with this mixed lineage leukemia was part of a multicenter, prospective, non-interventional study of the PETHEMA group conducted by Vivia Biotech.
Aims: The objective of the study is to determine the validity of a personalized drug sensitivity test to predict clinical response based on the analysis of leukemic cell death *ex vivo* in rare leukemias.
Methods: A bone marrow sample was collected at diagnosis and processed in the Vivia lab. The sample was diluted in its entirety (retaining erythrocytes and plasma) and plated with the drugs, each at 8 concentrations. The plates were incubated 48-hours, then analyzed by our automated flow cytometry-based ExviTech© platform. Malignant cell death was determined via labeling with appropriate monoclonal antibodies and AnnexinV-FITC. Dose-response curves for 16 drugs were generated, including Cyt, Dau, Flu and idarubicin (Ida) were run. The key parameters are the efficacy of the drug to deplete cells, and the potency of the drug measured as the concentration at which 50% of the cells are eliminated (EC50). A survival index is computed for each drug, calculating the percentage of malignant cells remaining. The lower the survival index, the more effective the drug is in eliminating the malignant population. The Effective Maximum (Emax) is the maximum possible effect for the drug, thus an Emax of 0% indicates that there are no surviving malignant cells. Vivia's results were not used to drive treatment.
Case report: A 77 year old man presented with a WBC of 37.54 x 10^9/L , Hemoglobin 12.7 gr/dl and platelets 64 x 10^9/L. Bone marrow aspirate showed 80% blast cells, and a diagnosis of mixed phenotype acute leukemia, T/ myeloid, was established. Cytogenetic studies showed two clones: 46, XY, del(5)(q15q33)[3] and 46, XY, del(5)(q15q33), del(6)(q15q25)[17]. Furthermore, oligolonal T-cell receptor rearrangement (VjA/VjB) was found. Induction chemotherapy with fludarabine (Flu) plus cytarabine (Cyt) was initiated. Twenty days later the patient remained in grade IV pancytopenia. Bone marrow aspirate showed persistence of 60% blasts, demonstrating chemo resistance. A DAOP chemotherapy including daunorubicin (Dau), Cyt, vincristine plus prednisone was administered. After 20 days, the patient showed complete neutrophil and platelet recovery. A bone marrow aspirate showed absence of leukemic blasts indicating that CR was achieved.
Results: Figure 1A displays the dose-response curves from 121 patients to Cyt (grey). The response of the patient referenced above (blue) to this drug was much worse than the average (red) with the drug having a survival index of only 48.3%, indicating that it was able to eliminate only 50% of the malignant cells. However, the patient appeared to be particularly sensitive to Dau, as seen in figure 1B. Shown are Dau dose-response curves from 102 patients (grey), with the patient's response in blue and the average response in red. Dau was both more potent (lower EC50) and very effective (survival index = 0) than the average patient response. The patient's sample also tested moderately sensitive to Ida but resistant to Flu and all other drugs tested.
Summary / Conclusion: Vivia's *ex vivo* analysis was able to predict the clinical response in this patient. This type analysis could prove useful in cases such as this where incidence is rare and standard treatment protocols are not known



Cyt	EMAX	EC50
Average,Red	22.1%	6.03µM
Patient, Blue	48.3%	11.2µM

Dau	EMAX	EC50
Average, Red	12.3%	0.80µM
Patient, Blue	0%	0.21µM

Chronic lymphocytic leukemia and related disorders

B2003

SERUM FREE LEPTIN, LEPTIN RECEPTOR AND INSULIN IN RELATION TO B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA RISK

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Background: Excess weight is now considered to be a risk factor for many types of cancer, including leukemia and lymphoma. Leptin, an adipocyte secreted hormone, and particularly free leptin, the biologically important form of leptin, reflecting accurately the body fat mass, modulates glucose and fat metabolism by improving insulin sensitivity and reducing intracellular lipids. Leptin has also been proposed to have a role in hematopoiesis and was not studied in depth in B-cell chronic lymphocytic leukemia (B-CLL).
Aims: In this case-control study, we attempted to investigate the contribution of serum free leptin, leptin receptor and insulin to B-CLL risk, taking also into account potential confounders including the family history of lymphohematopoietic cancer (LHC) and the body mass index (BMI). We also attempted to ascertain whether a relationship between free leptin and prognostic markers exists amid patients with B-CLL diagnosis.
Methods: Blood samples were collected from 95 cases with incident B-CLL, and 95 hospital controls, admitted for non-neoplastic and non-infectious conditions, matched on gender, age and year/month of diagnosis (±1 month) between 2001 and 2007. Serum insulin and leptin were determined by radioimmunoassay (Millipore and Linco Research, Inc. respectively). Serum leptin receptor levels (sOB-R) levels were measured using a commercially available ELISA (BioVendor Laboratory Medicine). Free Leptin Index (FLI) was calculated as the ratio of leptin to sOBR. Furthermore, serum lactate dehydrogenase (LDH), β_2-microglobulin (BMG), lymphocyte morphology and the surface expression of CD38 in >30% of B-CLL lymphocyteswere assessed. The statistical analysis of the data was performed using IBM-SPSS® version 20 for Windows.
Results: Patients with B-CLL presented on average a higher BMI as compared to control participants (27.8 vs. 26.7 kg/m^2; P<0.01). Significantly, more cases than controls had a family history of LHC (13 vs. 3 controls; P<0.01). Circulating leptin was significantly lower in cases as compared with controls (10.03 vs. 13.89 ng/mL, P<0.01). FLI was lower in cases than in controls (P=0.003). Cases with B-CLL exhibited higher sOB-R levels though not statistically significant at α=0.05. Among patients, FLI was positively associated with insulin (r=0.26, P=0.01). Also, FLI was negatively correlated with Binet stage, BMG, total lymphocyte count, LDH and atypical lymphocyte morphology (P<0.05). Finally, lower FLI was associated with B-CLL risk in unadjusted analyses as well as after controlling for age, gender, date of diagnosis, family history of LHC, BMI and insulin levels (OR: 0.23, 95% C.I.0.09-0.57; P=0.001).
Summary / Conclusion: FLI was found to be lower among cases as compared to controls, despite cases exhibiting a higher BMI. It has been recently shown that hypoleptinemia but not hyperleptinemia is linked to the risk for several malignancies. These results need to be confirmed in larger study populations, but if reproduced, they may suggest that leptin becomes dysregulated, or that other factors such as inflammatory cytokines secreted by lymphocytes suppress leptin expression at later stages of B-CLL process.

B2004

THE RESPONSE TO CHEMOTHERAPY AND IMMUNOCHEMOTHERAPY AMONG PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA ACCORDING TO THE NUMBER OF CELLS WITH A DELETION OF 13Q14

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Background: Nowadays, among patients with chronic lymphocytic leukemia (CLL), the response to the modern therapy depends on its type and the various prognostic factors, including chromosomal abnormalities. It is well-known, that the number of cells with a deletion of 13q14 (del13q14) affects the clinical course of CLL.
Aims: Assess the response to chemotherapy and immunochemotherapy in patients with CLL according to the number of cells with del13q14.
Methods: We identified 77 patients with CLL with the only del13q14. The median age was 61 years. Patients were divided into 2 groups according to the type of received treatment. The first group (n=35) was treated by the regime FC (fludarabine, cyclophosphamide), the second one (n=42) – by the regime RFC (rituximab, fludarabine, cyclophosphamide). Chromosomal abnormalities were determined by FISH during the time of diagnosis before specific therapy. Patients were divided into 2 subgroups according to the number of cells with del13q14. The first subgroup included 41 (53%) patients with del13q14 in ≥ 60% of cells, the second one – 36 (47%) patients with del13q14 in < 60% of cells.
Results: A complete remission (CR) was achieved in 7 (44%) patients among the 16 patients with del13q14 in < 60% of cells, received treatment FC, a partial remission (PR) – in 8 (50%) patients, no effect was observed in 1 (6%) patient. CR and PR were achieved among 3 (16%) and 7 (37%) patients accordingly from 19 ones of the first group with del13q14 ≥ 60% of cells. The lack of therapeutic effect was observed in 9 (47%) patients (p=0.021). Median follow-up of patients was 68 months. Median overall survival (OS) in patients with del13q14 in < 60% of cells, received treatment FC, has not been achieved, and in patients with del13q14 in ≥ 60% of cells it was 53 months (P=0.014). Median time to progression (TTP) in patients with del13q14 in < 60% of cells was 30 months, and with del13q14 in ≥ 60% of cells – 19 months (P=0.021). The effect of the therapy RFC was independent of the number of cells with del13q14. CR was achieved in 15 (75%) patients among the 20 patients with del13q14 in < 60% of cells, received treatment RFC, PR – in 4 (20%) patients; CR and PR were achieved in 14 (64%) and 6 (27%) cases respectively (P=0.644; p=0,849) among 22 patients with del13q14 in ≥ 60% of cells. Medians OS of patients received treatment by RFC, and having a different number of cells with del13q14, have not been achieved during the follow-up (P=0.438). However, the TTP was dependent on the number of cells with del13q14. Thus, median TTP in patients with del13q14 in < 60% of cells was 62 months; among patients with del13q14 in ≥ 60% of cells it was 43 months (P=0.041).
Summary / Conclusion: Determination of the number of cells with del13q14 among patients with CLL has an important clinical significance and can be used as additional prognostic factors of response to the therapy, as well as for the selection of appropriate treatment programs.

B2005

SERUM FETUIN-A/A2HS-GLYCOPROTEIN LEVELS IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Obesity and overweight may be considered as risk factors for many types of cancer, including leukemia and lymphoma. A significant association of excess body weight with insulin resistance (IR), characterized by hyperinsulinemia has been well documented. There is also evidence that IR is involved in several malignancies related to obesity. Fetuin-A, also known as α2HS-glycoprotein, a hormone synthesized mainly in the liver reflecting ectopic hepatic fat deposition, could cause IR via inhibition of insulin signaling; modulate adipocyte function by downregulating adiponectin expression; and interact with various growth factors influencing tumor initiation and progression.
Aims: In this case-control study, we explored the role of serum fetuin-A in conjuction with insulinemia in relation to B-cell chronic lymphocytic leukemia (B-CLL) risk. We also investigated its association with several established prognostic factors for B-CLL.
Methods: Blood samples were collected from 95 cases with incident B-CLL, and 95 hospital controls, admitted for non-neoplastic and non-infectious conditions, matched on gender, age and year/month of diagnosis (±1 month) between 2001 and 2007. Serum insulin was determined by radioimmunoassay (Millipore). Serum fetuin-A levels were measured using an enzyme linked immunosorbent assay (Biovendor R&D). Moreover, serum lactate dehydrogenase, β_2-microglobulin, lymphocyte morphology and the surface expression of CD38 in >30% of B-CLL lymphocyteswere determined. The statistical analysis of the data was performed using IBM-SPSS® version 20 for Windows statistical software package.
Results: Patients with B-CLL had a higher body mass index (BMI) compared to controls (27.8 vs. 26.7 kg/m^2; P=0.01). Circulating fetuin-A was significantly lower in cases as compared to controls (241.9 vs. 288.8 μg/mL, P=0.005). In multivariate analysis, the highest tertile of circulating fetuin-A levels is associated with an odds ratio of 0.21 (95% CI: 0.08-0.52) compared with the lowest tertile adjusting for age, gender, date of diagnosis (matching factors), body mass index (BMI), family history of LHC, and serum insulin levels. Because BMI is a significant risk factor for B-CLL in this dataset, stratifying by BMI revealed that only among overweight/obese subjects (BMI≥25 kg/m2), those with hypofetuinemia presented a higher risk for B-CLL before and after adjustment with matching factors, family history of lympohematopoietic cancer and insulin levels. Finally, no significant association was observed between fetuin-A and B-CLL prognostic markers (P>0.05).
Summary / Conclusion: This study suggests that low serum fetuin-A may be associated with B-CLL risk in the context of overweight/obesity. In B-CLL patients, fetuin-A levels were lower, possibly due to a compensatory response to the upregulation of other inflammatory cytokines/factors which may be ontologically linked to B-CLL. The mechanisms underlying fetuin's role in B-CLL etiopathogenesis require further investigation.

Myeloma and other monoclonal gammopathies – Clinical

B2006

THE POTENTIAL ROLE OF DEXA-BEAM IN MULTIPLE MYELOMA FOCUSING ON EXTRAMEDULLARY DISEASE – A CASE SERIES

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Background: Extramedullary (e) disease in multiple myeloma (MM) is characterized by an aggressive biology and an adverse prognosis especially when occurring at relapse. Published therapeutic regimens are inconsistent and results contradictory, especially regarding use of the "novel compounds". Due to the high proliferation found in eMM lesions we analyzed outcome data of patients treated with a non novel-agent-based therapy, the Dexa-BEAM protocol which is usually used in relapsed high grade lymphoma.

Aims: Evaluating the potential role for applying lymphoma-directed treatment to extramedullary multiple myeloma.

Methods: Retrospective analysis of MM patients having received Dexa-BEAM (including dexamethasone, carmustine, cytarabine, etoposide and melphalan) at a single center in the years 2007-2012. In all, 18 patients were identified, 11 of whom had extramedullary disease.

Results: Objective responses (≥PR) after Dexa-BEAM were achieved in more than half of patients with extramedullary disease (6/11); furthermore, a high-dose consolidation strategy with autologous or allogeneic stem cell transplantation improved upon the depth of remission in two-thirds of eMM patients (4/6) with ongoing remissions in three patients. In contrast all patients without consolidation relapsed. Progression-free survival after Dexa-BEAM was short in both patient groups with intramedullary or extramedullary myeloma with a median of 3 and 4 months, respectively. Toxicity was relevant with one treatment-related death and grade 3 and 4 toxicities in all 18 patients.

Summary / Conclusion: Dexa-BEAM may be an effective induction regimen in medically fit patients with extramedullary manifestations to regain disease control prior to an intended auto or allogeneic transplantation. For Patients who are not eligible for transplantation, alternative therapies should be chosen.

Myeloproliferative neoplasms – Clinical

B2007

COMPASSIONATE USE PROGRAM (CUP) WITH RUXOLITINIB IN PATIENTS WITH PRIMARY MYELOFIBROSIS (PMF), POST–POLYCYTHEMIA VERA MYELOFIBROSIS (PPV–MF), AND POST–ESSENTIAL THROMBOCYTHEMIA MYELOFIBROSIS (PET-MF)

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Background: Myelofibrosis (MF) is a myeloproliferative neoplasm (MPN) characterized by ineffective hematopoiesis and symptomatic burden such as cytopenias, and splenomegaly. The prevalence of MF symptoms is relatively uniform across the 3 main subtypes: primary MF (PMF), post – polycythemia vera MF (PPV MF), and post – essential thrombocythemia MF (PET MF). The only potentially curative therapy is allogeneic hematopoietic stem cell transplantation (HSCT) but treatment-related mortality remains high. Recently ruxolitinib an oral Janus kinase 1/2 inhibitor (JAK-1/2) has been approved to treat patients with MF. The safety and efficacy of Ruxolitinib has been evaluated 528 patients from COMFORT I and COMFORT II trials

Aims: To report the first clinical experience in a CUP with an oral JAK–1/2 Inhibitor (ruxolitinib) in 88MF patients in Mexico

Methods: PMF patients and PPV/PET MF eligible for the ruxolitinib CUP, were diagnosed according to 2008 WHO criteria, irrespective of JAK2 mutation status; classified as high risk; intermediate risk level 2; or, intermediate risk level 1 with an enlarged spleen; with a peripheral blood blast count of < 10%; adequate renal and liver function, platelet count >100x10^9/L. Therapy with ruxolitinib was administered to all patients. Doses were adjusted according to the platelet counts.

Clinical and demographic characteristics were assessed at baseline and during the follow-up. The analyzed characteristics were: Demographic: age, gender; Clinical: Risk group, Bone marrow fibrosis grade, spleen size, ECOG, MPN subtype, JAK mutation, CBC counts; Spleen size at baseline and at week 52 of treatment

Results: The median age was 64.2 (Interval 41 – 75). Gender distribution was 46% female and 54% male. IPSS risk category distribution: 10% low risk; 53.3% intermediate – 1 risk; 26.7 % intermediate – 2 risk; and 20% high risk. Bone marrow fibrosis grade distribution was: Grade I 46.15%; Grade II 23.07%; Grade III 30.76%. Spleen length below costal margin: <10 cm, 80.7%; 10 to ≤20 cm, 11.5% and > 20 cm, 7.7% of patients. Spleen Average size was 9.58 cm below costal margin at the beginning of treatment. ECOG 0 was present in 15% of patients; $ECOG_1$, 73%; and $ECOG_2$, 12% of patients. The disease subtype distribution was: PMF 45%; PPV-MF 19% and PET-MF 36%. JAK mutation was positive for 46% of patients, 19% were negative, and 35% did not have mutation analysis.

The findings from the baseline to week 52 were: Splenomegaly decreased from 9.58 cm (avg) to 2.8 cm (avg) (70.77% of reduction); Median Hemoglobin level was 13.2 gr/dL at baseline versus 10.24 gr/dL (22.42% of decrease). Median Platelet count was 437,000/mm^3 at baseline versus 341,503/mm^3 at week 52 of treatment

Summary / Conclusion: Data showed demonstrate principal characteristics of subjects. This analysis shows that continuous oral ruxolitinib therapy can reduce splenomegaly in MF patients. Besides, efficacy of treatment on low risk and

intermediate-1 patients is shown for the first time with good results.
Further follow-up is needed to assess the long-term outcomes with respect to efficacy and safety symptoms in this cohort of Mexican patients.

B2008

SAFETY PROFILE OF RUXOLITINIB IN PRIMARY MYELOFIBROSIS, POST-POLYCITEMIA VERA MYELOFIBROSIS AND POST-ESSENTIAL THROMBOCYTHEMIA MYELOFIBROSIS IN A COMPASSIONATE USE PROGRAM (CUP) IN MEXICO

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Background: Since the discovery of JAK2 in 2005, JAK inhibitors have been the subject of multiple clinical trials in primary myelofibrosis (PMF), post-essential thrombocythemia myelofibrosis (PET-MF) and post polycythemia-vera myelofibrosis (PPV-MF). Ruxolitinib (INCB018424) is a JAK1, JAK2 and JAK3 inhibitor. Phase III studies (COMFORT-I and COMFORT-II) have demonstrated its effectiveness and a suitable safety profile, being anemia and thrombocytopenia the most common adverse events, but they rarely led to discontinuation of the drug.
Aims: Describe the safety profile of ruxolitinib in CUP for patients with PMF, PET-MF and PPV-MF in Mexico.

Table 1. Hematologic and nonhematologic adverse events observed in patients treated in Compassionate Use Program with Ruxolitinib in Mexico.

Event	Ruxolitinib (N=88) Total events = 21 (23.8%)			
Grade	All grades (%)	Grade 3 or 4 (%)	Intervention	Final outcome
Nonhematologic	(14.7)	(4.5)		
Nausea	1 (1.13)	1 (1.13)	DS	W
Alopecia	1 (1.13)	0	None	Resolved
Septic shock	2 (2.27)	2 (2.27)	DS	W/Death
Pruritus	1 (1.13)	1 (1.13)	O	Resolved
Tuberculosis	2 (2.27)			
Pulmonary	1 (1.13)	NA	TS	Resolved
Nodal	1 (1.13)	NA	DS	W
Pneumonia	1 (1.13)	NA	O	Death
Superior respiratory tract infection	1 (1.13)	NA	O	Resolved
Zoster reactivation	2 (2.27)	NA	O	Resolved
Complicated urinary tract infection	1 (1.13)	NA	O/DS	W/Death
Basocellular carcinoma of the ear	1 (1.13)	NA	¥	Resolved
Hematologic	(9)	(2.27)		
Cytopenias	4 (4.54)	2(2.27)		
Thrombocytopenia	2 (2.27)	2 (2.27)	TS	Resolved
Anemia	2 (2.27)	0	None	None
Disease progression	4 (4.54)			
AML	3 (3.4)	NA	DS	W/Death
CMML	1 (1.13)	NA	DS	W

AML: acute myeloid leukemia. CMML: chronic myelomonocytic leukemia. TS: temporal suspension of ruxolitinib. NA: Not applicable. DS: definite suspension. W: withdrawal from the program. ¥ Topical treatment with 5-fluoracil. O: Other treatment (antihistaminics, antibiotics).

Methods: Descriptive, retrospective and multicentric study addressing the safety profile of 88 patients with primary or secondary myelofibrosis (WHO criteria), intolerant or non-responding to previous treatments, not eligible for allogenic transplantation and enrolled in a CUP with ruxolitinib was analyzed.
Results: Eighty-eight patients that received at least one dose of ruxolitinib were included in the analysis, from September 2011 to February 2013. In this group, the incidence of adverse events was 23.7% (20/88 patients) and both hematologic and non-hematologic grade III and IV events were present in 6.8% (6/88 patients). Grade III thrombocythopenia developed in 2.27% (2/88 patients) and self-resolved with temporarily suspension of treatment. Grade I and II anemia also developed in 2.27% (2/88 patients) requiring transient transfusion support. Infectious complications presented in 10.2% (9/88 patients), 2 cases of septic shock (without microbiologic isolation), 2 cases of tuberculosis reactivation (pulmonary and nodal), 2 cases of zoster reactivation, 2 cases of respiratory tract infections and last a case of pyelonephritis. Disease progression during treatment was present in 4.54% (4/88 patients), 3 patients progressed to acute myeloid leukemia (AML) and 1 patient to chronic myelomonocytic leukemia. At last follow up the rate of withdrawal from the program was 10.2% (9 patients), being disease progression the main cause. Seven deaths (7.9%) have been reported so far (three cases of AML progression and four associated to infectious complications).
Summary / Conclusion: Adverse events were monitored continuously during the program. According to these results safety profile of ruxolitinib in CUP proved to be manageable, associated with few grade 3 or 4 adverse events and the percentage of patients who discontinued treatment due to adverse events was small (10.2%). Infectious complications were the most frequently reported non-hematologic adverse events. Interestingly in this population there were two cases of reactivation of tuberculosis (nodal and pulmonary), one resolved with conventional treatment and was able to continue in the study, and the other (nodal) had to discontinue the drug and was withdrawn from the program. In summary, this data showed that oral ruxolitinib therapy has a tolerable safety profile in patients with myelofibrosis, as previously reported in other international clinical trials.

Non-Hodgkin lymphoma – Clinical

B2009

EFFICACY AND SAFETY OF THERAPY WITH 90Y IBRITUMOMAB TIUXETAN, IN B CELL NHL PATIENTS OVER 65 YEARS OLD.

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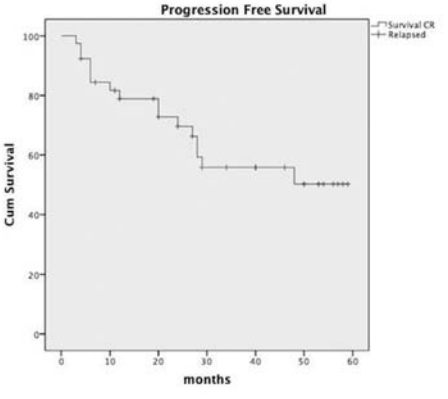
Background: 90Y Ibritumomab tiuxetan (90Y-IT) has become an efficient option to therapy in B-cell non-Hodgkin Lymphoma (NHL).
Aims: To analyse our updated information of patients treated with 90YIbritumomab/tiuxetan in a prospective study according clinical practice setting and to analyse treatment outcome in elderly patients.
Methods: A total of 39 B cell NHL patients were included in a clinical protocol conducted by a multidisciplinary team and treated in the same centre. According to the inclusion criteria: patients over 65 years old diagnosed as CD20+ NHL with neutrophils ≥1,5 x 10^9/L, platelets ≥ 100 x 10^9/L, bone marrow lymphocytes CD20+ ≤ 25%. All patients received 0,3 or 0,4 mCi /kg IV (88%) of 90YIbritumomab/tiuxetan and response evaluation was performed 12 weeks after. Period of study: September 2005/February 2013. The 90Y-IT was administered as consolidation of first line therapy (Rituximab alone, R-COP, R-CHOP21) or in relapsed/refractory status. Endpoints: objective response rate (ORR), time to relapse (PFS) overall survival (OS) and safety. Other clinical prognostic factors were observed to assess their possible influence upon treatment value.



Results: At the end of February 2013, 39 patients over 65 years old, had received treatment with 90YIbritumomab/tiuxetan and completed the evaluation protocol and were considered to analysis; M/F: 18/21, mean age 72.8 years (65-87); ECOG 0-1 92.3%. According OMS classification: NHL-follicular 27 (69.2%), mantle cell Lymphoma 7 (17.9%), DLBCL 4 (10.3%) and 1MALT (2.6%). Score distribution: low risk 19 (48.7%), intermediate 12 (30.8.2%) and advanced 8 (20.5%). Previous therapy schedules ≤2 (66.7%), >2 (33.3%). The median follow-up time: 46.0 months (95% CI: 4.0; 88.0), mean PFS: 39.5 months (95% CI: 32.2; 46.8) median NR (see Fig 1.). 13 patients received 90Y-IT as consolidation of first line therapy (33.3%) and 26 relapsed/refractory (66.6%). ORR was 84.6 % CR: 29 (74.3%); PR 4 (10.2%) and 6 failures (15.4%) in relapsed/refractory disease. Mean estimated OS since ^{90}Y-IT: 63.1 months (95% CI: 51.7; 74.4) and mean estimated OS since diagnosis 158 months. Median PFS was NR. The mean PFS for patients in consolidation therapy was 52.1 months (95% CI: 44.4; 59.7), but any NHL-follicular patients in consolidation (11) have been relapsed even dead. Safety: thrombocytopenia being the most frequent, G3-4 (35.9%), median time to developed haematological toxicity: fourth week, and neutropenia G3-4 (41.0%), the median time to recover normal values was 4.2 and 2.6 weeks respectively. In 5 (12.9%) of patients red blood cell transfusion was required, and 10 platelet transfusions (25.6%). The most frequent non haematological toxicity was asthenia. One patient developed a severe mucositis. Four patients have concomitant associated tumours (colon, breast, lung and prostate) and two patients over 77 years developed a rectum carcinoma after 18 months of 90Y-IT and another prostate and renal tumour after 8 years. Non-mortality related therapy was registered, at the end of study 10 patients have died, 6 of them in relapse.
Summary / Conclusion: In our experience 90Y Ibritumomab tiuxetan is a safety and effective therapy in patients with NHL over 65 years. According to obtained PFS results, it seems like the use of this kind of therapy as used in early part of therapy offers good and maintained response rate with lower toxicity in this fragile population. The OS in this population was not inferior to observed in younger NHL patients.

Stem cell transplantation – Clinical

B2010

ALLOGENEIC VERSUS AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR NON PROMYELOCYTIC ACUTE MYELOID LEUKEMIA: 30 YEARS EXPERIENCE IN A SINGLE CENTER

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Background: The optimal post-remission treatment for Acute Myeloid Leukemia (AML) remains uncertain. Traditional comparisons of Allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT) versus Autologous Hematopoietic Stem Cell Transplantation (auto-HSCT) noted higher TRM (Treatment Related Mortality) but lower CIR (Cumulative Incidence of Relapse), with discordant results in Overall Survival (OS).
Aims: Retrospective study of 274 patients diagnosed of AML (Acute Promyelocytic Leukemia excluded) who underwent Hematopoietic Stem Cell Transplantation (HSCT) in our center between 1982 and 2011. We compared CIR, TRM, OS and Disease Free Survival (DFS) when receiving allo or auto-HSCT.
Methods: Cumulative incidences were used for CIR and TRM whereas Kaplan Meier curves and log-rank test were used for OS and DFS. Multivariate analysis with Cox Regression was used for controlling confounding factors.
Results: Patient and disease characteristics in allo (162p) and auto-HSCT (112p) were respectively: median age of 37,8(IQR 30-52) and 44,5(IQR 36-56), secondary AML in 20,2% and 9,8%, failure after Induction course in 15,8% and2,8%, complete remission pre-HCST in 87,1% and 97,3% and year of HSCT ≥2005 in 46,9% and 14,3% .There was no statistical difference in any other characteristic (cytogenetic risk and WBC count at diagnosis, included).
The CIR in allo and auto-HSCT was respectively: 17,7%(CI 12,1-24,2) and 31,7%(CI 23,3-40,5) at 1 year and 24,2%(CI 17,5-31,5) and 50,0%(CI 40,2-59,0) at 5 years, with no statistical difference if the HSCT was perfomed before or after 1997(start to quantify the pre-HSCT minimal residual disease by flow citometry). The cumulative incidence of TRM in the earliest period (≤1996) for allo and auto-HSCT was respectively: 30,0%(IC 16,8-44,4) and6,5%(IC1,7-16,1) at 1 year and 35,0%(IC 20,8-50,0) and8,7%(IC2,8-18,9) at 5 years. However, the cumulative incidence of TRM in the latest one (>1997) was respectively: 16,4%(IC 9,8-24,4) and1,7%(IC 0,1-8,0) at 1 year and 25,2%(IC 14,3-37,7) and3,5%(0,7-10,8) at 5 years. The OS in the earliest period for allo and auto-HSCT was respectively: 39,6%(CI 26,6-52,4) and 60,8%(CI 46,1-72,6) at 1 year and 28,3%(CI 17,0-40,7) and 45,1%(CI 31,2-58,0) at 5 years. And the OS in the latest one was respectively: 65,6%(CI 55,8-73,8) and 70,3%(CI 57,1-80,2) at 1 year and 46,8%(CI 34,1-58,5) and 47,7%(CI 34,0-60,1) at 5 years. Any significant results in the multivariate analysis neither in the log-rank test regarding OS differences between allo and auto-HSCT were observed. Covariates such as elderly, elevated WBC count at diagnosis, high cytogenetic risk, early period of HSCT or secondary AML showed worse prognosis, but only MRD was a strongly outcome predictor. The DFS in the earliest period for allo and auto-HSCT was respectively: 50,0%(CI 33,8-64,2) and 65,2%(CI 49,6-77,0) at 1 year and 37,5%(CI 22,9-52,1) and 45,6%(CI 30,8-59,1) at 5 years. And the DFS in the latest one was respectively: 66,9%(CI 56,5-75,3) and 63,2%(CI 49,2-74,2) at 1 year and 51,7%(CI 37,3-64,3) and 46,5%(CI 32,6-59,4) at 5 years. 74% of the patients who underwent auto-HSCT have ECOG 0 the date of the last follow-up and 6 patients over 14 who relapsed after auto-HSCT were rescued with allo-HSCT.
Summary / Conclusion: In our series, we have found no statistical difference between allo and auto-HSCT in OS and DFS in any period. Despite the higher CIR, we have observed a higher survival in auto-HSCT in the earliest period which turns to get similar nowadays because of the decrease in TRM, more significative in allo-HSCT.

Hematopoiesis, stem cells and microenvironment

B2011

LOCAL RENIN-ANGIOTENSIN SYSTEM IN NORMAL HEMATOPOIETIC AND MULTIPLE MYELOMA-RELATED PROGENITOR CELLS

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Background: Local hematopoietic renin-angiotensin system (RAS) affects the essential steps of hematopoiesis in the bone marrow (BM) microenvironment. Malignant blood cells, including multiple (MM) myeloma cells, are derived from the clonal neoplastic stem cells within a complex series of pathological proliferative steps. Local BM RAS could affect neoplastic tumoral blood cell production. Local RAS is effective even in the embryonic hematopoiesis.The prominent functions of local RAS in primitive hematopoiesis further support the hypothesis that local autocrine BM RAS could also be active in the neoplastic hematopoiesis.
Aims: The aim of this study is to search critical RAS elements in normal CD34+ hematopoietic stem cells and multiple myeloma-related progenitor cells. For this aim, CD34+ hematopoietic cells obtained from the healthy allogeneic hematopoietic stem cell transplant (Allo-HSCT) donors and from the MM patients undergoing autologous stem cell transplantation were analyzed via qRT-PCR. Normal BM cells obtained from hematologically normal people were also searched to detect the impact of precursor cell compartment on the RAS expressions. Elucidation of the status of the local RAS molecules in the early and neoplastic hematopoiesis represents a clinically relevant basic research area for better understanding the biology of the diseases.
Methods: The study group comprised the total bone marrow cells (CBM) and the CD34+ stem cell samples (CD34+CBM) of 9 healthy donors for allogeneic peripheral stem cell transplantation, and the CD34+ stem cell samples (CD34+MM) of 9 multiple myeloma (MM) patients undergoing autologous peripheral stem cell transplantation. The diagnoses of multiple myeloma were reached based on the criteria of the International Myeloma Working Group. At the time of the sample collection, all of the patients were in good health and well hydrated. We searched for the gene expression of the major RAS components (ACE I, ACE II, RENIN and ANGTS) in healthy hematopoietic cells and myeloma cells by qRT-PCR.
Results: RENIN, ANGTS, and ACE I mRNA expression levels of CBM were significantly higher than those in myeloma patients (P=.03, P=.002, and P=.0008, respectively). Moreover, RENIN and ANGTS mRNA levels were significantly higher in CD34+ stem cell samples of healthy allogeneic donors compared to those in myeloma patients (P=.001 and P=.01). However, ACE I expression levels were similar in CD34+CBM and CD34+MM groups (P=.89). Other 'RAS pathway members' (ACE II, AGTR I and AGTR II) expressions were also examined and expressions of those were not at detectable levels, and no significant differences were determined between all groups.
Summary / Conclusion: RENIN and ANGTS mRNA expressions were significantly higher in normal CD34+ hematopoietic stem cells of in comparison to the myeloma-related progenitor cells (P=0.001 and P=0.01, respectively). Likewise, RENIN, ANGTS, and ACE I mRNA expression levels of CBM were significantly higher than those in the myeloma patients (P=.03, P=.002, and P=.0008, respectively). However, ACE I expression levels were similar in CD34+CBM and CD34+MM hematopoietic cells (P=.89) . Those findings support our original hypothesis that there is a biologically active local RAS in the hematopoietic system in normal and pathological states. BM AT1r expression levels of myeloma patients showed a positive correlation with their BM infiltration pattern and tumor load, indicated by serum Beta 2 Microglobulin levels.Our results about myeloma-related progenitors in this study provide an additional clue for the local RAS effects in the pathobiology of multiple myeloma.

B2012

LOCAL HEMATOPOIETIC RENIN-ANGIOTENSIN SYSTEM IN MYELOID VERSUS LYMPHOID HEMATOLOGICAL NEOPLASTIC DISORDERS

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Background: There is preliminary evidence that local BM RAS could affect neoplastic hematopoiesis. Over-expression of the angiotensin-converting enzyme (ACE) (CD 143) surface antigen in leukemic myeloid blast cells have been detected by flow cytometric analyses. Moreover, a positive correlation has been found between the ACE and BM blast count. ACE and p53 expressions were detected in the CD34+cells of patients with acute leukemia during and after induction chemotherapy. ACE-expressing macrophages in lymph nodes of Hodgkin's disease have been detected. The renin system is present in the K562 leukemic cell line in vitro model. Multipotential, hematopoietic malignant K562 leukemic blast cells also exhibited significant expressions of *RENIN, ANGIOTENSINOGEN (ANGTS)* and *ACE*.
Aims: The purpose of this study is to research mRNA expressions of the essential RAS elements (*RENIN, ANGTS, ACE1* and *ACE2*) in myeloid and lymphoid hematological neoplastic disorders. Elucidation of the presence of local RAS in the neoplastic myeloid and lymphoid pathological hematopoiesis is important because targeting the actions of local RAS may represent a valuable therapeutic option for the management of cancer.
Methods: Forty-six patients with newly diagnosed myeloid (AML, biphenotypic leukemia, CML) or lymphoid (CLL, NHL, B-ALL, T-ALL) hematological disorders were included in the study. Samples from BM were collected prior to the chemotherapy administered to each patient.We searched for the gene expression of the major RAS components (*ACE1, ACE2, RENIN* and *ANGTS*) in myeloblasts and lymphoid hematopoietic cells by qRT-PCR.
Results: In the lymphoid group, the median expression values of *RENIN, ACE1, ACE2* and *ANGIOTENSINOGEN (ANGTS)* mRNAs were 1.96%, 0.42%, 0.00% and 0.00%, respectively; in the myeloid group, 0.73%, 1.55%, 0.04% and 0.006%, respectively. In the lymphoid group, *RENIN* levels were significantly higher (*P*=0.001), whereas *ACE1* and *ACE2* levels were significantly higher in the myeloid group (*p* values were 0.013 and 0.010, respectively). *ANGTS* levels were similar in both groups. In patients with non-ALL lymphoid malignancies, *RENIN* expressions were significantly higher when compared to ALL patients (*P*=0.004). All patients with active disease had significantly higher *RENIN* mRNA expression levels than patients without active disease (2.03% vs 0.30%) (*P*=0.034).
Summary / Conclusion: These data support the original hypothesis that there is a local RAS in the BM affecting physiological and pathological hematopoiesis. The result indicates that the activities of local RAS may differ in distinct disease states such as leukemia and lymphomas.

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